Novel approaches to an automated decision support system for on-farm management of internal parasites of small ruminants

by

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A thesis submitted to the University of Pretoria, South Africa, in accordance with the requirements of the degree of Doctor of Philosophy in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science.

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February 2016

DEDICATION

This thesis is dedicated to the memory of my father, **Babayani James Nyewa**, who unfortunately didn't stay in this world long enough to share with me this milestone achievement.

AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Pretoria and was approved as Project Number V075-11 by the University of Pretoria (UP) Animal Ethics Committee.

The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted neither for any other degree nor to any other University in the world for examination.

Signed: 

ACKNOWLEDGEMENTS

Special thanks go to my supervisors: **Dr Eric René Morgan**, for his generosity, guidance and straight-laced humour throughout my PhD. **Dr Jan Aucamp van Wyk**, for selfless sharing of his data, knowledge and wisdom and for his unparalleled attention to detail that I endured to finally cope by learning to punctuate prose.

Thank you to **Mr L.J. van Rensburg** and **Mr N. Masuluke** for assistance in carrying out relevant field and laboratory tests as well as validation of results during the trials. Also, thank you to **Ms R.R. Coetzee** for data capture and acquisition of supplies during the trials and **Ms Maria Mtsweni**, Librarian at Jotello F Soga Library (UP), for her knack in finding me reference material after having exhausted all my MeSH terms.

I gratefully acknowledge the support and generosity of the UK Government through the Department for International Development (DFID) and the Biotechnology and Biological Sciences Research Council (BBSRC, grant number BB/H00940X/1), the National Research Foundationl (NRF) of South Africa, and the Red Meat Research and Development of South Africa, without which the present study could not have been completed.

And last but certainly not least, thank you to my: (i) **Wife**, for supporting me always in all my endeavours; (ii) **Mother**, for raising me to be the person I'm today; and (iii) **Kids**, for dealing with me understandably throughout my postgraduate studies, at times worlds away.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

THESIS SUMMARY

The global problem of anthelmintic resistance (AR) in small ruminants has prompted a paradigm shift in the approach to anthelmintic-based control of parasites, in order to delay the onset and propagation of AR. This shift is from the conventional approach of dosing the whole flock on a regular basis, either routinely or in response to observed effects of worm infection, to a sustainable integrated parasite management (**s**IPM) approach. This approach uses targeted selective treatment (TST), which consists of treating only those individuals within a flock or herd that are in need of treatment, and / or targeted treatment (TT) strategies, whereby the flock or herd is treated in response to indicators of high risk of disease or production loss. However, at present, the paradigm shift risks is still largely a theoretical concept due to limited practical adoption by farmers, attributed mainly to implementation complexity and labour demands associated with application of TST and TT, in the face of conflicting advocacy for their implementation. A set of tools involving epidemiological modelling and monitoring of animal activity were explored in this study, to facilitate the efficient application of TST and TT strategies, by supporting farmer treatment decisions. The outcomes are expected to contribute positively towards a global effort to increase practical adoption and sustenance of TST and TT strategies amongst livestock farmers.

A novel predictive epidemiological model framework was developed for haemonchosis in sheep. The model was parameterised and tested using meta-data covering one transmission season on a commercial Merino farm in South Africa that applied a FAMACHA©-based TST strategy for *Haemonchus contortus* control. The model incorporated farm management practices and *H. contortus* transmission dynamics. After model parameterization from the literature and expert opinion, and model fitting to observed data, the model was applied to analyse the application of FAMACHA©-based TST strategy. Sensitivity analysis was used to identify potential areas for optimisation. It was deduced from the model that sustained application of FAMACHA©–based TST strategy led to a decrease in *H. contortus* cases over time. Thereafter, observed cases did not reflect short-term changes in infection pressure well enough to optimise the model for within-season decision support. Also, the model suggested that to avoid significant production losses, all the animals in a flock

needed to be evaluated competently at relatively short intervals of approximately seven days. The predicted risk of infection with *H. contortus* at each of the serial FAMACHA© evaluation events was correlated with weather elements (rainfall, temperature and entropy) to determine which elements were the most significant drivers of infection at each evaluation interval on this particular farm. From this it was deduced that the risk of infection was significantly associated with total rainfall and average temperature from five weeks up to one week before each FAMACHA© evaluation event. The model showed adequate flexibility to capture the practical complexities of the FAMACHA©-based TST strategy as manifest on different farms. Its wider application could therefore yield insights into optimisation of the system for local conditions.

The second part of the thesis developed the concept of animal activity monitoring as an indicator of the need for anthelmintic treatment, as part of TST and TT strategies. This focused on developing and optimising a practical system for field use, including data management and interpretation. A prototype relatively unsophisticated remote activity level monitoring system was evaluated to establish key performance measures when placed on sheep, compared with baseline performance under controlled conditions. The system was based on accelerometers, intended to be attached to sheep, and a means of data transmission, storage and analysis to enable tracking of activity levels over time from a remote location, without the need to capture or handle the animals. Data transmission rate (DTR) was measured as a function of (i) Common obstacles to transmission found on farms; (ii) Distance between the telemeter tags and tag reader; (iii) Movement of telemeter tags and (iv) Disposition and number of tags within the reader's read range. This was done initially through hand simulation movements of telemeter tags. The activity monitoring system was then deployed on sheep on study farms with the set-up optimised, based on established performance measures. Subsequently the system was explored, using data from the tagged animals, for its potential to detect changes in activity associated with health status. More specifically, the system was evaluated for the potential of relayed activity scores to act as a risk indicator for clinical infection with *H. contortus*. This was done by characterising changes in animal activity between observed changes in behavioural and health states.

The activity monitoring system was able to detect changes in activity associated with: (i) Transition from resting to running at individual animal level; (ii) Onset of lameness and recovery from it at individual animal level; and (iii) A defined daily husbandry management routine on a farm at flock level. However, individual telemeter tags were found to perform differently from each other. These differences led to random measurement error in activity level scores, which confounded associations between activity level and natural *H. contortus* infection. This resulted in activity level score, as measured using this particular generation of tags, being judged to be a non-discriminatory predictor of clinical infection with *H. contortus*. However, an evident decrease in mean activity level score with increase in natural *H. contortus* infection burden was discernible even though the association was not statistically significant. Remote activity monitoring using such relatively unsophisticated systems therefore holds promise as a detector of haemonchosis in sheep, and warrants further investigation.

Outcomes of this work support the case for continued exploration and application of case-based predictive epidemiological models as an alternative to more traditional, burden-based model frameworks in the field of veterinary parasitology. Cases of disease matter directly to the application of TST strategy, while parasite burden matters indirectly, as high burdens do not necessarily translate directly into either disease or production loss in resilient animals. The model framework developed in this thesis could, after further validation, be applied to the prediction of future cases. This can be done in a form of a ‘black-box’ decision-support tool, needing few easily measured variables to be input at farm level. An output of predicted future case numbers could be presented as a risk index and linked to practical recommendations for risk mitigation.

Continued work on the remote activity level monitoring system is also justified. Particularly important will be improved telemeter sensitivity to animal movements and quality control in telemeter assembly, as well as incorporation of a streaming data analysis interface. The prospects of a risk-based evaluation of an animal or flock, using activity level as a risk indicator for clinical infection with parasites, appears feasible. Activity level recording with unsophisticated systems, as used here, also hold promise as a universal risk indicator for almost all types of malaise because

it is dependent on degree of lethargy, a clinical manifestation of almost all forms of infectious disorder. This system also has the potential to identify those individual animals showing the effects of parasite infection, prompting treatment as and when the need arises, thus reducing labour and production costs when compared to present recommendations for scheduled whole-flock FAMACHA© evaluations at set intervals.

Both the epidemiological model and remote activity monitoring provide novel technological solutions for supporting TT and TST in a **s**IPM framework. To this end, further refinement and scientific evaluation, coupled with translation of such tools to the farm, are needed in a way that simplifies, rather than complicates, adoption of **s**IPM by farmers, in support of sustained parasite control and global food security.

CHAPTER 1

General Introduction

Helminthosis is one of the major constraints to the successful production of livestock throughout the world (Bath and De Wet, 2000; Waller and Thamsborg, 2004; Jabbar et al., 2006; Jackson et al., 2009; Hussain et al., 2010). From a wide consultation with resource-poor farmers of South-East Asia and Africa, south of the Sahara, Perry et al. (2002) reported helminth parasitism to have the highest global index as an animal health constraint to the rural poor. Also, Bath and De Wet (2000) reported that "…worms form the most prevalent, and most important group of diseases in sheep and goats. More money is spent on combating these diseases, and more economic losses are experienced by farmers than for any other grouping of diseases" (Njuki and Sanginga, 2013). As livestock populations are projected to increase in the coming years to meet demand for livestock and livestock products in the developing world (Thornton, 2010), the problem of helminth parasitism will become more pressing due to high production losses. These could render futile global efforts centred on livestock production to alleviate poverty amongst the rural poor.

1.1 Economic impact of nematode infection

A number of gastrointestinal (GIT) helminths of the family Trichostrongylidae infect domesticated ruminants and make a significant contribution to the animal health constraints associated with helminth parasitism. Economic impacts are felt by both the rural poor (Perry et al., 2002) in developing countries and commercial farmers in the industrialized countries of the world (Perry and Randolph, 1999; Jackson et al., 2009). In the United Kingdom (UK), for example, nematode infection in sheep alone is estimated to cost the sheep industry more than GBP 84 million per year (Nieuwhof and Bishop, 2005) and this level of impact is likely to be globally typical (Morgan et al., 2013).

In tropical/subtropical regions, *Haemonchus contortus* (Rudolphi, 1803) is predominant (Levine, 1963; Perry et al., 2002) due to its high biotic potential of up to 10 000 eggs per female per day, and rapid development of infective larvae under favourable warm and moist conditions (Gordon, 1981). It is also ranked the most economically important parasite of small ruminants in these regions due to its high

pathogenicity (Perry et al., 2002), resulting in acute disease outbreaks with high levels of mortality, particularly in young animals (Gordon, 1948). As an example, a drop of up to seven percent in haematocrit within seven days at peak season has been reported by Malan et al. (2001). In Kenya and South Africa annual financial losses directly attributable to *H. contortus* alone are between US$ 26 million and US$ 45 million (Krecek and Waller, 2006), while in India the annual costs associated with control of this parasite have been estimated at US$ 103 million (McLeod, 2004).

Ostertagiasis by the nematode *Ostertagia ostertagi* is the most important of the GIT helminth infections of cattle in temperate climates (Myers and Taylor, 1989). Even at sub-clinical infections, where the effects can be subtle, non-obvious and not easily measurable, it can have a profound economic impact on the performance of the whole farm, as illustrated by Van Der Voort et al. (2014) in dairy cattle.

These estimates excluded other society-level economic variables, such as uncertainty with regards to social security as a result of helminthosis, which has a huge impact on societies' states of well-being as noted by Wood (2003) and Perry and Grace (2009).

1.2 Risk factors for nematodosis

1.2.1 Ecological factors

These are environmental factors which determine the success or failure of the free–living phases of the parasite life cycle. Knowledge and understanding of these factors makes it possible to predict the spatial and temporal variation in the level of risk of infection of small ruminants (Gordon, 1948, 1973; Thomas, 1974; O'Connor et al., 2006). Hence, the exploitation of this knowledge is an integral part of parasite management.

1.2.1.1 Temperature, rainfall and sunlight

Temperature and moisture in the form of rainfall are the dominant variables influencing the free-living stages of trichostrongyloid nematodes (O'Connor et al., 2006). The developmental success of all trichostrogyloid nematodes in the free–living L1/L2 phase is highly influenced by temperature, with *Haemonchus contortus* most susceptible to low temperatures, followed by *Trichostrongylus. colubriformis*

and then *Teladorsagia circumcincta* (Levine, 1963; Crofton, 1965; Donald, 1968; Gibson and Everett, 1972; Jasmer and Wescott, 1986). Due to the influence of temperature on the first (L1) and second (L2) larval stages of the early free-living phase, the developmental cycle shortens with an increase in temperature, although higher temperatures within the development range also tend to increase mortality. However, the optimum temperature range for success of development to L3 varies for different species (Gibson and Everett, 1972; Hsu and Levine, 1977; Young et al., 1980; Salih and Grainger, 1982; Smith, 1990b; O'Connor et al., 2007b).

Moisture is also a crucial requirement for larval development to the infective larval stage (Chiejina and Fakae, 1989; Besier, 1992; Besier and Dunsmore, 1993b; Rossanigo and Grüner, 1995; Cheah and Rajamanickam, 1997; O'Connor et al., 2007a, b). Thereafter, both moisture and temperature become less important to survival (Thomas, 1974) due to protection from a double sheath comprising the L3's own sheath, together with the retained L2 cuticle. On the other hand, moisture is essential for migration of the L3 to more favourable microenvironments (O'Connor et al., 2006). The migration is facilitated by softening of the faecal crust by rain and a continuous film of moisture on herbage due to rainfall or dew (Silangwa and Todd, 1964; Levine and Andersen, 1973; Stromberg, 1997), as demonstrated by Wang et al. (2014).

Tromba (1978) implicated the ultraviolet (UV) component of sunlight in the survival rate of free-living L3 parasitic stages and this has recently been confirmed through trials by Van Dijk et al. (2009) on L3 of *Te. circumcincta*, *Nematodirus battus* and *H. contortus*. As to be expected, the latter species, being predominant in the tropics, showed more resilience to ultraviolet light than the other two species tested. Van Dijk et al. (2009) also suggested that the migratory behaviour of L3 on pasture, observed by Crofton (1948), is a means of defence against the harmful effects of UV.

Haemonchus contortus is predominant in summer rainfall regions (warm and moist) and less common in winter rainfall regions (cold and moist) with hot and dry summers. As with all other GIT nematodes, it is most susceptible to desiccation during the pre-infective stages (Rose, 1963; Waller and Donald, 1970; Rossanigo

and Grüner, 1995), indicating the potentiating effect of interaction between temperature and moisture on helminthosis in warmer regions. In these regions the high temperatures normally prevalent at the faecal and soil surface level, lead to rapid drying of faecal pellets (Young, 1983; Berbigier et al., 1990), with the potential to limit development of free-living stages of the parasite, due to lack of moisture in the microclimate (Hsu and Levine, 1977; Rossanigo and Grüner, 1995), particularly in the first 1-4 days after deposition of faecal pellets (O'Connor et al., 2007a). Thereupon, the hardened outer crust of the pats or pellets prevents emergence of the L3 that do manage to develop.

From bioclimatographs, early researchers estimated approximate 50 mm of rain to be the minimum per month required for outbreaks of haemonchosis (Gordon, 1948; Levine, 1963). In South Africa, McCulloch et al. (1984) suggested that a "…minimum amount of rain ….. over a 4-week period" was required for heightened *H. contortus* pasture infectivity. Also, Barnes et al. (1988) defined dependency of *H. contortus* on effective rainfall as in excess of 16 mm of rainfall over seven days, but added that the probability of an egg developing to an infective larva would decline with increase in length of time between egg deposition and effective rainfall.

Studies conducted subsequent to the above showed that it was more the temporal spread of rainfall on a given faecal deposit, rather than its total amount, which influenced *H. contortus* development (Besier, 1992; Besier and Dunsmore, 1993b; O'Connor et al., 2007a; Reynecke, 2007). From trials by O'Connor et al. (2007a) on the effect of amount, distribution and timing of rainfall relative to faecal deposition on development success of *H. contortus* eggs to L3 stage, it was shown that even though the faecal pellets are normally deposited with adequate moisture to allow for some development, early addition of moisture to faeces after deposition was essential for development to the infective stage. On the other hand, once developed, L3 can survive for considerable periods of time in the faeces, in relation to both the worm and host species. For instance, recovery of some L3 of *H. contortus* and *Haemonchus placei* (*H. placei*), which are known to be relatively short-lived on pasture, was shown up to 10 and 16 months, respectively, after deposition of faeces on irrigated pasture in South Africa (R. C. Krecek, unpublished results, 1990), quoted by Krecek et al. (1991).

While numerous studies have shown L3 to be considerably less sensitive than pre-infective stages, their survival is also influenced mainly by temperature and moisture (Barger et al., 1972; Besier and Dunsmore, 1993a). For instance, Barger et al. (1994), Gruner et al. (1989), Bang et al. (1990), and Cheah and Rajamanickam (1997) showed that, in general, survival times for infective larvae under tropical conditions were considerably shorter than under temperate conditions. In addition, once the crust has formed over faecal pats or pellets, the larvae which have developed are trapped until such time as they either die, or rain or other forms of moisture have softened the crust sufficiently for them to migrate out onto pasture. In this way, faecal pats or pellets act as a larval reservoir in dry conditions. For instance, from a field study conducted in a semi-arid area of South Africa, Reinecke (1960) reported that infective larvae of *H.placei* of cattle can survive for 105 days in autumn and winter, both in the dung and after migration to pasture, and result in serious verminosis after effective rain in the ensuing summer. Concerning migration of *H. contortus* from faecal deposits, Wang et al. (2014) reported from a series of experiments involving different amounts and patterns of simulated rainfall and ecological conditions that included relative humidity and hydration status of faeces, that, while rainfall is required for migration, relative humidity appears to act through faecal moisture content to modify the effect of rainfall on larval migration out of faeces.

1.2.1.2 Pasture type and slope

These can influence survival and migration of L3 in a variety of ways, for instance: (i) Irrigation of pasture results in reduced soil temperatures and increased humidity in the microclimate occupied by free-living trichostrongylid stages, relative to non-irrigated pastures. Together, these conditions are generally conducive to development and survival of *Haemonchus contortus* (*H. contortus)* free-living stages (Bullick and Andersen, 1978), as well as to migration of L3 onto herbage (Uriarte and Gruner, 1994); (ii) Pasture herbage green matter has in some circumstances been found to be a good predictor of *H. contortus* L3 availability to the host (Barger et al., 1972; Besier and Dunsmore, 1993b); and (iii) Topography of the pasture can also influence the distribution of infective larvae on pasture (McCulloch et al., 1984), particularly after heavy, high intensity rain where run-off down the slope tends to wash away faeces and potentially L3, mostly into pans or streams, where they are

diluted and less available to their hosts. Alternatively, lighter rainfall, with less run-off, may concentrate the infective larvae in localised depressions on pasture and constitute a higher degree of risk, on the one hand for verminosis due to a tendency of vegetation to grow more profusely on such patches, and, on the other, for dissemination of resistant worm populations to other properties

1.2.2 Host factors

1.2.2.1 Reproductive status

Reproductive status has an important bearing on susceptibility to endoparasitism. For instance, in trials by Malan et al. (2001), only 44.6% of lactating ewes were able to manage *H. contortus* challenge without a need for drenching, compared to 83% of dry and 70.6% of pregnant ewes, which shared the same pasture as a single flock. In addition, Vlassoff (1976) and Hoste at al. (2002a; 2002b; 2002c), reported respectively that ewes and high producing dairy goat does in their first lactation led to high levels of contamination of pasture with infective larvae. This could be due to nutritional stress during lactation (Whitlock et al., 1943), with protein-supplemented animals having been documented to have an increased ability to withstand the effects of parasitism (Knox, 1996; Coop and Kyriazakis, 2001; McClure, 2012). Also, the phenomenon of peri-parturient relaxation of resistance (PPRR), which occurs 3-4 weeks before and after lambing, is known to increase susceptibility to endoparasites (Barger, 1993; Houdijk et al., 2000; Houdijk et al., 2001; Huntley et al., 2004; Sargison et al., 2012).

Malnutrition from sub-optimal nutrient (especially protein) supply may result in immunosuppression, hence increased susceptibility, i.e. reduced resilience, to nematode parasite infection (Adams, 1982, 1988; Greer et al., 2005). On the other hand, experiments by Adams and Davies (1982) and Morley and Walkden-Brown (2006), indicated that corticosteroids, known for reducing immunocompetence, had no significant effect on worm burden. This contradiction may indicate that immunosuppression is not the only mechanism through which malnourished animals become increasingly prone to endoparasitism. Straightforward energy resource allocation, pertinently protein, where the functions of growth and production are prioritised over the expression of immunity in times of sub-optimal supply, can lead to increased susceptibility to endoparasites (Coop and Kyriazakis, 1999).

1.2.2.2 Breed

Due to genetic variation, different breeds of sheep and goats, have shown varying levels of genetic susceptibility to worm infection, with indigenous tropical sheep breeds like East Africa Red Maasai, Florida Native, Barbados Blackbelly and St. Croix sheep showing more resistance and resilience towards nematode infection than breeds developed for intensive production (Loggings et al., 1965; Knight et al., 1973; Rege and Baker, 1993; Baker, 1996). Among dairy goats in north-western France, Alpine goats had consistently lower faecal egg counts than the more developed Saanen breed (Richard et al., 1990). Such breed effects are presumably due to natural and artificial selection of indigenous breeds over centuries, in the face of climatic stress, under-nutrition and high levels of parasite challenge in the absence of effective anthelmintics, compared to more highly developed breeds (Waller, 1997b; Waller and Thamsborg, 2004).

1.2.2.3 Age

Although adult animals are generally more resistant and resilient than young animals, particularly with respect to haemonchosis (Gordon, 1948; Morley and Donald, 1980; Malan et al., 2001; Waller and Thamsborg, 2004), in the field it is usually difficult to separate age from immune effects due to previous infection (Gordon, 1948). However, it has been shown that aged animals become more susceptible to worms and their pathological effects, and this can be of epidemiological significance where lambs are raised in a flock composed of aged ewes (Gordon, 1948; Miller and Horohov, 2006) or does (Mahieu et al., 2007).

1.3 Anthelmintic resistance (AR)

The global problem of helminths in livestock is made worse by high levels of anthelmintic resistance (AR), which is said to have become established when a previously effective drug ceases to kill an exposed parasitic population to an expected extent at the therapeutically recommended dosages (Jabbar et al., 2006). AR has now become the *status quo* in almost all the intensive sheep-rearing countries of the world (Kaplan and Vidyashankar, 2012). Resistance of various species of GIT nematodes has been reported to all of the five major older activity groups of anthelmintics that are, *a priori*, highly effective against *H. contortus* (benzimidazoles, macrocyclic lactones, substituted salicylanides, organophosphorus

and imidazothiazoles / tetrahydropyrimidines), including the different drugs within each of the groups (Van Wyk et al., 1997b; Jabbar et al., 2006). Of particular concern, however is that the first cases of resistance against monepantel, a novel amino-acetonitrile derivative anthelmintic, have been reported in New Zealand (Scott et al., 2013) and NSW Australia (Love, 2014), about four years after its launch in both countries. Also, *H. contortus* resistance to monepantel in sheep farms has been reported in the Netherlands (Van den Brom et al., 2015) and in Uruguay (Mederos et al., 2014). Currently, this state of affairs leaves derquantel, a spiroindole derivative, as the only drug from a group yet without a reported field case of resistance world-wide, but even in this case a reduced efficacy of 18.3% against larval stages of the June 2008 field isolate of the barber's pole worm *H. contortus* (Haecon-51, Australia), known to be resistant to benzimidazoles (BZs), levamisole and macrocyclic lactones (MLs), has been shown by Kaminsky et al. (2011).

1.3.1 How did AR arise?

In the extreme situation of subsistence farming, which is common in the resource-poor tropical/subtropical regions where anthelmintics are either unaffordable or of such inferior quality that the stock owners have given up on them, tragic mortalities of young stock due to helminth parasites, in particular *H. contortus*, are common, particularly in Sub-Saharan Africa (Perry et al., 2002; Bath et al., 2005). At the other extreme, where commercial farming is undertaken, the control of nematode parasites has depended largely on the ready availability of cheap and effective anthelmintics (Waller, 1997b), with the conventional practice of drenching all the animals when one or two die or when any show signs of heavy infection (Malan et al., 2001). In more intensive grazing systems, farmers are inclined to protect especially growing and reproductive animals from nematodes by regular, frequent treatments before any signs of infection are documented. This effectively controls parasites in the short term and decreases pasture contamination and subsequent infection pressure. However, since such systems rely very heavily on effective anthelmintics, AR represents a huge threat to their viability; as no provision is made for refugia. "Refugia" defines the proportion of the parasite population that is not exposed to a given control measure, mainly anthelmintic dosing, thus escaping selection for resistance (Van Wyk, 2001) and contributing genetically to subsequent generations.

Since the introduction of non-herbal anthelmintics based on hazardous concoctions of copper, arsenic and nicotine early in the last century by Green (1918), quoted by Van Wyk et al. (2006), the availability of new classes of highly effective and safe anthelmintics entering the market since the 1950's has been at a rate of one per decade (Kaplan, 2004). This resulted in a plethora of anthelmintics and livestock production targets particularly of small ruminants becoming based to a large extent on the improved effectiveness of these drugs (Van Wyk et al., 2006). Hence, with the exception of the very latest, unrelated anthelmintic activity groups (namely the spiroindole, derquantel, and the amino-acetonitrile derivative, monepantel), anthelmintic prices remained markedly low subsequent to the arrival of thiabendazole on the market in the early 1960s (Van Wyk et al., 1999; Waller, 1999; Kaplan, 2004). For example, between 1961 and 1999 a mean inflation rate of 367% was realised in South Africa for older groups of anthelmintics (BZs, salicylanilides, imidazothiazoles, substituted nitrophenols and organophosphorus compounds), compared with 2700% for general inflation in the country (Van Wyk et al., 1999).

It was generally expected that the stream of cheap, highly effective drugs reaching the market during the 1970s and 1980s would continue (McKeller, 1994; Waller, 1997a; Van Wyk et al., 1999). Drug manufacturers, but also helminthologists and practitioners, recommended year-round chemical management of endoparasites protocols to protect animals from exposure to any level of worm challenge whatsoever (Van Wyk, 2001). In the process, the new highly effective anthelmintics encouraged farmers progressively to rely almost exclusively on drenching for worm control (Van Wyk et al., 1999), often at increased frequency to maximize livestock health, productivity and profitability (Anderson, 1972, 1973; Brown et al., 1985; Kaplan, 2004). Consequently, some of the highest drenching frequencies on record occurred, up to approximately every three weeks during the *H. contortus* season or even throughout the year to protect sheep more fully (Van Wyk et al., 1989; Malan et al., 2001). Often this was coupled with a mentality of maximum kill against the maximum number of worm species, which prevailed amongst farmers, extension agents, sales representatives and researchers alike (Waller, 1999), with little or no heed to the imminent threat posed by anthelmintic resistance (Brunsdon et al., 1983). The situation was worsened by the introduction and recently expanding extensive use of long-acting products leading to even greater over-exposure of parasite

populations to the anthelmintics involved (Sutherland and Scott, 2010; Sargison et al., 2012).

The expectation of a plethora of highly effective anthemintics for time immemorial was not to be, as the pharmaceutical industry scaled down drug discovery efforts in animal health, largely for economic reasons (McKeller, 1994; Soll, 1997; Waller, 1997a; Geary and Thompson, 2003). As a consequence, no new classes of anthelmintic compounds applicable to ruminants were developed from the early 1980s until the amino–acetonitrile derivative, monepantel (Kaminsky et al., 2008a), and the first paraherquamide derivative compound for use in ruminants, derquantel (Ostlind et al., 1990), in combination with abamectin (Little et al., 2011), were respectively licenced and 'world launched' in New Zealand as Zolvix® (Novartis Animal Health Inc) in 2009 and as Startect®(Zoetis Animal Health) in 2010 (Kaminsky et al., 2011). The prolonged lack of introduction of new drugs from different activity groups into the market in the phase of over-exposure of parasite populations to anthelmintics led to greater selective forces for the development of AR (Van Wyk et al., 1999; Leathwick et al., 2001; Bath et al., 2005; Leathwick et al., 2009; Sutherland and Scott, 2010; Falzon et al., 2014).

1.3.2 Current global AR state of play

AR went from being documented as rare in early surveys in some countries like New Zealand (Kettle and Vlassoff, 1980; Kettle et al., 1982) to being commonplace (Echevarria et al., 1996; Eddi et al., 1996; Maciel et al., 1996; Nari et al., 1996; Waller, 1997a; Van Wyk et al., 1999; Kaminsky, 2003; O'Connor et al., 2006; Besier, 2007; Good et al., 2012; Kaplan and Vidyashankar, 2012), and progressively involving all anthelmintic activity groups available, even to the extent of a single worm population of *H.contortus* presenting with resistance to all five of the different anthelmintic activity groups available for controlling this worm species at the time (Van Wyk et al., 1997b).

1.3.2.1 AR situation in Europe

Reports from European countries indicated an increasing problem of AR mainly associated with resistance to the BZs and the imidazothiazoles, and subsequently increasing numbers of cases of resistance to the MLs (ivermectin in particular) have been reported (Papadopoulos, 2008; Papadopoulos et al., 2012). In the UK, resistance has been documented to the BZs in *N. battus* in lambs (Mitchell et al., 2011) and its resistance selection mechanism to BZs was recently characterised, both phenotypically and genotypically, by Morrison et al. (2014). Also, multiple resistance to the BZ, levamisole and avermectin anthelmintic groups in a population of *Te. circumcincta* has been documented (Sargison et al., 2010a). In Scotland, resistance to BZ, imidazothiazole and ML anthelmintics, separately and in combination, was diagnosed (Sargison et al., 2010b), while in Wales, 100 of 122 farms surveyed were positive for anthelmintic resistance to BZs, levamisole or both (Mitchell et al., 2010). In the Irish commercial sheep flock, Good et al. (2012) reported resistance of nematode populations to both BZs (>88% of flocks) and levamisole (>39% of flocks), while resistance of nematode populations to MLs was suspected on 11% of farms.

A recent study to determine level of resistance to benzimidazoles (BZ) in north-eastern Spain estimated a flock resistance prevalence of 11% out of 107 commercial sheep farms surveyed (Calavia et al., 2011). In another study, also in Spain, treatment failure was documented respectively in 40.8%, 20.8% and 9.6% of farms to levamisole, ivermectin and albendazole (Martínez-Valladares et al., 2011). Cernansta et al. (2006) found resistance respectively in 4% and 23% of the farms tested in the Slovak Republic to albendazole and ivermectin, while in The Netherlands, Borgsteede et al. (1997; 2010) reported resistance to BZs and ivermectin on sheep farms. In Italy, resistance to levamisole was recorded on all three of the farms tested and to ivermectin on two of them (Traversa et al., 2007), while in a survey by (Chartier et al., 1998) in 23 randomly selected sheep and dairy goats farms in western France AR to BZs was found on 83% of the farms and to levamisole on 50% of farms , mainly affecting *Teladorsagia*, *Trichostrongylus* and *Cooperia* spp. In a study conducted in Greece on sheep and goat flocks by Papadopoulos et al. (2001), a flock prevalence of 17 out of 107 flocks on small islands was found in *Teladorsagia* sp. populations studied, using in-vitro tests, while

recently in the same country, Gallidis et al. (2011) reported the presence of 100% homozygous BZ-resistant populations of *H. contortus* in dairy sheep flocks. Recently, the first field case of *H. contortus* resistance for the Netherlands to monepantel was reported by Van den Brom et al. (2015), and worryingly the efficacy of treatment was reported to be 0%.

While investigations on AR in the European Union (EU) historically focused mainly on the GIT parasites of small ruminants, problems were also recorded in cattle. For instance, in first-season-grazing calves on German dairy farms (Kleinschmidt and Samson-Himmelstjerna, 2008; Kleinschmidt et al., 2010) and the UK (Stafford and Coles, 1999; Taylor, 2010). In fact, a recent systematic review study by Rose et al. (2015) showed AR to be widespread in European farmed ruminants that included cattle, sheep and goats. In addition, it was concluded from a multinational anthelmintic efficacy investigation in cattle in Europe by Demeler et al. (2009) that anthelmintic efficacy testing should be intensified due to possible insufficient efficacy of current drugs, which can be as a result of either: (i) Substandard drug formulations in the face of susceptible parasites populations, as earlier found with generic rafoxanide products available on the South African market by Van Wyk et al. (1997c); or (ii) Increased drug resistance of parasite populations in the face of standard formulated drugs.

1.3.2.2 AR situation in Australasia

AR has also been reported to be a serious problem in Australasia for instance, two decades ago already regional surveys in Australia in areas receiving more than 500 mm rain per year showed approximately 80% of worm populations on the farms to have AR to both BZ and imidazothiazole groups of anthelmintics (Waller et al., 1995). Substantial, widely disseminated AR of *H. contortus* to salicylanilide and closantel has been documented by Rolfe (1993), possibly due to its extensive use during the much promoted “WormKill” programme (Dash et al., 1985) in the summer rainfall regions of Australia (Van Wyk, 2001, 2006). Furthermore, failure of the epidemiologically based “summer drenching” programs due to AR of *Tr. colubriformis* and *Te. circumcincta* resulted in considerable production loss which was inversely proportional to the level of effectiveness of the anthelmintics used

(Besier, 1996). The potential annual cost of worms in Australia has been estimated at up to AU$700 million by 2010, using the 2001 trends of resistance development and without a significant change in worm control practices (Welsman, 2001), and has recently been documented to amount to approximately 1 billion AU$ in sheep and cattle (Roeber et al., 2013). A current drench resistance study across Australia, after an interim of nearly 20 years, has revealed unexpectedly high and rising levels of resistance to most widely used drench actives, at 54% for moxidectin and 96% for BZs and levamisole (Playford et al., 2014). Worryingly, the first case of resistance to monepantel, a new drug from a novel anthelmintic group which was launched in Australia in spring 2010, has been confirmed against *Tr. Colubriformis* and *Te. circumcincta* in goats in that country (Love, 2014). In New Zealand AR against BZs, imidazothiazoles and macrocyclic lactones is now widespread on sheep farms (Hughes et al., 2005; 2007; Waghorn et al., 2014) and worse on goat farms (West et al., 2004), where the first case of resistance against monepantel was recorded in *Tr. colubriformis* and *Te. circumcincta* only four years after its launch in that country (Scott et al., 2013). Historically, AR was reported to be generally less of a problem in cattle in Australasia when compared with sheep and goats (Waller, 1997a; Kenyon et al., 2009) but lately AR in nematodes of beef cattle in Australia was reported to be common by Cotter et al. (2015). Also, there have been reports of resistance of *Cooperia* spp. in cattle herds to benzimidazoles in New Zealand (Vermunt et al., 1995; Hosking et al., 1996; Pomroy, 2006; Waghorn et al., 2006).

1.3.2.3 AR situation in Asia

Throughout south-east Asia and south Pacific regions, with rapid development and intensification of small ruminant industries, resorting to intensive use of anthelmintics for controlling nematode parasites resulted in a corresponding increase in prevalence and level of AR (Waller, 1997a). This resulted in the phenomenon being considered one of the greatest threats to the future of small ruminants in the south Pacific regions (Banks, 1988), an opinion vindicated by subsequent surveys showing combined AR to BZ and levamisole in Fiji, with emerging AR to ivermectin (Le Jambre, 1994), similar to the situation in Malaysia (Dorny et al., 1994; Chandrawathani et al., 2011) and also the sub-continent of India where

multiple resistance in GIT nematodes to BZs, ivermectin, albendazole and levamisole was documented (Gill, 1993; Easwaran et al., 2009).

1.3.2.4 AR situation in North America

In North America, although the small ruminant industries are small, limited surveys conducted in the 1990s showed high level AR to the BZs (Uhlinger et al., 1992), while, in the southern states of Louisiana and Florida, AR was already at such a high level for all anthelmintics by the turn of the century that sheep farming using imported breeds had become difficult to manage because of mortalities due to *H. contortus* (Waller, 1997a). In the south-eastern United States, for example, an AR prevalence study on sheep and goats farms showed *H. contortus* populations from 45, 25, 35 and 11 farms out of 46, respectively to be resistant to BZs, levamisole, ivermectin and moxidectin, with resistance to all three anthelmintic activity groups being detected on 22 farms (Howell et al., 2008). These findings supported earlier reports of multiple AR in sheep and goats by Zajac and Gipson (2000) and Terrill et al. (2001), to the extent that it led to the founding of the multi-disciplinary ACSRPC (American Consortium for Small Ruminant Parasite Control) group, dedicated to development of sustainable systems of worm management, amongst others through use of FAMACHA© and pastures with anthelmintic properties. The AR situation in the southern United States led to specific recommendations on control of nematodes of small ruminants, including off-label anthelmintic use particularly in goats (Kaplan, 2010; Mobini, 2010). Where applicable, the FAMACHA© method is applied for sustainable management of *H. contortus* to increase the proportion of pararasites in refugia for improving the sustainability of those anthelmintics, which are still reasonably effective (Kaplan et al., 2004; Leathwick et al., 2008; Leathwick et al., 2012). In cattle, multi-species anthelmintic resistance has been documented respectively in the western (Edmonds et al., 2010) as well as the south-eastern United States (Gasbarre, 2010). Although reports in Ontario, Canada, initially showed AR to be scarce in sheep flocks as a result of: (i) Limited need for treatment as a result of extreme temperatures commonly limiting larval development in Canada; (ii) Diversity in management practices; (iii) Less frequent use of anthelmintics compared to other countries; and (iv) The small size of individual farms and sheep flocks compared to other countries (Guthrie et al., 2010), a recent

study by Falzon et al. (2013) in Ontario recorded ivermectin drench failure in 88% of the 39 farms studied. Furthermore, a subsequent investigation on the same farms indicated 97% (28/29), 95% (19/20) and 6% (1/17) resistance to ivermectin, fenbendazole and levamisole, respectively, with *H. contortus* commonly isolated from post-treatment faecal cultures.

1.3.2.5 AR situation in South America

In the late 1990s South America recorded arguably the highest and most spatially widespread levels of AR in the world (Waller, 1997a). For instance, surveys conducted in northern Argentina (Eddi et al., 1996), southern Brazil (Echevarria et al., 1996), Paraguay (Maciel et al., 1996) and Uruguay (Nari et al., 1996) indicated that the BZs and imidazothiazoles had almost reached the end of their therapeutic lifespan in these countries and have continued to the present times. In Brazil many products containing combinations of different anthelmintics were found to be almost ineffective as a result of heightened AR (Da Cruz et al., 2010; Molento et al., 2011), as recently shown from studies conducted by Almeida et al. (2010) and Cezar et al. (2010) where populations of *Haemonchus* spp. and *Trichostrongylus* spp. with resistance to multiple drugs, with some drugs like ivermectin and moxidectin yielding trial-based approximate efficacy of 1% (Cezar et al., 2010), were reported. Also, populations of *Haemonchus* spp. resistant to both macrocyclic lactones and BZ, as well as *Cooperia* spp. resistant to macrocyclic lactones and ricobendazole, have been documented in cattle in Argentina (Anziani et al., 2004; Fazzio et al., 2012). Of particular concern to South America as a whole is the recent demonstration of poor efficacy of monepantel against *H. contortus* on two farms in Uruguay by Mederos et al. (2014), four years after the drug had become commercially available in that country in 2010.

1.3.2.6 AR situation in Africa

Although, as noted by Vatta and Lindberg (2006), there is a paucity of AR surveillance studies in Africa, the few documented studies suggest cause for concern. In Kenya, 50% of 42 farms surveyed showed AR to at least one anthelmintic group (Wanyangu et al., 1994), while in a study conducted on six commercial sheep farms

in Zambia, albendazole resistance was found on five (Gabriel et al., 2001). In South Africa, with a comparatively well-developed commercial small ruminant sector compared to the rest of the continent, particularly in sheep farming, the first record of AR is amongst the earliest for the continent (Berger, 1975). AR has been described variously as widespread and rapidly increasing (Van Wyk et al., 1998), entering a crisis situation (Van Wyk et al., 1999) and alarming as by then it was already affecting practically all anthelmintics available at the time in South Africa (Van Wyk, 2001). The worm species found to be affected were *H. contortus* (Berger, 1975; Van Wyk and Malan, 1988), *Tr colubriformis* (Van Wyk et al., 1990), *Te. circumcincta* (Van Schalkwyk et al., 1983) and *Moniezia expansa* (Visser et al., 1987). Some farmers were reported to have abandoned sheep farming altogether when it became evident that worm control was no longer sustainable (Van Wyk et al., 1989; Van Wyk, 1990).

Of special concern and relevance to this study are the levels of resistance exhibited by *H. contortus* populations in South Africa and that the first reports of resistance of the macrocyclic lactones (ivermectin), salicylanilides (both rafoxanide and closantel), and the substituted phenols (nitroxynil and dinitrophenol) concerned field cases in the country (Van Wyk and Geber, 1980; Van Wyk et al., 1982; Carmichael et al., 1987; Van Wyk and Malan, 1988; Van Wyk et al., 1997b). In the case of the macrocyclic lactones, two different *H. contortus* populations were reported to be resistant to ivermectin within three years of the drug having been registered for use in sheep in South Africa (Carmichael et al., 1987; Van Wyk and Malan, 1988; Van Wyk et al., 1989). This led to a hypothesis of cross-resistance between anthelmintic groups by Van Wyk et al. (1989), with that between the BZs and macrocyclic lactones being the most likely candidate, for which possibility there now seems to be mounting evidence (Mottier and Prichard, 2008), even though Leathwick et al. (2009) and Bartram et al. (2012) judged this to be far from a clear indication of true cross-resistance. In addition to the above, a South African population of *H. contortus* was reported to be resistant to compounds from all five of the activity groups of anthelmintic present at the time for nematode control in small ruminants (Van Wyk et al., 1997b). Also, a subsequent survey in the country (Van Wyk et al., 1999) reported AR to at least one drug out of the four used (namely albendazole, rafoxanide, levamisole and ivermectin, representing four different activity groups)

on 79 farms out of the 80 involved. On two farms out of 26 in one of the districts where the survey was conducted, every one of the above four drugs was shown to be less than 40% effective against the populations of *H. contortus* involved. In consequence, these authors suggested that unless conventional suppressive anthelmintic treatment practices to maintain animal productivity were discontinued, new anthelmintic groups would bring probably only a temporary short-lived relief to the AR crisis (Van Wyk et al., 1999), and this appears to be happening, with severe resistance to monepantel having developed practically as rapidly as to ivermectin (Scott et al., 2013; Love, 2014). While the above references to AR in Africa are relatively outdated and have not been followed by more recent surveys, (Van Wyk and Reynecke, 2011) emphasise the fact that the uptake of alternative, more sustainable methods of worm control has been poor among farmers. Hence it is reasonable to infer that AR in Africa has become considerably more severe in the meantime.

1.3.3 Cause for concern about AR

In theory, reversion to susceptibility should occur on withdrawal of a given drug before the resistant genes are widespread in parasite populations, since the resistant worms could be expected genetically to suffer from a decrease in fitness. However, there seems to be little evidence of this in the field (Kaplan, 2004). And where reversion to susceptibility was documented, for example, with levamisole against *H. contortus* (Rowan et al., 1996) and the BZs against *Teladorsagia* and *Trichostrongylus* spp. (Waller et al. (1988), it turned out to be short-lived upon the drug being re-introduced (Leathwick et al., 2001). This could, on the whole, mean that AR is permanent, as observed by Guinan and Kieran (1980), Kelly and Hall (1979), Webb and McCully (1979), Waller et al. (1989) and also suggested by Kaplan (2004). On the other hand, there exists what seems to be an exceptional finding by Rowan et al. (1996), who documented efficacious performance (>99%) of levamisole with regular use against *H. contortus* and without decline for 12 years post reintroduction after three years of withdrawal.

1.3.4 Consensus on arresting AR

Although the conventional approach to anthelmintic usage was highly effective as regards reduction in losses in animal production to worm challenge while it lasted,

the history through the current crisis-level resistance clearly suggests that this approach was short sighted and unsustainable (Morley and Donald, 1980; Kaplan, 2004). There is also general agreement amongst parasitologists that the conventional practice of all-out chemical–based control of parasites in small ruminants must be changed, from one of maximum, to one of optimal worm management compatible with sustainability (Van Wyk et al., 2006), by animal producers learning to: "farm with 'good worms'" (Van Wyk, 2000), or at least to live with worms (Coles, 2002), through adoption of and persistence with sustainable integrated parasite management strategies (Morley and Donald, 1980; Barger, 1997, 1999; Waller, 1999; Krecek and Waller, 2006; O'Connor et al., 2006; Van Wyk et al., 2006; Bath and Van Wyk, 2009; Kenyon et al., 2009; Leathwick et al., 2009; Morgan et al., 2013). This practice encompasses both chemotherapeutic and non-chemotherapeutic baskets of options that are practical, affordable, available and appropriate whether to the commercial producer, or the resource-poor farmer (Krecek and Waller, 2006). These strategies have been researched, documented, reviewed and largely validated for implementation. However, implementation can only be considered when the particular strategy is able to reduce selection for resistance while maintaining an acceptable level of worm control and productivity at acceptable profit margins to the producer (Morley and Donald, 1980; Leathwick et al., 2009; Besier et al., 2010).

The recent release and first 'world launching' into the market in New Zealand of two unrelated new drugs from different drug activity groups, that is the amino–acetonitrile derivative (AAD), monepantel (Kaminsky et al., 2008b) in 2009, together with derquantel in association with abamectin (Little et al., 2011), for use in ruminants in 2010 (Kaminsky et al., 2011; Scott et al., 2013; Love, 2014), after three decades of none and only one new drug every decade between the 1950s and early 1980s before that (Kaplan, 2004). These novel drugs offered all small ruminant producers and other stakeholders an almost unique opportunity for a new start as regards AR. But while it was to be expected that the opportunity would have been seized with both hands by all through adoption of and persistence with sustainable integrated parasite management in an attempt to engineer maximal sustainability of efficacy in relation to the rate of development of AR, compared to the rate at which it developed against older drug classes (Besier, 2007), this was not to be. Despite serious attempts by the respective manufacturers of Zolvix® and Startect® to

stimulate farmers and their advisors to adhere to the principles of sustainable use, resistance has been documented within a similar record period of time as that set for ivermectin. (Scott et al., 2013; Love, 2014).

1.4 Approaches to sustainable integrated parasite management (sIPM)

In the light of the global seriousness of the phenomenon of AR, Van Wyk et al. (2006) suggested it a shortcoming that there was no mention of sustainability in the term "Integrated Parasite Management", and that this should be rectified through changing the term and its acronym respectively to "**S**ustainable Integrated Parasite Management", and "**s**IPM". The latter comprises application of modified management decisions intended to reduce the extent and severity of helminthosis by reduced anthelmintic treatments in the context of holistic helminth management (Bath, 2006) and should include approaches such as provision of safe pastures, while making adequate provision for refugia (Van Wyk, 2001; Van Wyk et al., 2006; Kenyon et al., 2009; Besier et al., 2010).

1.4.1 Refugia-based strategies

Refugia, as defined by Georghiou and Taylor (1977), quoted by Van Wyk (2003b), refers to a proportion of a given parasite population that is not exposed to a given control measure, in particular drenching with anthelmintics, thus escaping selection for AR (Van Wyk, 2001), and successfully establishing in a host, and producing viable offspring to contribute to subsequent generations (Leathwick et al., 2009). This includes, but is not limited to nematode free-living stages on pasture when animals are dewormed, as it commonly includes parasites inaccessible to the effects of drugs administered, for instance in the host's tissues (Van Wyk, 2003b). Refugia-based strategies are those, which maximise the proportion of helminths in refuge, without compromising acceptable animal production margins and welfare (Leathwick et al., 2009). For convenience, these have been split as discussed below, into targeted selective, and targeted treatment, the latter of which is ideally combined effectively with movements between pastures, and good on-farm biosecurity.

1.4.1.1 Targeted selective treatment (TST)

Targeted selective treatment (TST) is a part-flock treatment strategy which makes use of the realisation that parasitic burdens are highly aggregated and grossly over-dispersed in farm animals (Barger, 1985; Hoste et al., 2001), with as much as 80% of the worm parasitic stages having been reported to be carried in only 20-30% of the hosts (Sreter et al., 1994). Thus, treatment under this strategy is directed towards those animals which are unable to cope with the parasitic burden unaided, as opposed to the conventional approach of whole-flock treatment when parasitosis occurs, and a tendency to increase use of prophylaxis, either through increased frequency of treatment, or by strategic treatment at times when there are low proportions of parasites in refugia (Van Wyk, 2001; Van Wyk et al., 2006; Kenyon et al., 2013).

The implementation of TST strategies requires the ability to identify the overly susceptible individuals within a flock for treatment (Kenyon et al., 2009). The Five Point Check© system of Bath and Van Wyk (2009) has been proposed as a tool to help identify such overly susceptible animals to endoparasites, and involves examination of the: (i) Nose for exudates, (ii) Submandibular region for oedema (bottle jaw); (iii) Eyes for anaemia; (iv) Lumbar region for body condition score; and (v) Perineum for dag (diarrhoea) score. However, the most extensively validated field-based TST indicator is the FAMACHA© system developed in South Africa for haematophagous parasites like *H. contortus*, by assessing conjunctival membrane colour for levels of anaemia (Malan and Van Wyk, 1992; Bath et al., 1996; Malan et al., 2001; Vatta et al., 2001; Van Wyk and Bath, 2002; Kaplan et al., 2004; Ejlertsen et al., 2006; Burke et al., 2007; Mahieu et al., 2007; Reynecke et al., 2011a; 2011c; 2011b). This strategy was found considerably to reduce drenching (Malan et al., 2001), with consequent savings on anthelmintic costs. However, its main advantage is an increased proportion of unselected worms in refugia, in that the worms in the untreated animals have not been exposed to the drugs involved, and void relatively large numbers of unselected eggs, compared to the few eggs from effectively dewormed animals (Van Wyk et al., 2006). Granted successful natural infection of their hosts, the unselected, more highly susceptible worm majority in refugia from untreated animals (in relation to those surviving in those that have been treated) will ultimately breed with and thus dilute the resistance genes from the few eggs voided

by the effectively dewormed animals. And in the process, selection for AR is delayed, as shown in trials aimed at deliberate dilution of resistance genes through artificial or manipulative means by Van Wyk and Van Schalkwyk (1990) for *H. contortus* and Moussavou-Boussougou et al. (2007) for *Te. circumcincta*, as well as in various treatment regime trials by Kenyon et al. (2013).

1.4.1.2 Targeted treatment (TT)

This is a tactical whole-flock, blanket treatment of animals, which is applied when an impending or imminent increase in worm burden is foreseen from a knowledge of parasite epidemiology in the area (Gordon, 1948), or in response to evidence of increasing infection or decreasing performance, bearing in mind the need to maintain refugia (Kenyon et al., 2009). It differs from strategic treatments which are given prophylactically at more or less fixed times as part of the seasonal plan of parasite control, based on historical epidemiological information of the parasites in a given area, to protect animals and prevent diseases over a long period (Gordon, 1948; Kenyon et al., 2009; Kenyon et al., 2013). This tactical use of anthelmintics reduces the frequency of drenching and increases the interval between drenching (Besier and Love, 2003; Cringoli et al., 2008). In this way it leads to refugia benefit, hence less AR selection pressure, by allowing susceptible genotypes to establish on pasture and limit the risk of heterozygotes mating to produce homozygous resistant offspring (Kenyon et al., 2009).

While presently not as well-developed as FAMACHA©, there are other potential indicators for TST and TT to be explored besides those suggested under the Five Point Check© system of Bath and Van Wyk (2009). These include: (i) Production indices such as milk yield, body condition score, wool and live weight gain (Barger and Southcott, 1975; Coop et al., 1977; Hubert et al., 1979; Morley and Donald, 1980; Coop et al., 1988; Cottle, 1991; Hoste et al., 2002a; 2002b; 2002c; Van Wyk et al., 2006; Broughan and Wall, 2007; Besier, 2008; Bath and Van Wyk, 2009; Kenyon et al., 2009); and (ii) Parasite-based markers such as faecal egg counts (Roberts and Swan, 1981; Coadwell and Ward, 1982; Beriajaya and Copeman, 2006; Kenyon et al., 2013). A novel production index-based decision support model, named Happy Factor™, that relied on calculation of the efficiency of nutrient utilization to identify the overly susceptible individuals within a flock for treatment

as part of TST regime, was developed by Greer et al. (2009) in South East Scotland. Recently improved for field application as a TST indicator for managing the parasites population in refugia (Kenyon et al., 2013), the Happy Factor™ has been shown to: (i) Successfully discriminate between animals which are likely to respond favourably to anthelmintic drenching and those that are not (Greer et al., 2009); and (ii) Appear to maintain animal performance and conserve anthelmintic efficacy when compared with a neo-suppressive anthelmintic treatment regime (Kenyon et al., 2013).

1.4.1.3 Treat-and-Stay strategy

In reaction to the "Treat-and-Move" strategy that has been shown severely to select for AR, a Treat-and-Stay strategy (Bath et al., 2005) is suggested to enhance re-infection of the treated animals with helminths that have not been exposed to the drug(s) involved. In contrast, the drench-and-move strategy was designed to reduce re-infection of low worm pastures (Stromberg and Averbeck, 1999), but, as said, it has been shown to select very severely for AR (Martin, 1989). Drenching animals and leaving them to become re-infected on the same infected pasture, that is to treat-and-stay (Bath et al., 2005), leads, through interbreeding, to dilution of the resistant worm gene pool of the survivors of the drug treatment (Van Wyk, 2006). The duration of stay needs sufficiently to overlap the residual effect of the drench used, to ensure substantial re-infection of the treated animals, thus to ensure a build-up of a preponderance of unselected worms (from the re-infection before the move to "clean" pastures) in relation to the relatively small numbers of selected worms which have remained in the animals after treatment (Bath et al., 2005). Also, the post-treatment duration of stay necessary to accord sufficient re-infection of animals, can be affected by acquired partial immunity in the animals involved or through season hypobiosis of the L3 picked up. An alternative to the treat-and-stay strategy is the move-then-treat approach (Molento et al., 2004), that is, after movement of the animals, to allow repopulation of the new pasture with worms in refugia before the animals are treated. In this way it is ensured that the cleaner pasture is populated first by unselected worms which, given highly effective deworming after the interval on the new pasture, will later lead to progressively higher levels of dilution of resistance genes as a result of a preponderance of unselected-, in relation to selected worms on the new pasture.

1.4.1.4 Farm-gate bio-security measures

Due to the high prevalence of anthelmintic resistance, the possibility of imported stock carrying anthelmintic resistant parasites has become a significant risk, hence the need to quarantine-treat all imported stock before being allowed to mix with resident animals (Van Wyk, 1990; Van Wyk et al., 1991; Coles and Roush, 1992; Sager et al., 2010). After treatment, the incoming stock then needs to be placed on pasture harbouring sufficient worms in refugia so as to genetically dilute any resistant worms that could have survived the prescribed and usually intensive, quarantine drenching (Dobson et al., 2001; Van Wyk, 2002; Love, 2007).

1.4.2 Grazing management

Evasive grazing management strategies are based on the understanding that the longevity of free-living parasite stages is reduced under exposure to unfavourable environmental elements (Banks et al., 1990; Barger et al., 1994; Waller, 1999; Waller and Thamsborg, 2004). This understanding led to design of various evasive grazing management strategies designed to incorporate rotational grazing in one form or another for **s**IPM, as discussed below.

1.4.2.1 Classical pasture spelling

Classical pasture spelling is the practice of resting contaminated pasture by removing all domestic ruminants and delaying their re-introduction long enough for the pasture to become safe for susceptible animals. Although the development of free-living parasite stages is generally faster and more successful in the tropics/subtropics than in temperate regions, their longevity is much more reduced due to exposure to unfavourable environmental elements, hence there is greater potential for classical pasture spelling to succeed in the tropics/subtropics than in temperate regions. Examples are *H. contortus* and *Trichostrongylus* spp., which appear on pasture within as short a period as a week post contamination and drop to almost non-detectable levels within 4-6 weeks. This situation led to design of effective rotational grazing regimes of less than week's grazing to avoid re-infection and 4-6 weeks' spelling to make the pasture relatively safe without having to incorporate anthelmintic treatment (Barger et al., 1994; Sani and Chandrawathani, 1996). However, in contrast, the spelling period needs to be prolonged for cooler and/or drier environments than those in the tropical Pacific Islands like Malaysia where the

above experiments were conducted, in relation to increasing periods of survival on pasture (Waller, 1999; Krecek and Waller, 2006).

Under drier conditions worm free-living stages accumulate in faecal deposits, where they are shielded to a large extent from unfavourable conditions. For instance, after a severe fifteen-month drought in Australia many *Ostertagia ostertagi* (*O. ostertagi*) L3 survived and appeared on pasture contaminated by calves up to a year previously (Barger et al., 1984), and in South Africa the only report of a severe, widely disseminated outbreak of helminthosis in cattle occurred after severe, debilitating droughts was in a study by Reinecke (1960). The latter study included *Haemonchus placei*, the survival of L3 of which in faecal pats of calves was found to be similar on (irrigated) pasture to that of *H. contortus* in sheep pellets (Krecek et al., 1991). A grazing system involving pasture spelling referred to as the 50/50 system was promoted in South Africa (Kirkman and Moore, 1995). In this system, half of a given farm is rested while animals are grazed on the other half and this is rotated annually between the two halves, enabling provision of relatively worm-free pastures on an annual basis (Van Wyk et al., 1998). Granted absolute avoidance of a Treat-and-Move situation, this system has the potential dramatically to enhance the sustainability of anthelmintic efficacy, especially with strategically planned pasture alternation after the animals have arrived on the new paddocks, which have been spelled for a period lasting up to a year. Yet another strategy, as an adjunct to classical pasture spelling, mixed ages of adult (more resistant and resilient) animals and susceptible young animals are grazed together and rotated through paddocks. The adult animals remove more larvae from pasture than they contribute through worm egg deposition on pasture and this leads to a reduced rate of pasture contamination, and hence lower worm burdens in the hosts (Michel, 1976; Leathwick et al., 2008).

With improved pastures, especially those under irrigation, classical pasture spelling is costly for spelling lasting longer than even a couple of weeks, which is hardly long enough to make the pasture safe; due to a lack of economic use, coupled with the suboptimal pasture growth occurring during the spelling periods (Morley and Donald, 1980). As pointed out by Michel (1983), pasture spelling needs, reasons of economics, of pasture primarily to comply with optimal pasture growth, and only

secondarily with requirements for worm management. Each type of pasture has its own growth curve and utilisation should start when this is at its peak and stop before the rate of re-growth is reduced in relation to withdrawal at the optimum point in time.

The shortcomings of classical pasture spelling have necessitated modification to alternate grazing in instances where it is not economical to rest pasture long enough for worm free-living stages on pasture spelled from grazing by a given host species, to die before the animals return.

1.4.2.2 Alternate grazing by age

This involves alternation of hosts of the same species, but of disparate ages, taking advantage of the resistant and resilient character of older animals compared to younger ones (Morley and Donald, 1980). When alternating such animals of the same species, the young susceptible animals graze in paddocks ahead of the more resistant and resilient older animals and in turn follow in the wake of these older animals. This alternation can be modified, particularly for suckling lambs, with special fencing to allow them access to ewes on adjacent paddocks, the so-called forward creep grazing system (Michel, 1969), which has recently been evaluated in New Zealand by Moss et al. (2009) and found to increase lamb weaning live-weight at 13 weeks by 4 kg without affecting lamb resistance to endoparasites.

1.4.2.3 Alternate grazing by species

In this practice, where cross-infectivity is limited or not effective, host species are alternated through the paddocks (Morley and Donald, 1980; Barger, 1996). Ingested larvae are then unable to reproduce in the alternate host and die in the absence of their definitive host. Thus grazing by the alternate hosts leads to reduction in pasture contamination without risk to their own health and productivity. Alternation of equids or ostriches with ruminants on pasture is ideal as regards the effect on worm management, but it is probably only on small numbers of farms where it would be practical to balance pasture utilization between the species. In contrast, alternation of small ruminants and cattle, while still useful and practical as regards pasture management (Morley and Donald, 1980), suffers from some cross-susceptibility of some host species to certain major worm species, making it less effective for worm

management. The most notable exception to host specificity is *Tr. axei*, a parasite that can infect a number of herbivores (Lindqvist et al., 2001). Also of importance are *H. contortus* of small ruminants and *H. placei* of cattle; despite being highly susceptible to one another's species, each host develops more effective immunity to the alternative species than to its own and can thus play an important role in the management of the said alternative species. In addition, while *O. ostertagi*, a nematode parasite of economic importance in extensively grazed cattle in temperate regions, is generally unable to mature in sheep, there have been reports of it being able to adapt to and cause clinical manifestations in sheep (Herlich, 1974; Coop et al., 1985; O'Callaghan et al., 1992).

1.4.2.4 Alternate grazing and cropping

Alternation of crops and grazing can ensure provision of safe pasture, while at the same time ensuring optimal economic use, for instance through production of hay or silage during periods of spelling, growing fodder or grain crops and subsequently using the aftermaths and stubbles to provide safe pasture. However, the cost implications of this parallel adventure need to be thoroughly considered before being embarked upon (Morley and Donald, 1980). Furthermore, it can lead to serious drug resistance if the animals are drenched at the time of the move, since there are generally few or no worm stages on such aftermath, which is usually grazed for a short while only, with the result that the resistant adult worms can be expected to survive the period and contribute substantially to future generations of worms that will populate the next pasture to be utilised after the crop aftermath (Martin, 1989).

1.4.3 Vaccination

Vaccination has met with limited success in field application. In fact, despite a variety of vaccines having been shown to be highly effective, as discussed below, the only one with a track record of very successful application over decades is that against the lungworm *Dictyocaulus viviparus*, comprising live larvae attenuated by irradiation (Jarrett et al., 1960). Also, for some parasite species where tangible leads towards an effective vaccine have been discovered, poor uptake by stakeholders (animal owner, drug companies and their advisors) has constrained further development of those promising leads. For example, an *Ancylostoma caninum* vaccine never penetrated the canine market meaningfully, despite its proven

effectiveness through trials (Miller, 1965). Furthermore, even though the recombinant antigen vaccine from the cestode parasite *Taenia ovis* by Johnson et al. (1989) had shown effective immune responses in sheep (Lightowlers and Rickard, 1993; Harrison et al., 1999) and was even registered by the New Zealand Animal Remedies Board in February 1994, a commercial product was never produced from it (Rickard et al., 1995), most probably owing to the economics of mass-vaccination of sheep as the intermediate host, despite the fact that the parasite is a stumbling block to Australia regarding exporting of sheep meat (Love, 2008).

Native antigen extracts from adult *H. contortus* in sheep have long shown high levels of protection through a series of trials conducted under field conditions (Newton and Munn, 1999; Smith, 2001), but it took almost 25 years before a combination of a novel method of presentation of minute amounts of the native antigen to sheep for development of effective immunity, and mass production of antigen made it possible for the Moredun Research Institute in Edinburgh, UK, to develop and launch such a native antigen vaccine (Knox et al., 2003). The vaccine has subsequently been shown to be effective (Le Jambre et al., 2008; Besier et al., 2012) and has since been commercially launched as Barbervax in October, 2014, in Australia, the only country where it is available as yet (Besier et al., 2015). Although this is a positive step, the lag time between its development and commercial launching is typical of the slow progress with vaccine endeavours against helminths in the field of parasitology, despite the known severity of global economic impact associated with helminths in sheep and goat production (Perry et al., 2002).

The advent of mathematical modelling has enabled theoretical evaluation of the effectiveness of these erstwhile discounted vaccines against GIT parasites, resulting in the realisation that perhaps the expected impact of worm vaccines was set too high, by expecting these vaccines to perform almost on a par with modern anthelmintics (Barnes et al., 1995) and discounting anything less. It has been deduced from such models that substantial economic benefits could be obtained with 60% efficacy of a vaccine against GIT nematodes in 80% of the flock (Waller, 1997b, 1999). Hopefully, such projections may stimulate increased uptake of nematode vaccine development and usage in future.

1.4.4 Breeding approaches

The genetic ability of native, "unimproved" tropical breeds of animal hosts to tolerate or resist diseases is often superior to their "improved" counterparts from the temperate regions (Waller, 1997b, 1999; Waller and Thamsborg, 2004; Krecek and Waller, 2006) and has been well established in sheep breeds for resistance or resilience to nematode (Baker, 1996; Bishop et al., 1996) and trematode (Roberts and Suhardono, 1996) parasites. Although indigenous breeds often possess inferior productivity and performance traits, when compared to 'improved' exotic breeds, they should be considered with favour in cross-breeding and breed substitution programmes, as they possess unique adaptive traits which enable them to survive and remain productive in the face of worm challenge. For example, in a trial to compare the performance of the indigenous Red Maasai sheep breed, with the 'improved', exotic Dorper breed and their crosses, the Red Maasai outperformed the Dorper in terms of dependence on anthelmintic treatment and mortality (Baker et al., 1999).

Planned breed substitution should be considered where excessive dependence on anthelmintics for lucrative production in the tropics/subtropics has led to development of multiple drug resistance. For instance, in Paraguay (Maciel et al., 1996), such breed substitution offers a simple, quick and economically viable preventative measure under these circumstances (Waller, 1997b), especially for resource-poor farmers. On the other hand, although research evidence suggests that genetic variation in resistance to nematode infections is likely to be great between breeds (Baker, 1996; Waller, 1999; Amarante et al., 2004), it is consistently lower than that between individuals within the same breed (Gray et al., 1987; Barger, 1989). And this has been demonstrated in practice in various investigations, such as those of Karlsson and Greeff (2006), who, through breeding only with rams with consistently low faecal worm egg counts, developed the well-known Rylington Merino Stud, which has such substantial resistance/tolerance to high levels of field challenge with *Teladorsagia* and *Trichostrongylus* spp in Western Australia, that no targeted treatment (TT) is required any longer (Karlsson and Greeff, 2006). Pathophysiological markers such as FAMACHA©, just like production indices and parasite-based markers for helminthosis, offer a practical, low cost means of selecting for hardiness within breeds by culling those animals which consistently

require treatment to be able to produce well under conditions of seasonal parasite challenge (Van Wyk and Bath, 2002; Riley and Van Wyk, 2009).

1.4.5 Supplementary feeding

Strategic feed supplementation, particularly to the most vulnerable classes of stock, including the young, old, peri-parturient and especially lactating animals, can have a high beneficial effect in managing helminthosis (Waller, 1999; Waller and Thamsborg, 2004; Krecek and Waller, 2006); halfway through the 20th Century it was already pronounced by Whitlock et al. (1943) that parasitism is a nutritional disease and this has been confirmed by a large body of evidence, from investigations, amongst others, by Knox (1996), Coop and Kyriazakis (1999) and Marley et al. (2005), who showed that better nourished animals are better able than animals on lower plane of nutrition to withstand the effects of worm infection. Similar positive results were reported with peri-parturient sheep on protein supplement (Houdijk et al., 2000; 2001; Huntley et al., 2004), after Leng (1991), Donaldson (1997) and Van Houtert (1997) had investigated practical ways of incorporating supplementary feeding into routine farm management. On the other hand, this brooks further investigation, since a study conducted in South Africa by Van Rensburg (2002), has cast doubt on the financial viability of such protein supplementation on a commercial scale.

1.4.6 Biological Control

Biological control measures are directed at preventing the development of free-living larval stages within the faecal deposit, and in many cases seek to separate hosts from infested dung (Waller, 1997b). Such measures include bovine dung collection for fuel, or fertiliser, and the not so dependable dung dispersal and removal by coprophilic arthropods, as discussed previously by Grønvold et al. (1996), Waller (1997b), Vlassoff et al. (2001) and Waghorn et al. (2002). Biological control offers a possibility of chemical-free livestock production, as demanded by the modern day "green" consumer. A number of micro-organisms potentially effective against free-living stages of nematodes have been identified (Waller and Faedo, 1996), with the nematophagous fungus *Duddingtonia flagrans* found to be the most promising (Waller and Larsen, 1993; Larsen, 1999). However, the need for extremely large

numbers of the fungal spores of the organism to be continuously fed to the animals involved, in synchrony with parasite egg pasture contamination (Larsen, 1999), has made it difficult to implement in practice (Waller and Thamsborg, 2004), despite abundant research into ways and means of doing so (Waller et al., 2001a; Waller et al., 2001b; Chandrawathani et al., 2003; Waller, 2003; Paraud et al., 2005; Fitz-Aranda et al., 2015).

1.4.7 Ethno-veterinary medicines and condensed tannins

Owing to the fact that plants represent an unparalleled source of molecular diversity for drug development (Waller, 1999; Chamuah et al., 2010), there has been a resurgence of interest in traditional health practices (Hussain et al., 2010; Mahima et al., 2012; Tyasi et al., 2015) as an alternative under mounting parasitic resistance to modern anthelmintics, and as an inspirational source with potential for discovery of novel, unrelated effective anthelmintic groups. Condensed tannins extracted from tannin-containing plants have been shown to have anthelmintic properties (Molan et al., 2000; Butter et al., 2001; Molan et al., 2002; Paolini et al., 2003; Min et al., 2004; Hoste et al., 2012; Williams et al., 2014), the more so against cattle nematodes (Novobilsky et al., 2011). Particular emphasis has been placed on the tanniferous legume, sericea lespedeza (SL; *Lespedeza cuneata*), as a natural dewormer after it was shown to significantly: (i) Reduce faecal egg count (FEC) and increase packed cell volume (PCV); (ii) Lower the percentage of ova in faeces that developed into infective L3 larvae; and (iii) Lower the numbers of both abomasal and small intestinal nematodes in small ruminants, when compared to different species of grass pasture (Shaik et al., 2006; Anon., 2010). Also, a recent study by Kommuru et al. (2014) showed a significant reduction ($P<0.01$) in *Eimeria* spp. oocysts per gram in goats on the SL pellet diet when compared with animals fed the control pellets, seven days after initiation of feeding. However, scientific validation of the purported anthelmintic activity of most of these ethnic products and identification of the active compounds contained therein are lacking (Waller, 1999; Williams et al., 2014) and this has led to limited uptake by livestock producers (Waller, 1999).

1.4.8 Copper therapy

Low doses of 2-5 g of copper oxide wire particles (COWP), in capsules administered orally, have been shown to potentiate anthelmintic effect against *H. contortus*, as

well as to have a protective effect against incoming infection with *H. contortus* (Bang et al., 1990; Reid, 1995; Nyman, 1999; Canto-Dorantes et al., 2004; Waruiru et al., 2004; Burke et al., 2010). Although COWP has been found to be cheap, relatively safe (Burke et al., 2005) and highly efficacious against *H. contortus*, its curative use has largely remained limited in practice due to risk of copperiedus (Burke and Miller, 2006), particularly if there are relatively high levels of copper in the herbage.

1.4.9 Anthelmintic usage

The drenching that is undertaken as part of **s**IPM (Morley and Donald, 1980; Waller, 1997b; Van Wyk et al., 2006) can be managed better to reduce selection pressure for resistance by: (i) Limiting drenching frequencies (Prichard et al., 1980; Martin et al., 1982; Barton, 1983; Falzon et al., 2014); (ii) Avoiding under-dosing (Besier and Hopkins, 1988; Prichard, 1990; Scott et al., 1991; Hazelby et al., 1994; Hennessee, 1994; Kieran, 1994; Conder and Campbell, 1995; Waller et al., 1995; Chartier et al., 1998; Geerts and Gryseels, 2000); (iii) Avoiding long-acting anthelmintic products (Le Jambre, 1981; Dobson et al., 1996; Leathwick et al., 2001; Van Wyk, 2001; Besier and Love, 2003; Leathwick et al., 2009; Falzon et al., 2014); (iv) Using anthemintics of high efficacy (Prichard, 1990; Barger, 1995b; Van Wyk, 2001); (v) Annual alternation of anthelmintics (Hall and Kelly, 1979; Donald et al., 1980; Prichard et al., 1980; Waller et al., 1988; Barger, 1995a; Conder and Campbell, 1995; Lloyd and Soulsby, 1998). In contrast, Le Jambre et al. (1978) recommended using one group of anthelmintics after the other until no longer effective, a strategy also proposed for adoption in South Africa by Van Wyk (2001), mainly due to lack of capacity to progressively monitor AR in South Africa and other countries, in the absence of which resistance may not be detected before all the anthelmintics involved are affected (Van Wyk et al., 1997b). Also, Smith (1990a) showed no theoretical advantage between sequential and rotational use of anthelmintics as a strategy to arrest resistance development; and (vi) Using combination anthelmintics with similar ranges of activity (Smith, 1990a; Barnes et al., 1995; Dobson et al., 2001; Leathwick et al., 2009; Dobson et al., 2011a; Dobson et al., 2011b; Bartram et al., 2012; Leathwick et al., 2012; Leathwick, 2013)**,** although with reservations voiced by some due to the fact that, in the absence of routine anthelmintic efficacy testing and failure to comply with essential precautions such as to drench and move

to "clean" pasture, selection for AR will occur simultaneously to all the ingredients in the combination (Van Wyk et al., 1997a; Van Wyk, 2001).

1.5 Challenges associated with implementation of sIPM

In the past there has generally been a limited to well-nigh absolute failure by livestock owners to accept, adopt and persist with **s**IPM, as pointed out by Waller (1997b), and concluded during two international electronic conferences on sustainable worm management (Van Wyk et al., 2002; Van Wyk, 2003a) despite the promise held by **s**IPM towards arresting the problem of AR. However, even though the global adoption level of the sustainable parasite management systems is still not satisfactory (Besier, 2012), in some countries and regions where resistance has reached crisis proportions, farmers are beginning to accept the inevitability of having to adopt **s**IPM strategies if their enterprises are to remain profitable in the long term (Torres-Acosta et al., 2012). The hurdles associated with uptake of **s**IPM are many and varied between countries and regions within countries but have long been recognised and documented by Besier (1997, 2012) and Bath (2006) and to include the following:

1.5.1 Inapparent nature of AR problems

The inapparent nature of the anthelmintic resistance problem results in delayed appreciation of the problem by the farmer until severe production losses have occurred, as illustrated by losses of over 10% in wool production and weight gain in sheep due to anthelmintic resistance, before the effects were clinically obvious to the farmer (Besier et al., 1996). Often it is only after multiple drug resistance has established that the problem is discovered, at which time it is difficult to implement corrective measures (Van Wyk et al., 1997b).

1.5.2 Complexity of implementation of sIPM

The complexity of practical implementation of **s**IPM strategies like FAMACHA© and Body Condition or Dag Scores to determine treatment, time to next evaluation, host class and proportion of flock to examine next and drugs to use/not to use (Van Wyk and Reynecke, 2011) tends to reduce the likelihood of adoption of **s**IPM strategies. Nevertheless, these relatively complex, yet effective strategies cannot be

compromised for simplicity (Van Wyk, 2003a; Van Wyk et al., 2006), as is underlined by the fact that the conventional anthelmintic drenching as well as the hugely popular "Wormkill" programs aimed at *H. contortus* in northern New South Wales and adjacent portions of Queensland (Dash et al., 1985; Dash, 1986) that were simplified in their application to encourage adoption by small ruminant producers, led to widely-disseminated, high levels of AR.

1.5.3 Costs associated with sIPM

The costs of **s**IPM are potentially higher in the short term than complete dependence on drenching alone (Van Wyk, 2001). This is worsened by the generally perceived increased risk of parasitism leading to production loss from the deliberate retention of parasites in refugia, necessary under **s**IPM. Furthermore, increased labour costs associated with frequent monitoring of animals, for example every seven days at the peak of *H. contortus* season (Van Wyk and Bath, 2002), are a disincentive to adoption, with the exception of conditions of general, intractable resistance, as has occurred in the Southern States of the USA (Terrill et al., 2012).

1.5.4 Ineffective communication of sIPM

Effective communication of **s**IPM as a way of knowledge transfer to farmers and back-up services to convince farmers not only to adopt but also to persist with **s**IPM, generally lacked in the past (Waller, 1999). This was mainly due to the declining number of parasitologists and other scientists involved with parasite management, as well as experienced extension personnel to provide tailor-made advice directly to individual farmers (Van Wyk et al., 2006). As a result, research results and even often repeated and stressed recommendations therefrom were largely unused by farmers as the intended beneficiaries (Waller et al., 1995; Bath, 2006). Furthermore, communication of **s**IPM was often selective and conflicting in substance due to the opposing views propagated by advisors who had not heeded advances in **s**IPM (Van Wyk, 2003b). This, coupled with promotional material by some members of the pharmaceutical industry that was often misleading (Van Wyk, 2003b; Bath, 2006), contributed to lack of wide uptake of **s**IPM strategies by farmers. However, owing to the current global consensus on the value of implementing **s**IPM strategies, adoption levels, though still patchy, have improved in some regions and countries of the world where deliberate and concerted efforts have

been made to educate the farmers. For example, in Australia 65% of participants in a study by Cornelius and Besier (2015) were aware of the TST concept and 25% of them had implemented it in some form, which is a considerable improvement on the situation in the past.

1.6 Aims of the thesis

As a step towards optimal production with sustainable control of helminth parasitism, with the emphasis on *H. contortus*, this thesis aims to improve the basis for extensive acceptance, adoption and persistence with **sIPM** strategies, in particular TST and TT, to manage the problem of AR. The major target is the hurdle presented by the complexity and labour requirements of **sIPM**, when based on frequent clinical evaluation of the flock, e.g. using the FAMACHA© system. The central theme of the work presented here is to leverage advances in information technology to support TT and TST for *H. contortus*, through improved predictive epidemiology and also by using a novel indicator of parasite impact through monitoring of animal activity.

The first objective was to: (i) Develop a novel case-based predictive model of *H. contortus* natural transmission dynamics and apply it to a commercial Merino sheep farm in South Africa, where FAMACHA©-based TST strategy was routinely applied to monitor *H. contortus* infection; (ii) Thereafter, to conduct sensitivity analysis to determine how the predicted incidence of haemonchosis was affected by different parameters; (iii) Support optimisation in the application of the FAMACHA©-based TST strategy (Chapter 2); and, (iv) Correlate the predicted risk of infection at each FAMACHA© evaluation event in the dataset used in Chapter 2 with independently sourced weather element values (average temperature, total rainfall, and rainfall entropy or evenness) at each FAMACHA© evaluation event over the period of investigation (Chapter 3), in order to assess the influence of climate on transmission risk, with a view to underpinning decision support as part of **sIPM** for haemonchosis in South Africa.

A second objective was to set up a prototype remote activity level monitoring system, employing radio frequency identification (RFID) technology, that has a potential to identify abnormally reduced activity in groups and individuals and evaluate it to determine its performance in artificially constrained settings, as

described in Chapter 4. Thereafter, it was aimed to deploy the prototype system on study farms and optimise it to remotely monitor animal activity level at individual and flock levels in a longitudinal (Chapter 5) and a cross-sectional (Chapter 6) study design. Furthermore, to use the data captured from the longitudinal study to evaluate the potential of the prototype system to transmit recognisable signatures of transition events in animal activity (Chapter 5) and to use the transmitted activity level scores from the cross-sectional study to evaluate the potential to act as a risk indicator for clinical infection with *H. contortus* (Chapter 6). This could facilitate risk-based 'as-and-when-required' Five Point Check© evaluations with a view to reducing labour intensiveness of TT/TST and the associated labour costs.

The third objective was to synthesise, based on the results obtained, the future potential of modelling and remote animal activity level monitoring as on-farm decision support systems that could encourage adoption and persistence with **s**IPM for sustainable production and parasite control in support of global food security (Chapter 7).

CHAPTER 2

A dynamic infection model to optimise targeted selective treatment (TST) application against *Haemonchus contortus* in South Africa

2.1 Introduction

Anthelmintic resistance (AR) among small ruminant GIT parasites has become spatially widespread and intractable in many major sheep production countries of the world (Waller, 1997a; Van Wyk et al., 1999; Kaminsky, 2003; O'Connor et al., 2006; Leathwick et al., 2009). This is so despite the existence of a number of tried and tested sustainable integrated parasite management (**s**IPM) systems, which are aimed at delaying the onset of AR, as well as at reversing AR through dilution of resistance parasites (Van Wyk and Van Schalkwyk, 1990) where it has already set in through dilution of resistance parasites. However, recently two new drugs from different anthelmintic activity groups, namely monepantel and derquantel in association with albamectin (Ostlind et al., 1990; Kaminsky et al., 2008b; Little et al., 2011), were introduced into the market after almost three decades of none. Although resistance to these two new drugs has already been realised in some countries (Scott et al., 2013; Love, 2014; Mederos et al., 2014; Van den Brom et al., 2015), as judged from published results, it is not yet widespread within and between countries of the world. This should give impetus to adoption of practical alternatives and measures aimed at avoiding mistakes of the past, with the hope of extending the useful shelf life of these new drugs, particularly under circumstances where resistance against these drugs has not yet set in.

Targeted selective treatment (TST), is part of the **s**IPM "basket of options" (Krecek and Waller, 2006), and is a part-flock treatment strategy that includes use of short acting anthelmintics, and is aimed mainly at delaying the onset of AR by ensuring increased proportions of unselected worms in refugia (Van Wyk, 2001). The strategy makes use of the phenomenon of over-dispersed parasite burdens in farm animals (Barger, 1985; Sreter et al., 1994; Hoste et al., 2001), to treat only those individual animals which cannot manage their current worm burdens unaided (Van Wyk, 2008). TST implementation requires the ability to identify such overly susceptible (or more challenged) individuals within a flock for treatment at an early stage, before there is a significant production effect on the animals concerned (Kenyon et al.,

2009). Use of the FAMACHA© system (Bath et al., 1996; Malan et al., 2001) for detection of the anaemia of haemonchosis has enabled validation and successful implementation of the selective treatment approach to controlling this disease.

Despite thorough field testing having shown the FAMACHA© system to be effective at farm level and the system having been disseminated widely in the world, the rate of uptake by farmers in place of the conventional practice of drenching all the animals when one or two die or when any show signs of heavy infection (Malan et al., 2001) is limited, due amongst others to the complexity of optimal **s**IPM and its labour intensiveness (Van Wyk and Bath, 2002; Van Wyk, 2003a, 2006). As a consequence, TST is presently being used principally in relatively small farming enterprises (Van Wyk et al., 2006). Of critical importance for optimal implementation of the FAMACHA©-based TST, is decision support on: (i) Interval between FAMACHA© evaluation events; (ii) Proportion of animals to evaluate within and between the different groups of animals on the farm; and (iii) Customised fit-for-purpose anthelmintic usage strategies to adopt (Van Wyk and Reynecke, 2011). Despite the importance of such decision support for optimal FAMACHA©-based TST implementation strategy, there have been few attempts to base these decisions on critical data analysis, for optimal application. This leaves a possibility for less than optimal FAMACHA©-based TST strategies being recommended on farms in South Africa and elsewhere, without sufficient background knowledge for any degree of certainty concerning the outcomes to be expected. Moreover, the high labour costs of the 'default option' of weekly checks during the transmission season (Van Wyk and Bath, 2002) are a disincentive to wider uptake of FAMACHA©-based **s**IPM.

In the present study, a micro-simulation technique (Habbema et al., 1996) using a group-structured deterministic model of differential equations was applied to data from a commercial Merino sheep farm, where FAMACHA©-based TST was routinely applied for *H. contortus* infection, to predict the reported number of cases of haemonchosis. The grouping was based on reproductive status of ewes and the model incorporated parasite transmission dynamics and on-farm animal husbandry management practices. Thereafter, sensitivity analysis of the model was conducted using different FAMACHA©-based TST decision values to predict how the overall

number of haemonchosis incidence cases reported on this particular farm would have been affected. This was done to explore opportunities for optimised FAMACHA©-based TST implementation in South Africa. In particular, evidence-guided reduction in frequency or extent of evaluation using FAMACHA© could result in considerable labour savings and improve the uptake of TST and hence the efficiency of production and the sustainability of drug efficacy.

2.2 Methods

2.2.1 Model purpose and application

The model was designed to simulate flock management and *H. contortus* infection dynamics, based on meta data from a farm in Mpumalanga Province of South Africa, in the summer season, from 10th November 1997 to the 13th April 1998. Firstly, the general structure of the model and its application in the estimation of unknown parameter values of interest will be described. Then, with a fully parameterized model, the effect of varying the values of parameters relating to application of FAMACHA©-based TST was investigated through the predicted incidence of cases on the farm concerned. The purpose of this stage is to optimise the way in which FAMACHA© is applied, and the parameters examined are: (i) Interval between FAMACHA© evaluation events; (ii) Proportion of flock to examine within and between the three ewe classes; (iii) Anthelmintic strategy to adopt; and (iv) Competency level in conducting FAMACHA© evaluations.

2.2.2 Husbandry practices on data-source farm

Over the period of the investigation an average of 212 ewes consisting of 81 dry, 61 lactating and 70 pregnant were present and grazed in three groups according to reproductive status (dry, pregnant and lactating) on separate paddocks on the 100 ha farm. Every ewe lambed yearly in synchronised groups, in April, August or December. After having lambed, the ewes remained on the same irrigated pasture for about three months (90 days), whereupon they were weaned onto unirrigated natural grassland-type veldt (Acocks, 1988). The irrigated pasture was then rehabilitated for a maximum of one month before the next group of highly pregnant ewes was introduced. Movement of ewes between the grass veldt paddocks was determined by herbage availability, as opposed to pasture infective levels. Replacement ewes were from a single source outside the farm, mostly as pregnant healthy ewes and off-take

from the farm resulted from deaths and culling, estimated to be 20% of ewes per annum. Control of *H. contortus*, the predominant worm species on the farm, was based on weekly FAMACHA© evaluation of all animals on the farm, with treatment of diagnosed cases (≥3 FAMACHA© score) undertaken using levamisole (Ripercol-L, Bayer Animal Health) at 7.5 mg kg^{-1}.

2.2.3 Model general structure

The flock used for model evaluation was divided into three reproductive-status group strata of: (i) dry; (ii) pregnant; and (iii) lactating ewes. Within each of these groups, the flock was further divided into categories based on *H. contortus* infection status: (i) susceptible to infection with *H. contortus* (S, susceptible); (ii) infected but not yet infectious (E, exposed); (iii) infectious (I, infectious); (iv) infectious, diagnosed by FAMACHA© evaluation as cases and reported as such (D, diagnosed); (v) previously infectious and subsequently immune through having acquired active immunity (P, protected); and (vi) earlier diagnosed as cases that subsequently recovered (R, recovered) after treatment. These category abbreviations combine to SEIDPRS for the model structure as a whole.

The model structure is case-based, in common with models of infectious micro-parasites (viruses etc.), rather than based on parasite abundance, as is more usual for models of macroparasites (e.g. helminths). Burden-based models are generally appropriate for GIT nematodes, because infection between animals is transmitted through pasture and, since almost all animals sharing an infective pasture are expected to have some level of infection practically all of the time (Gordon, 1981). Prevalence or case-based models would therefore have no power to predict variation in infection status in this system. However, burden-based models also have limitations: predictions are difficult to validate because of parasite overdispersion and wide variation in the relationship between indirect measures of parasite abundance such as fecal egg counts (FEC) and actual parasite burden. For the purpose of the present exercise it was assumed that parasite burden could be estimated from FAMACHA© score, to the extent of being able to regard only the seriously affected ewes as “infected” (exposed), and the rest then as “uninfected or unexposed” (susceptible). It was the proportion of ewes seriously affected that was assumed to contaminate the pasture more severely, thus leading to infection of

susceptible ewes, hence considered infectious through pasture in the model. This was done by allowing the model to approximate the proportion of seriously affected ewes in each reproductive status group. This approach has the advantage of being centred, and calibrated, on the prevalence of anaemic animals, indicated by FAMACHA© scores ≥3. This is an outcome of key interest to farmers and one that, in TST systems, leads directly to treatment, unlike decisions based on parasite burden in the absence of clinical signs of anaemia. Similarly, most of the cases (FAMACHA© scores ≥3) carry the heaviest burdens of adult *H. contortus* and are responsible for most of the eggs produced onto pasture, hence contagion in the model from cases to cases via pasture is realistic, and subsumes the need to track parasite population abundance explicitly.

It was assumed that the pre-infective period for pasture grazed by animals, ranged from zero for persistently infective pasture, to a maximum of 28 days [pre-patent period of (19 – 21 days) plus developmental period (5 – 7 days) from egg to infective L3] for clean pasture, where initial contamination was seeded by the newly introduced animals harbouring various stages of parasitic infection (Veglia, 1915; Herlich et al., 1958; Urquhart et al., 1995). The infectious period in days for the infectious ewes which ended up as cases in each group because of limited resistance and resilience was assumed to be equal to the average interval between FAMACHA© evaluations plus time lapse between treatment and maximum drug effectiveness against *H. contortus*. Thus, reported cases (≥3 FAMACHA© score) in each group were treated and assumed to cease to be infectious, hence recovered, in the time interval it takes the levamisole drug used to reach maximum effectiveness against the prevailing parasitic burden. However, the other resilient proportion of infectious ewes which did not experience clinical signs was assumed to be infectious for the duration it takes to acquire immunity against *H. contortus* (Levine, 1980; Smith, 2001; McClure, 2012). Recovered ewes in each ewe group were assumed to become susceptible to re-infection after waning of the direct and indirect residual effect of the levamisole drug, while those that acquired immunity, became susceptible to re-infection after immunity had lapsed. These differential rates of infection were assumed to be preserved during all re-infection cycles. The unspecified parameters listed above were fitted heuristically by minimising deviance

of model predictions form observed data, and values used in the final model are given in the results section.

The reproductive-status group categories (dry, pregnant and lactating) in the model were also linked by an equation between corresponding infection states (susceptible, exposed, infectious, diagnosed, protected, and recovered) to enable transition of ewes between reproductive-status categories. This transition was assumed to take place at: (i) Conception at a rate proportional to the inverse of the dry period; (ii) Parturition at a rate proportional to the inverse of the gestation period; and (iii) Weaning at a rate proportional to the inverse of the lactation period. From all the infection states, ewes were assumed to be removed through culling and deaths at a rate equal to the replacement rate, with the latter carried out through pregnant susceptible ewes. Further details of the model, including equations and parameters, are shown in Appendix 1A.

2.2.4 Reproduction class group-dependent contact

On this trial farm animals were grouped and managed separately according to reproductive status. Owing to the above husbandry practice, which is common in most commercial ewe farming enterprises, and also to the well documented variability in susceptibility to parasite infection between ewe reproductive classes (Vlassoff, 1976; Malan et al., 2001; Hoste et al., 2002a; Hoste et al., 2002b; Hoste et al., 2002c; McClure, 2012), the mixing pattern between individual ewes in different ewe reproductive status groups was assumed to be heterogeneous (Emilia and Richard, 2010).

Heterogeneous mixing assumes that individuals in one reproductive status group mix intensively via pasture within their own group and less intensively with individuals in other groups. Implicit in heterogeneous mixing pattern assumptions is that the number of cases occurring in the ewe group that has recently been introduced on a particular pasture will be dependent on the contamination levels of that particular pasture by the immediate previous group that grazed on it. The contact patterns within and between the ewe groups was estimated indirectly in the form of a Who-Acquired-Infection-From-Whom (WAIFW) matrix (Anderson and May, 1985b; 1992).

Matrix one (M1) assumed simultaneous presence of different groups, as was the case on this particular farm, but strictly grazed and rotated separately through designated non-contiguous pastures or grazed and rotated separately through shared pasture, but with a follow-up group allowed onto pasture previously grazed by another group only when the pasture is deemed clean from *H. contortus* contamination. Hence, only within-group effective contact parameters were predicted and zeros were attributed to the between-group effective contact parameters in M1.

With matrix two (M2), concurrent existence of different reproduction-class groups on the farm as well as separate grazing were also assumed. However, the groups' sequential rotation between the different paddocks was assumed to be based on herbage condition, such that the follow-up group could be put on pastures judged to be nutritious for their reproductive status even though still infective due to contamination from the immediate previous group which grazed on the same pasture. Because infection is pasture-mediated, i.e. signifying indirect transmission, asymmetric matrices are permissible. M2 was assumed to be asymmetric and populated by imposing key epidemiological features to reduce the number of parameters so that the matrix can become identifiable (Edmunds et al., 1997; Kanaan and Farrington, 2005), as shown in Table 2.1. Therefore, the model assuming M1 matrices and that assuming M2 matrices are non-nested.

Table 2.1: Summary of model contact pattern assumptions. Matrix M1 assumes complete segregation of ewe reproductive classes by separate grazing or co-grazing after elapse of the infectious period of pasture. Matrix M2 assumes mixing of groups, with greater sharing of pastures within groups and less between groups. Based on the on-farm husbandry management routine being in place, the probability of an effective contact between individuals in the dry group and those in the pregnant group was assumed to be 20% of that between individuals in the dry group while for individuals in the pregnant group and those in the lactating group it was assumed to be 10% of that between individuals in the lactating group. Transition rates are in units of 'per day'.

	Matrix M1			**Matrix M2**		
	Infecting group			**Infecting group**		
Susceptible group	*Dry*	*Pregnant*	*Lactating*	*Dry*	*Pregnant*	*Lactating*
Dry	β_1	0	0	β_1	β_2	β_3
Pregnant	0	β_2	0	$0.2*\beta_1$	β_2	$0.1*\beta_3$
Lactating	0	0	β_3	β_1	β_2	β_3

β1 represents the assumed effective contact amongst dry ewes.

β2 represents the assumed effective contact amongst pregnant ewes.

β3 represents the assumed effective contact amongst lactating ewes.

2.2.5 Data source for model fitting and validation

The analysis was based on data obtained from routine weekly FAMACHA© evaluations of an initial flock of approximately 212 ewes in number for the period spanning 10th November 1997 to 13th April 1998, i.e. during the course of one entire *Haemonchus* transmission season. Data for pregnant ewes was extracted as a subset of the whole dataset, by concentrating on a period spanning 150 days (average gestation period) before the recorded lambing date of each individual ewe up to the 10th November 1997. For lactating ewes, data for the next 90 days (average on-farm lactation period) from the recorded lambing date were used, while for dry ewes, data for the following 30 days (average period ewes stayed dry, i.e. not pregnant and not suckling) after the 90 days, were extracted up to the 13th April 1998.

Minor data manipulations were carried out by merging and deleting of data where necessary in each ewe group, as explained below, to ensure data capture time interval compatibility between all three of the groups, which is a requirement for this chosen format of modelling. It was ensured that the interval between subsequent FAMACHA© evaluation events was at least six days, by deleting all records which were less than six days from the previous record if they were of the same animal (as deduced from the recorded animal ID) or by merging all records, which were less than six days apart if they were of different animals. This was done in order to minimise repeat observations of ongoing clinical cases, in which erythropoiesis had not yet compensated for prior anaemia. It takes on average of five to seven days to land mature erythrocytes into circulation from stem cells in the bone marrow (Reece and Swenson, 2004; Michael and Denbow, 2013), therefore to cure anaemia subsequent to dosing, a six day minimum interval should elapse. Also records with five or less animals during an evaluation event were excluded from the analysis to avoid targeted evaluations of selected sick animals, as that would lead to biased incidence rates. The average dry period was ≤30 days, as the ewes were allowed to suckle lambs for a long period, which resulted in less FAMACHA© evaluation events in the dry group when compared to the other groups. The 14 records for the dry group satisfying the adopted inclusion criteria were used in the analysis, whereas only the FAMACHA© evaluation records of the pregnant and lactating groups corresponding time- and interval-wise to the FAMACHA© evaluation records of the

dry group, were included in the analysis. In one evaluation event the lactation group had no record corresponding directly time-wise to that for the dry group and instead the nearest previous record for the lactation group that corresponded with that for the dry group was used to approximate the case numbers that could have been reported, had the lactation group been evaluated during that particular evaluation event.

2.2.6 Model data fits and parameter estimations

Due to limited and short-term immunity against *H. contortus* in infected sheep, repeat infections do occur and can result in reported cumulative incidence cases at the end of the period of investigation exceeding the physical count of animals on the farm. To manage that problem, the cumulative number of animals evaluated in a particular group at each FAMACHA© evaluation event over the period of investigation was taken to be the total population size of that particular group instead of the physical count of animals. The approach also had the advantage that it automatically took into account any sudden increase (growth) or decrease in ewe population size. Standardisation of the observed proportion of cases in each group across the period of investigation was done by scaling the observed proportion of cases out of 100 ewes at each FAMACHA© evaluation event for comparative purposes. This resulted in a total of 1400 standardised population of ewes in each group at the end of the period of investigation (10th November 1997 to 13th April 1998).

The unknown in-situ model baseline parameter values were estimated by maximum-likelihood fitting. These were: (i) Within-group pasture-mediated effective contact parameters (beta); (ii) Time to maximum effective levels of levamisole in the animals; (iii) Average residual drug activity period in each group; (iv) Time to acquisition of immunity by ewes in each group after becoming infectious; (v) Protective period of the acquired immunity in each group; (vi) Removal rate of ewes through culling and deaths from the whole flock; (vii) Proportion infected (exposed), i.e. the proportion of ewes carrying heavy burdens of parasites in the whole flock; (viii) Proportion of infectious ewes which experienced clinical signs of haemonchosis due to limited resilience and resistance in each group; (ix) Effectiveness of the Ripercol-L drug used to treat cases diagnosed at FAMACHA©

evaluation events in the whole flock; (x) Average evaluator competency in conducting FAMACHA© evaluation on this farm over the period of study, i.e. the fitted agreement between the observed cases (≥3 FAMACHA© score) by the evaluator and the exact number of cases; and (xi) Number of ewes in each of the three groups which were: (a) infected; and (b) infectious at the start ($t = 0$).

Maximum likelihood fitting of model-predicted numbers of cases to the observed numbers of cases per 100 ewes per evaluation event in the three reproductive status groups simultaneously was conducted by simultaneous variation in all unknown parameters listed above. Model fitting was done in Berkeley Madonna™ v. 8.0.1 software by minimising the negative (Poisson) log likelihood using optimize function, with fourth order Runge–Kutta method used to numerically solve the equations at a time step of 0.001 days and 0.02 days output steps (Macey et al., 2000; Emilia and Richard, 2010). The optimize function concurrently varies the values of the input parameters to be estimated within a constrained range of probable values to pick the best parameter estimate values that best fit the model to the data. Maximum likelihood data fitting method was based on the simplex method for function minimisation (Nelder and Mead, 1965; Emilia and Richard, 2010) and the goodness of fit statistic, namely the log likelihood deviance, which describes how far the model predictions deviate from the observed data, was calculated using the expression shown in Appendix 1B. The larger the deviance the poorer the model fit to the data.

2.2.7 Modelling the "IF" scenarios for potential TST optimisation

A heuristic evaluation of the efficiency of the FAMACHA©-based TST strategy as implemented on the farm was done by running "what if" scenarios using only the statistically selected best-fitting model to the observed case (FAMACHA© ≥ 3) incidence data. Analysis was based on the total cumulative number of cases across the three ewe groups from 10th November 1997 to 13th April 1998. This was done by running scenarios on: (i) Competency level in conducting FAMACHA© evaluation, by looking at various assumed on-farm FAMACHA© sensitivities; (ii) Various intervals between FAMACHA© evaluation events; (iii) Various group-based proportions of ewes to evaluate with treatment of all cases diagnosed; and (iv) Anthelmintic application strategy of whole-flock treatment instigated at different

cross-sectional incidence rates. Risk (R) or attack rate was calculated for each of the above scenarios and the effect of each scenario was judged by comparing the calculated R to the baseline (unexposed) R as a risk ratio (RR). From the above sensitivity analysis, potential areas for FAMACHA©-based TST optimisation in general, and with specific reference to the study farm, were assessed.

2.3 Results

2.3.1 Analysis of observed case numbers and parameter value estimates

Fits of the model to the weekly numbers of observed cases in the three ewe reproductive groups are shown graphically in Figure 2.1 and the corresponding WAIFW matrices are summarized in Figure 2.2. The best-fitting versions of each model, M1 and M2, using heuristically fitted parameter values, are summarized in Table 2.2. By visual inspection of Figure 2.1, model M1, which assumed assortative mixing within stock classes and none between classes, appeared to fit the data better than the alternative model M2. The deviance was also much smaller for model M1 (660) when compared with model M2 (1056).

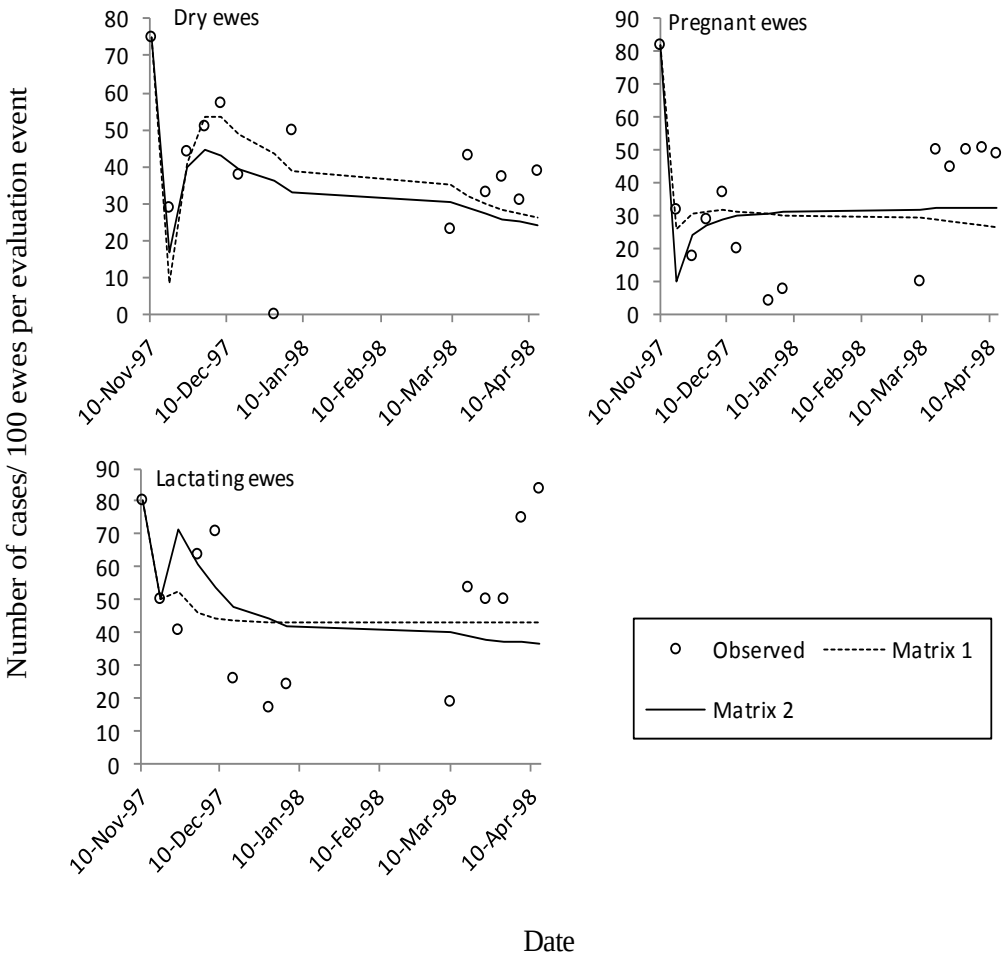


Figure 2.1: The observed data (circles) and the best-fitting model predictions of numbers of cases per 100 ewes per evaluation event in ewes of different reproductive status (lines), assuming FAMACHA© evaluation was conducted every seven days in all three of the groups. A case is defined as a FAMACHA© score ≥ 3.

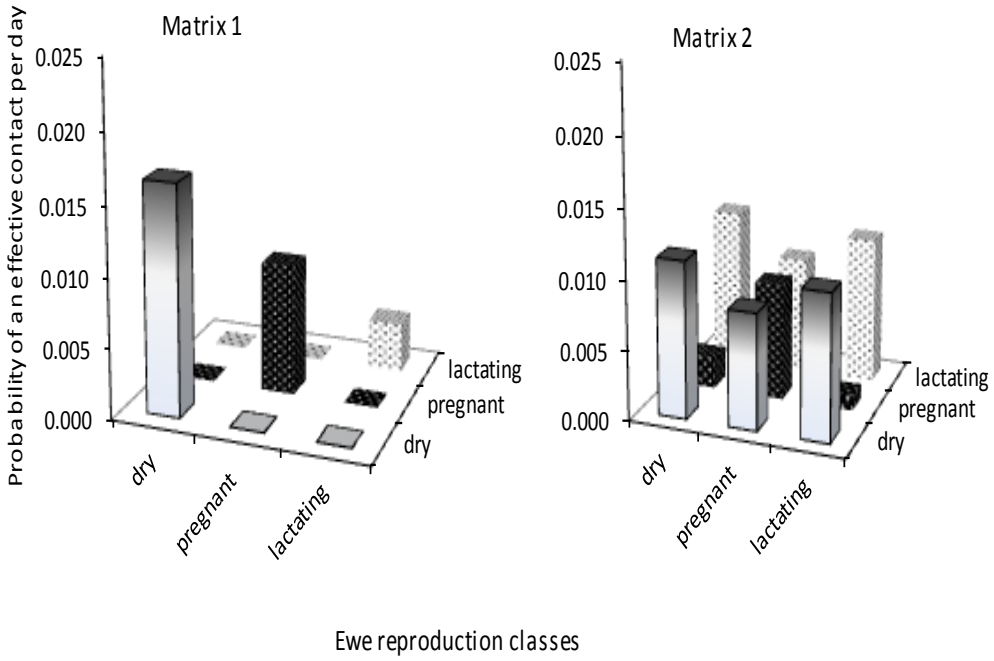


Figure 2.2: Fitted matrices of "Who-Acquires-Infection-From-Whom", obtained by assuming either without inter-group contact through pasture (Matrix 1, M1), or with inter-group contact (Matrix 2, M2). Susceptible ewe groups are displayed on the z-axis and infecting ewe groups on the x-axis.

Table 2.2: Maximum likelihood baseline parameter estimates obtained by fitting model predictions of weekly numbers of cases to the observed incidence cases per 100 ewes per evaluation event in each group.

		Model 1*			Model 2*		
		Groups			Groups		
Parameter †	Description	Dry	Pregnant	Lactating	Dry	Pregnant	Lactating
B j _ j	within group effective contact between animals per day	$1.65x10^{-2}$	$9.36x10^{-3}$	$3.60x10^{-2}$	$1.13x10^{-2}$	$8.35x10^{-3}$	$1.05x10^{-2}$
residual_period_ j	protective period of drench used to treat cases in days	5.97	2.62	6.81	$4.45x10^{-1}$	$7.38x10^{-1}$	1.34
period_to_immunity_ j	time interval from infectious status to immune status in days	35.58	74.85	7.89	15.15	2.97	34.06
immune_period_ j	protective period of acquired immunity in days	56.88	101.74	9.23	$4.58x10^{-3}$	4.06	119.81
Preinfous_ j0	number of infected ewes at the start, $t = 0$	13.03	28.88	10.50	2.62	38.31	3.26
Infous_ j0	number of infectious ewes at the start, $t = 0$	$1.17x10^{-1}$	$9.22x10^{-1}$	$1.70x10^{-2}$	$3.34x10^{-3}$	$5.55x10^{-1}$	$3.78x10^{-1}$
preinfective_period_ j	average pre-infective period for pasture grazed by ewes in days	27.70	$4.66x10^{-4}$	$4.49x10^{-4}$	32.91	15.80	$4.49x10^{-4}$
period_to_drug_eff_ j	time period for dosing drug to reach maximum effective level in days	$6.26x10^{-1}$	$8.74x10^{-1}$	$8.56x10^{-1}$	$4.56x10^{-1}$	1.00	$6.24x10^{-1}$
frac_infous_clin_ j	proportion of infectious ewes which experience haemonchosis per day	$4.01x10^{-1}$	$4.34x10^{-1}$	$1.59x10^{-1}$	$2.83x10^{-1}$	$1.75x10^{-1}$	$1.27x10^{-1}$
dose_eff	proportion of cases cured as a result of drenching per day		$9.58x10^{-1}$			$5.01x10^{-1}$	
famacha_sen	proportion of FAMACHA©-diagnosed cases that are truly anaemic		$4.50x10^{-1}$			$5.36x10^{-1}$	
infected_prop	proportion of ewes carrying heavy burden of worms in the flock per day		$6.32x10^{-1}$			$4.66x10^{-1}$	
removal_rate	removal of ewes through culling and death per day		$1.33x10^{-3}$			$2.17x10^{-3}$	
Deviance	How far model predictions deviates from the observed data		660			1056	

***** Model 1: Matrix M1 is adopted; Model 2: Matrix M2 is adopted

† For detailed parameter description see Appendix 1A, Tables 1A (i) and 1A (ii)

j denotes dry, pregnant and lactating ewe groups

According to the best-fitting model estimates, the average proportion of ewes with heavy parasite burdens over the period of study, i.e. considered infected or exposed, was 63.2% per day while the average proportion of infectious ewes which were predicted to show clinical signs of *H. contortus* varied between the three groups with the highest of 43.4% per day in pregnant ewes, followed by 40.1% per day in dry ewes and 15.9% per day in lactating ewes. The average competency level in conducting FAMACHA© evaluations for all the evaluators who did the evaluations on the farm during the period of investigation was predicted to be 45.0%, i.e. 45.0% of cases reported as such during FAMACHA© evaluation events were true cases of haemonchosis. levamisole drug effectiveness was 95.8% (fitted value) and it took <1 day after dosing for the drug to reach maximum effective levels against the *H. contortus* parasitic burden in all three ewe groups. Average residual activity period for levamisole was approximated to be 6.0, 2.6 and 6.8 days for dry, pregnant and lactating groups of ewes, respectively.

Estimates of time to acquisition of immunity for the infectious but resilient proportion of ewes in each group ranged from 74.9 days for pregnant ewes to 35.6 and 7.9 days for dry and lactating ewes, respectively, while the period of immunity for that proportion, which acquired immunity in each group ranged from 9.2 days for lactating ewes to 56.9 and 101.7 days for dry and pregnant ewes, respectively. The pre-infective period was <1 day for the pastures grazed by lactating ($4.5x10^{-4}$ days) and pregnant ($4.7x10^{-4}$ days) ewes while it was 27.7 days for pastures grazed by dry ewes. The estimated *H. contortus* transmission rates via pasture, i.e. effective contact rates (beta), ranged from the highest amongst lactating ewes ($3.6x10^{-2}$ per day) followed by that amongst dry ewes ($1.7x10^{-2}$ per day) to the lowest rate predicted, which is that amongst pregnant ewes ($9.4x10^{-3}$ per day). Removal rate through culling and deaths on the farm was estimated to be $1.37x10^{-3}$ per day.

2.3.2 Analysis of "IF" scenarios for potential optimisation of FAMACHA©-based TST

From the best-fitting model (M1) baseline predictions (Table 2.3), the cumulative overall predicted haemonchosis incidence rate (40%) was very close to the observed incidence rate (41%) over the period of study, as expected by the fact that the model was fitted to the data. The group-specific incidence rates reported and predicted over

the period of investigation were highest amongst the lactating group (50% and 48%, respectively), followed by the dry group (both 39%) and lastly by the pregnant group (35% and 33%, respectively).

Table 2.3: Baseline prediction summary of number of cases recorded on the farm over 14 evaluation events, assuming 100 ewes in each reproductive status group presented for evaluation at each evaluation event (every seven days) over the period of investigation. The best-fitting model (M1) parameter value estimates are assumed.

		Observed cases		Predicted cases	
	Group	Number	% of group population	Number	% of group population
Matrix 1	dry	550	39	542	39
	pregnant	485	35	463	33
	lactating	705	50	667	48
	Overall	1740	41	1672	40

Comparison of the predicted overall cumulative cases to observed cases showed high model sensitivity to changes in: (i) Competency level in conducting FAMACHA© evaluations; (ii) Interval between FAMACHA© evaluations; and (iii) Proportions of ewes evaluated within and between the ewe groups. A ≥5% decrease and a ≥10% increase in FAMACHA© evaluation competency led to a significant decrease and increase in the overall cumulative number of diagnosed cases predicted ($p < 0.001$ and $p < 0.001$ respectively, and Table 2.4). Also, a ≥1 day decrease and ≥1 day increase in FAMACHA© evaluations interval led to a significant decrease and increase in the overall cumulative number of diagnosed cases predicted ($p = 0.001$ and $p < 0.001$, respectively and Table 2.5). Evaluation of ≤90% of animals in either the dry, pregnant or lactating group and 100% in the other two groups was predicted to lead to a significant decrease in diagnosed cases over the period of investigation ($p = 0.019$, $p = 0.018$ and $p = 0.005$, respectively and Table 2.6) at $p < 0.05$ level of significance.

The model showed poor sensitivity to whole-flock treatment strategy at the different cross-sectional incident rates assumed for its instigation. Whole-flock treatment intervention at daily disease rates of one, two, three, four and five cases per 5000 ewes, assuming that whole-flock treatment hypothetically reduces risk of infection by a factor of 1000, showed a significant decrease in overall predicted cumulative number of cases when compared to no application of whole-flock treatment over the period of investigation ($p< 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.008$ and $p = 0.043$, respectively and Table 2.7 and Figure 2.3).

Table 2.4: Effect of FAMACHA© evaluation competency (sensitivity) on the number of reported cases, as predicted by the model. Overall numbers of reported cases are listed, based on evaluation of all of the 100 ewes in each group per evaluation event, assuming different levels of FAMACHA© evaluation competency (sensitivity), for the whole period of investigation.

	FAMACHA© sensitivity (%)	No. of cases	†R	‡RR (95% CI)	*p-value
Matrix 1	40	1526	0.36	0.88 (0.83 – 0.93)	< 0.001
	50	1815	0.43	1.04 (0.99 – 1.10)	0.098
	60	2094	0.50	1.20 (1.15 – 1.26)	< 0.001

† R: risk or attack rate.
‡ RR: risk ratio (attack rate ratio) and the baseline (unexposed) value is the overall predicted case. numbers in Table 2.3 with FAMACHA© sensitivity of 45 %.
***** p-value: probability of getting the RR value obtained or more if RR equals one.

Table 2.5: Effect of FAMACHA© evaluation interval on the number of reported cases, as predicted by the model. Overall numbers of reported cases predicted with evaluation of all of the 100 ewes in each group per evaluation event, assuming different FAMACHA© evaluation intervals, for the whole period of investigation.

	FAMACHA© intervals (days)	No. of cases	†R	‡RR (95% CI)	*p-value
Matri x1	6	1886	0.45	1.08 (1.03 – 1.14)	0.001
	8	1508	0.36	0.87 (0.82 – 0.91)	< 0.001

† R: risk or attack rate.

‡ RR: risk ratio (attack rate ratio) and the baseline (unexposed) value is the overall predicted case numbers in Table 2.3 assuming an interval of seven days between FAMACHA© events.

***** p-value: probability of getting the RR value obtained or more if the null hypothesis that the RR equals one is true.

Table 2.6: Effect of evaluating a proportion of ewes on the number of reported cases, as predicted by the model. Overall numbers of reported cases predicted with evaluation of different assumed and randomly selected proportions of ewes within and between the 100 ewes in each reproductive group, for the whole period of investigation, with treatment of all diagnosed cases at each evaluation event.

	Percentage (%) of ewes examined	No. of cases	†R	‡RR (95 % CI)	*p-value
Matrix 1	90 in each group	1541	0.37	0.89 (0.84 – 0.93)	< 0.001
	90 dry group, 100 other groups	1635	0.39	0.94 (0.89 – 0.99)	0.019
	90 pregnant group, 100 other groups	1634	0.39	0.94 (0.89 – 0.99)	0.018
	90 lactating group, 100 other groups	1616	0.38	0.93 (0.88 – 0.98)	0.005

† R: risk or attack rate.

‡ RR: risk ratio (attack rate ratio) and the baseline (unexposed) value is the overall predicted case numbers in Table 2.3 with 100 % evaluation of all ewes in all the groups at each evaluation event.

***** p-value: probability of getting the RR value obtained or more if the null hypothesis that the RR value equals one is true.

Table 2.7: Effect of timed whole-flock drenching on the number of reported cases, as predicted by the model. Overall numbers of reported cases predicted with evaluation of the 100 ewes in each group at each evaluation event, treatment of all diagnosed cases at each FAMACHA© evaluation event and a once-off whole-flock treatment when the daily number of cases reported reaches a particular assumed rate. This was done to investigate the daily disease rate beyond which a once-off suppressive treatment (whole-flock drenching) will have no significant difference on the number of reported cases, when compared to weekly FAMACHA©-based TST, for the entire period of investigation. The target daily disease rates were worked out of a flock of 5000 ewes for amplification with a hypothetical value of 1000 assumed to represent the immediate reduction in risk of infection (FOI) with a once-off whole-flock treatment. Taken into account for the latter is the documented husbandry management on the farm, which included rotational grazing.

	Daily rate of overall reported number of cases per 5000 ewes ≤	No. of cases	†R	‡RR (95% CI)	*p-value
Matrix 1	1	1218	0.29	0.70 (0.66 – 0.74)	< 0.001
	2	1466	0.35	0.84 (0.80 – 0.89)	< 0.001
	3	1570	0.37	0.90 (0.86 – 0.95)	< 0.001
	4	1620	0.39	0.93 (0.88 – 0.98)	0.008
	5	1649	0.39	0.95 (0.90 – 1.00)	0.043
	6	1662	0.40	0.96 (0.91 – 1.01)	0.083
	7	1670	0.40	0.96 (0.91 – 1.01)	0.120

† R: risk or attack rate.
‡ RR: risk ratio (attack rate ratio) and the baseline (unexposed) value represent the overall predicted case numbers in Table 2.3 with no whole-flock treatment intervention done over the period of the investigation.
***** p-value: probability of getting the RR value obtained or more if the null hypothesis that the RR value equals one is true.

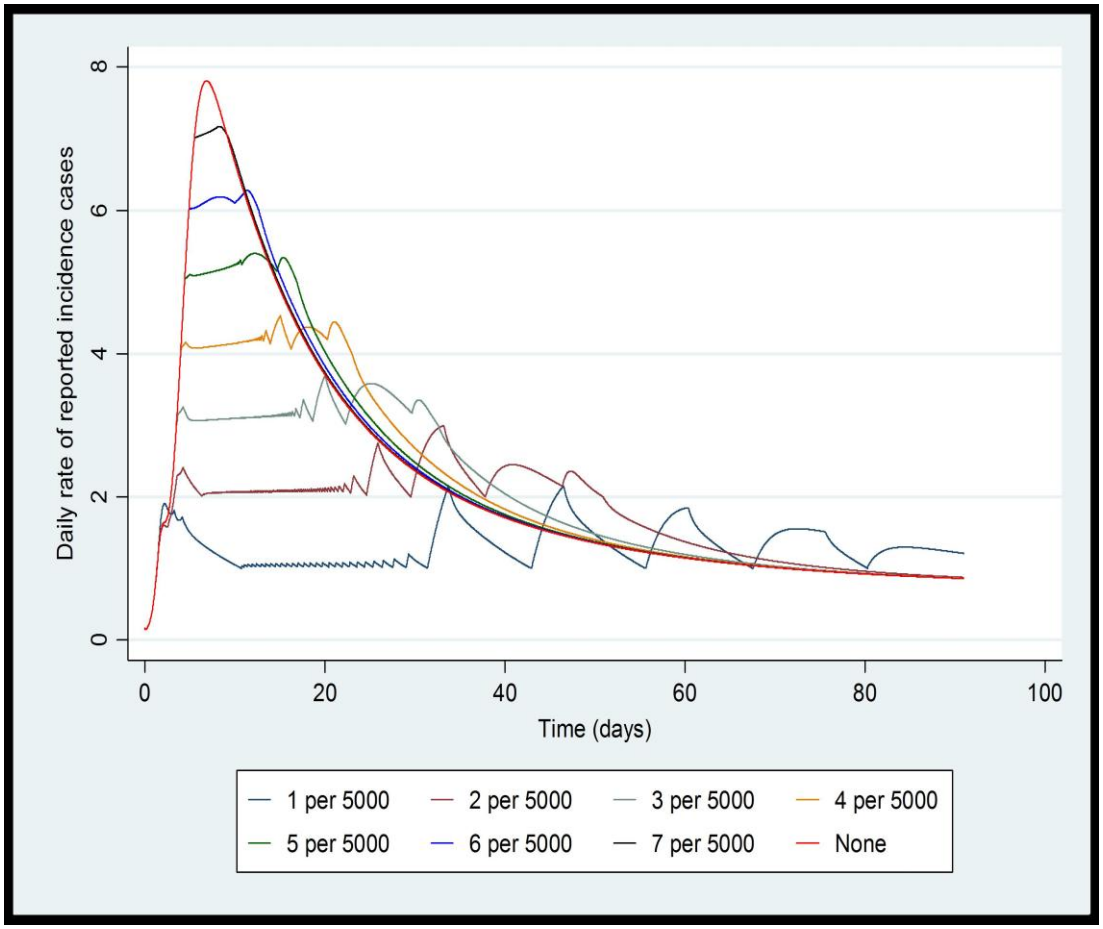


Figure. 2.3: Predicted rate of reported incidence per 5000 ewes per day during the period of investigation, with once-off whole-flock drenching interventions assumed in Table 2.7.

2.4 Discussion

The use of a SIR-type model framework for a macroparasite is novel, since previous models have invariably tracked parasite population abundance (Smith and Grenfell, 1994) rather than number of substantially infected individual hosts. This unusual approach was chosen to match the data collection modality, in that cases of haemonchosis were estimated using FAMACHA©, rather than parasite abundance. Therefore, a case-based model structure was suitable. Additionally, TST of *H. contortus* using FAMACHA© seeks to prevent clinical disease arising from a combination of parasite burden and host resilience, rather than aiming to target high worm burdens *per se*. Related to this point, the model incorporated the further novelty of simultaneous prediction of disease in different sheep classes, using a reproductive status group-structured model. This allowed for on-farm estimation of reproductive status group-dependent unknown parameter values and, notably, estimation of transmission rates (effective contact rates) for haemonchosis within reproductive status groups (dry, pregnant and lactating), something that has not been previously attempted, but could be useful in applying FAMACHA© efficiently on farms with multiple sheep classes of varying exposure and resilience.

The model predicted competency (sensitivity) in conducting FAMACHA© evaluations (FAMACHA© sensitivity) of 45 % on this farm, which is consistent with the results obtained in previous FAMACHA© validation studies (Kaplan et al., 2004; Burke et al., 2007; Reynecke et al., 2011c). Residual activity period of between three to seven days predicted for the short acting drug, levamisole, used to treat diagnosed cases, appears reasonable in modelling (a non-exact science), even though slightly higher than the quoted value of less than one day (Barragry, 1994). The slightly longer drug residual activity period could be attributed to the plausible potentiating effect of either evasive husbandry practices like rotational movement of animals around the farm particularly at time of dosing, or to the immunostimulant effect of levamisole (Renoux et al., 1976; Cabaj et al., 1995), both of which have potential to delay reinfection, but were not adjusted for in the model due to unavailability of relevant data. However, the times to maximum effectiveness for levamisole were the same across the groups at <1 day, which seems to suggest that the effectiveness of drugs is not dependent on parasitic burden. Judging from the predicted drug effectiveness of 95.8 %, it seems that levamisole was highly effective as per the

international guidelines (Wood et al., 1995). This prediction is consistent with the 94.3% levamisole effectiveness value obtained after genetic dilution trials were conducted on the same farm in 1998 by Van Wyk et al. (2001).

The best-fitting model to the data, which is that adopting M1 matrices, since it had a much lower deviance value when compared to that adopting M2 matrices, suggests that, as was to be expected from the nature of pasture utilization on the farm, mixing was more intense within a reproductive group, i.e. assortative, than between the reproductive groups, and that between-group contact did not explain any additional variation in number of cases over the season. Also, model parameter estimates imply that the pastures grazed by lactating and pregnant ewes on this farm were persistently infective when compared to pastures grazed by dry ewes, which could arise by being grazed more frequently leading to persistent contamination. Persistent contamination of pastures grazed by lactating ewes seems to be implied by the near persistent susceptibility of lactating ewes, relative to other groups, as they were predicted to be immune for approximately 9 days hence almost always infected with *H. contortus*. Continuous grazing on the same irrigated pasture was practised on the farm with the lactating group and this tallies with the model deduced persistent infectiveness of pasture grazed by lactating ewes. Also, probably owing to the persistently infective nature of pasture grazed by the lactating group, the highest transmission rate [effective contact rate (beta)] between ewes via pasture and the highest proportion of cases observed and predicted over the period of investigation was that for the lactating group.

The highest proportion of cases was observed and predicted by the model in the lactating group. This model deduction is consistent with findings from previous work done elsewhere (Vlassoff, 1976; Hoste et al., 2002a; Hoste et al., 2002b; Hoste et al., 2002c) and in South Africa (Malan et al., 2001), where it was shown, through trials, that lactating ewes are generally more susceptible (less resilient) to endoparasites than the dry and pregnant groups. The highest transmission rate, continuously infective pasture and shortest duration of protective immunity (near persistent susceptibility) was predicted for the lactating group, which probably explains the highest overall proportion of cases for the entire period of investigation being in this group. However, it is worth noting that the lactating group was also

predicted to have the lowest proportion of infected and infectious ewes, which ended up as clinical cases of haemonchosis per unit time. Better nourishment of lactating ewes could have led to the above scenario, since the lactating group on this particular farm was kept on irrigated pasture, hence a better plane of nutrition (energy and / or protein), leading to better defence against *H. contortus* infection. This is consistent with the statement of McClure (2012) to the effect that "…feeding a higher quality diet, in terms of digestibility, bypass protein and/or readily available carbohydrates" can counter the heightened susceptibility to parasites infection shown by ewes in late pregnancy and early lactation. And this is in line with earlier pronouncements by Whitlock et al. (1943) that "parasitism is a nutritional disease" and the generally enhanced performance of ewes supplemented with proteins as shown in the field by Knox (1996), Houdijk et al. (2000), Coop and Kyriazakis (1999, 2001) and Van Rensburg (2002).

Although the pregnant group, and similarly the lactating group, was predicted to be grazed on persistently infective pastures when compared to the dry group, it was estimated to experience the lowest transmission rate amongst the ewes via pasture and to have contributed the lowest proportion of cases to the overall cumulative cases observed and predicted on the farm. This could be explained by the predicted protective duration of acquired immunity against *H. contortus*, which was longest amongst pregnant ewes, intermediate amongst dry ewes and shortest amongst lactating ewes. Interestingly, the predicted immune period for pregnant ewes was 102 days, leaving approximately 48 days out of the 150-day gestation period when the ewes are expected to be vulnerable to infection due to limited immunity. The balance of 48 days falls within the peri-parturient relaxation of resistance (PPRR) period of 3-4 weeks before and after lambing (Barger, 1993; Houdijk et al., 2001). Therefore, it is plausible that the predicted 102 days of protective immunity out of the 150-day gestation period in the pregnant group indicates the phenomenon of PPRR. This is further supported by realisation that the period of investigation was from 10th November 1997 to 13th April 1998 and since the practice on this farm was to lamb ewes in December and April, thus with heavily pregnant ewes in their third trimester and scheduled to lamb in December 1997 and April 1998 would have been in the majority of the pregnant group. The PPRR could also partly explain the reason

why the shortest protective period of acquired immunity was in the lactating group, since PPRR overlaps into the early stages of lactation.

The fitting of model predictions to the observed case incidence data showed an evident overall downwards trend in the numbers of cases over the period of investigation. This could probably be an indication that sustained repeat FAMACHA©-based TST does not only afford benefits regarding sustainable worm management but also, by allowing animals to be naturally challenged, development of protective immunity is enabled and leads to reduction in future cases. Also, heritability of resistance and resilience to *H. contortus* infection could have been accelerated over time within the flock through the culling practice of getting rid of ewes which frequently presented with FAMACHA© scores ≥3 during routine evaluations. As this was as practised on this particular farm, it would progressively have led to reduction in future incidence cases as previously documented by Riley and Van Wyk (2009).

Analysis of the impact of TST implementation decision output, based on contact patterns inferred from the observed FAMACHA© data, implies, with respect to this farm, that: (i) Not evaluating almost all the animals at each FAMACHA© evaluation event would have led to a significant decrease in number of cases diagnosed over the period of investigation as a result of missed cases in the other proportion, which was not evaluated at each evaluation event; (ii) That either conducting FAMACHA© at long intervals, or by less skilled FAMACHA© evaluators, would have led to a significant number of cases being missed (false negatives), hence with a concomitant decrease in the predicted cases diagnosed over the period of investigation. On the other hand, assuming high competencies in conducting FAMACHA© evaluations, i.e. high FAMACHA© sensitivity, or conducting FAMACHA© at very short intervals resulted in increased predicted numbers of cases when compared to the baseline situation. This is probably due to false positive cases, which are probably more acceptable than high false negatives particularly for high value stud farms (Van Wyk, 2008; Reynecke et al., 2011a). The significant decrease in overall cumulative numbers of cases predicted with low competency in conducting FAMACHA© and long intervals between evaluations is explained thus: the missed cases, which initially would have led to an increased incident rate as a result of heightened pasture

contamination, are assumed dead in the model as the most probable outcome with untreated cases of haemonchosis. Therefore, at each iterative model time step (0.001 days) the disease rate, which applies to the susceptible flock population at a particular point in time and relates the number of new cases to the whole period of investigation, operated on an ever-diminishing population as time goes on (Kirkwood and Sterne, 2003). This means that the predicted number of new cases per unit time was steadily decreasing, which ultimately led to a significantly decreased cumulative output of predicted cases at the end of the period under investigation when compared to the baseline situation. As a result, for practical interpretation of the results at farm level, the significant decrease in case numbers should be taken to mean the opposite, i.e. an equally significant increase in cases with deaths of the undiagnosed cases resulting in a reduced flock size due to: (i) Low competency in conducting FAMACHA© evaluations; (ii) Not evaluating almost all the animals at each FAMACHA© evaluation event; and (iii) Long intervals between FAMACHA© evaluations.

With due consideration to the exceptions listed below, the deduced lack of significant difference from the baseline value of predicted cases when applying whole-flock treatment interventions (except when done at unrealistically low daily disease rates and high impact assumptions), seems to suggest that the observed cases played an insignificant role in the overall epidemiology of *H. contortus* on this farm. If correct, it seems to contradict what has always been believed of cases in the epidemiology of *H. contortus* and endoparasites in general, i.e. that cases perpetuate the disease due to high levels of contamination of the pastures with worm eggs, in relation to sub-clinically affected animals in the group (Coffey and Hale, 2012). This is believed to the extent that selective deworming in control of equine helminth infections targets horses that shed the majority of eggs, as there are judged to be responsible for the bulk of pasture contamination (Gomez and Georgi, 1991; Reinemeyer, 2009). However, this opposing deduction from the model seems to agree with conclusions from other studies, that the majority of parasites are outside the animals, on pasture or in dung at any point in time (Barger, 1978), such that treating cases is not expected in general, to affect the numbers of cases in the next cycle. It also re-enforces the concept of refugia under TST application in the respect that, had numbers of cases requiring treatment increased over time, a reciprocal

effect on proportions of worms in refugia was to be expected, i.e. progressively more selection for AR, given that all else was equal.

A simplifying assumption that contact parameter values (beta) did not change during the season was made. This could be too simplistic since beta is a function of both host behaviour and parasite biology, which are dynamic variables influenced respectively by the day-to-day on-farm husbandry practices and weather elements. However, beta and infectious-proportion variables in each group are functions of the risk or force of infection (FOI) experienced by each group in this model. FOI is the rate at which susceptible individual ewes acquire an infectious disease, in this case *H. contortus*, and it's a key measure of flock transmission (Vynnycky and White, 2010) that is largely dependent upon resistance and resilience status of individual animals within a flock. The FOI varied at each FAMACHA© evaluation event during the fitting of the model to the observed cases, mainly on account of the variable infectious-proportion parameter in each group at each FAMACHA© evaluation event. The infectious-proportion parameter absorbed the lack of variability in beta to enable a fit of the model to the data, hence the infectious proportion was most likely either over- or under-estimated at each evaluation event. The variability in FOI at each evaluation event allows for carrying out of further work to determine which, amongst the weather elements (temperature, rainfall and entropy) were significant drivers of infection at each FAMACHA© evaluation event. This is considered in the next chapter.

2.5 Conclusion

Although SIR-type models are currently not being much explored in the field of veterinary parasitology, it has been shown here that they can be a valuable tool in gaining useful insight into complex and dynamic farming systems and strategies which can lead to better implementation, through optimisation, of disease management systems. Further exploration of SIR-type models is recommended, particularly case-based ones because they model what matters most to FAMACHA©-based TST routine application, namely cases as opposed to parasite burden. Parasite burden in TST only matters in so far as the proportions of parasites in refugia relative to those that are not (Van Wyk, 2001). Whereas the described model structure is largely generalizable across sheep commercial farms, the expected

variability in husbandry practices and weather elements between farms, as known potential drivers of *H. contortus* risk of infection, makes the estimated input parameter values here most likely to vary between farms, hence less generalizable. Therefore, this necessitates further analysis of data within and between farms to reasonably estimate these parameter values across farms so that less farm-specific and more generalizable parameter values can be approximated. The dependence of the model on heuristically fitted model parameters means that a run-in period would be required before bespoke application of the model on any given farm, while conclusions on optimum application of TST might also reach no further than the farm in hand.

It appears from the model deductions in this chapter that for the most effective application of FAMACHA©-based TST, all the animals in the flock have to be evaluated at each evaluation event, at the highest possible competency and at short intervals of ideally seven days but not less to avoid repeat diagnosis (false positives) of previous cases as new cases. However, such a regime would be demanding in terms of resources, especially operator time. Therefore, the potential to use the model to limit such high-intensity monitoring practices to the highest risk times of year, based on climatic indicators, is considered in Chapter 3.

CHAPTER 3

Variation in modelled force of infection with *Haemonchus contortus* as a function of climate

3.1 Introduction

Ecological factors, particularly rainfall, temperature and sunlight are known determinants of the success or failure of the free–living phases of most GIT parasites lifecycles, including those of *H. contortus* (Gordon, 1948; Levine, 1963; Rose, 1963; Gibson and Everett, 1972; Levine and Andersen, 1973; Thomas, 1974; Rossanigo and Grüner, 1995; O'Connor et al., 2006; 2007b, a; Van Dijk et al., 2009; Van Dijk and Morgan, 2011; Khadijah et al., 2013). On-farm husbandry practices modify the climate-driven risks of exposure of susceptible hosts to infective parasite stages (Michel, 1969, 1976; Bullick and Andersen, 1978; Morley and Donald, 1980; McCulloch et al., 1984; Van Wyk et al., 1991; Kirkman and Moore, 1995; Waller, 1999; Van Wyk, 2001; Molento et al., 2004; Bath et al., 2005).

Although total rainfall in absolute terms has been shown to be a strong determinant of larval development success and pasture availability in *Haemonchus* spp. in a number of studies carried out in South Africa (Rossiter, 1961; Thomas, 1968; Horak and Louw, 1977), it was also concluded from concurrent experiments carried out in South Africa by Muller (1964, 1968), Viljoen (1964) and McCulloch et al. (1984) that it is the combined effect of the spread and amount of rainfall that has a much more decided influence on larval emergence and availability on pasture in this species, than rainfall amount *per se*. The importance of the temporal spread or distribution of rainfall on *Haemonchus* spp. development and availability on pasture was also described by Besier (1992) and by Besier and Dunsmore (1993b). This was after Barger et al. (1972) had deduced, through a predictive model, greater dependency of *H. contortus* development upon the time taken for cumulative precipitation to exceed cumulative evaporation than on total rainfall alone.

Barnes et al. (1988) described dependency of *Tr. colubriformis* transmission on effective rainfall, which was defined as the total rainfall over 7 consecutive days exceeding 16 mm, with decreasing probability of an egg developing to an infective larva as the length of time between egg deposition and effective rainfall increases.

On the other hand, McCulloch et al. (1984) suggested that a "minimum amount of rain was required to fall on a property over a 4-week period", for a meaningful *H. contortus* pasture infectivity. Furthermore, Shannon's entropy theory model (Shannon, 1948) has been used to describe spatio-temporal variability in rainfall (Sonuga, 1976; Kawachi et al., 2001; Maruyama et al., 2005) and was first used by Reynecke (2007) successfully to correlate temporal availability of rainfall (entropy) to risk of haemonchosis.

The aim of the work in this chapter is to describe and characterise the dependency of a model-predicted natural force-of-infection (FOI) with *H. contortus* on temporal variability in rainfall, temperature and entropy experienced on a farm where weekly FAMACHA©-based TST was applied. This would enable high risk periods to be identified from climatic variables, stimulating more intense monitoring using FAMACHA© and other tools. Effectiveness and efficiency of FAMACHA©-led TST would be improved by such a risk index, since intensity of focus of resources and vigilance could be maximised during periods of high risk, with reduced effort in times of low risk.

3.2 Methods

The model framework described in chapter 2 was used and extended to consider climate. The weather elements, being rainfall, temperature and rainfall entropy were summed (rainfall) or averaged (other variables) over a four-week (28 day) period up to different intervals (7, 14 and 21 days) before the day of each considered FAMACHA© evaluation event. The four-week period was chosen to take account of the pre-patent period and pathogenic lag of *H. contortus* infection, such that peaks of infection would be expected to lead to increased numbers of cases some four weeks later, following maturation of adults worms and loss of blood through feeding. The data used to test the model were obtained from the same intensively monitored farm as described in chapter 2. Input variables were obtained from the following sources:

3.2.1 Reproductive status group-specific force-of-infection (FOI) data

The FOI, also called the risk-of-infection or incident or hazard rate, was predicted at each weekly FAMACHA© evaluation event from the SEIDPRS model developed in

Chapter 2, and is a fitted parameter optimised on observed FAMACHA© cases, as already described.

3.2.2 Rainfall data

Daily total rainfall was recorded on the farm, using a standard rain gauge, from August 1989 to December 2002 and from these records, four-week (28 day) period total rainfall in mm was calculated up to 7, 14 and 21 days before the day of each considered FAMACHA© evaluation event, henceforth referred to as 7-day, 14-day and 21-day total rainfall values.

3.2.3 Temperature data

Daily temperature recordings from the nearest national weather station, approximately 20 km from the farm, were obtained from the South African Weather Service (SAWS) for the period from July 1997 to December 1998. Average temperature (°C) per four-week period was processed as for total rainfall to get the approximate on-farm 7-day, 14-day and 21-day average temperature values at each considered FAMACHA© evaluation event.

3.2.4 Calculation of rainfall entropy

Shannon's informational entropy (H), was calculated using the following formula, which relates the intensity of a random variable, in this case daily rainfall amount over a set period and its probability of occurrence, or frequency:

$$H = -\sum_{i=1}^{s} pi \, \ell n \, pi \qquad \textit{(Equation 1)}$$

where H is the entropy of the daily recorded four-week period rainfall in mm up to 7, 14 and 21 days before the day of each considered FAMACHA© evaluation event, pi represents the occurrence probability for the rainfall amount on the i^{th} day, ℓn is natural logarithm to the base e and s is the number of events, i.e. rainfall days (Kawachi et al., 2001).

Consideration of rainfall in a probabilistic sense was done as per the method described by Kawachi et al. (2001) and used by Reynecke (2007), for equating total rainfall in mm over the four-week period (28 days) to equal number of successful trials over the same period. All of the 28 days of the four-week period were assumed to have an equal probability of being selected, such that a day in which 10 mm of rainfall was regarded as having been successfully selected 10 times (10 successes) while the days with zero rainfall had zero successes. The resulting series generated represents the occurrence probability of daily rainfall from the first up to the s^{th} day of recorded rainfall with its distribution representing the probabilistic characteristic (variability) of the temporal apportionment of total rainfall over the four-week period. Substitution of daily rainfall occurrence probabilities for the four-week periods up to 7, 14 and 21 days before the day of each considered FAMACHA© evaluation event into equation (1), using Microsoft Excel (2010), gave the 7-day, 14-day and 21-day entropy values.

3.2.5 Statistical analysis

Data were plotted to visualize temporal trends and examine them for possible data entry errors such as values outside possible ranges and missing values. This being a correlated (dependent) time series data with reproductive status group as the cluster variable at level two and the repeated FAMACHA© evaluation events (waves) at level one, multi-level model analysis was used. For this analysis a random effects model was chosen over a fixed effects model because there was reason to believe that the differences in husbandry management and physiological predisposition to *H. contortus* across the three reproductive status groups on the farm had some influence on the FOI at each FAMACHA© evaluation event and also that the differences were uncorrelated with the predictors (temperature, rainfall and entropy) included in the model (Torres-Reyna, 2013). The regression model for the dependency of the interval FOI on the on-farm recorded four-week-period average temperature, total rainfall and entropy was firstly run in Stata™/IC 10.1 software (StataCorp, 2007) using the 7-day, 14-day and 21-day values with the cluster variable (reproductive status groups) included as a random effect in a stepwise selection procedure at a set p-value threshold of 0.3 (Kirkwood and Sterne, 2003). Subsequent tests were conducted on whether: (i) Choice of a random effects model over a fixed effects

model (Hausman test) was statistically justifiable under the null hypothesis that the preferred model is random effects, i.e. unique (between cluster group) errors are uncorrelated with the predictors against the alternative that it is the fixed effects model; and (ii) Time-random effects are required when running the random effects model under the null hypothesis that all FAMACHA© evaluation waves coefficients are jointly equal to zero.

Mean separation of significant effects was done by two-tailed t-tests at $p < 0.05$. Model diagnostic test, using standardized normal probability plot (normal P-P), was conducted on the mixed effects model that gave superior results to see if it satisfied the linear regression model assumptions. Thereafter, for the model that gave superior regression analysis results: (i) Formulation of an estimating equation for interval FOI given values of significant predictor variables over a four-week period; and (ii) Estimation of the average FOI across the three groups within the farm, was done. The analysis commands carried out are shown in Appendix 2A.

3.3 Results

The predicted FOI values and the recorded temperature, rainfall and entropy values are summarised for each reproductive status group in Appendix 2B, Tables 2B (i), (ii) and (iii). The predicted FOI experienced by the dry group ranged from a minimum of 1.93×10^{-3} to a maximum of 5.00 with a median FOI value of 2.96 per 100 ewes per evaluation event. For the pregnant group the predicted FOI values ranged between 8.63×10^{-3} and 15.15 with a median FOI value of 14.12 per 100 ewes per evaluation event, while for the lactating group, the predicted FOI ranged between 6.13×10^{-5} and 3.00 with a median FOI value of 2.16 per 100 ewes per evaluation event. The FOI values for the three groups are graphically illustrated in Figure 3.1 and show a right-skewed temporal pattern that nearly flattens out towards the end of the *H. contortus* season in all the groups, probably implying stability in risk of infection late in the season.

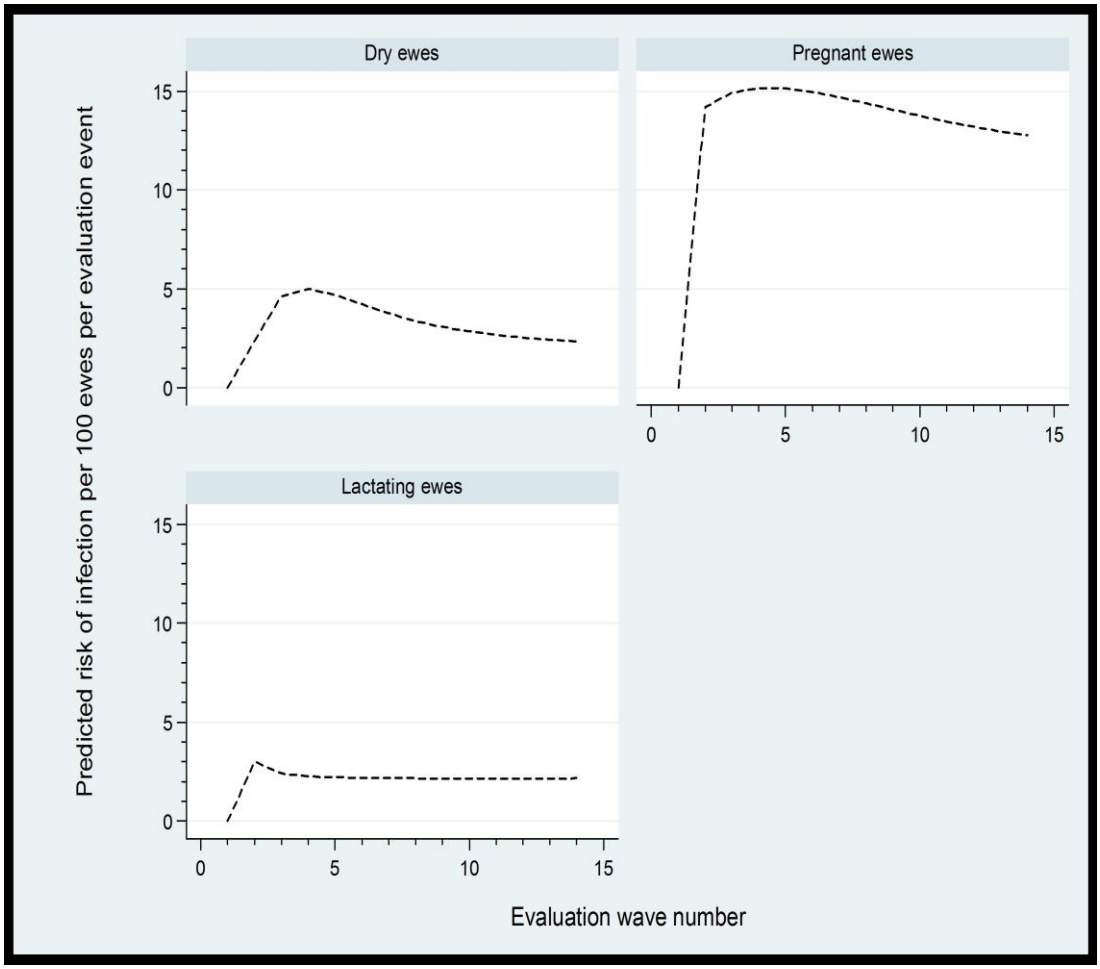


Figure 3.1: On-farm model predicted force-of-infection (FOI) per 100 ewes per evaluation event in the three reproductive status groups of ewes for the 14 FAMACHA© evaluation waves (events) conducted weekly during the period of investigation (see Chapter 2).

Average temperature for the 7-day and 14-day values ranged between 23.52 and 28.00 °C, while that for the 21-day values ranged between 23.52 and 28.85 °C. Entropy for the 7-day and 14-day values ranged between 1.02 and 2.00 *bit* while that for the 21-day values ranged from 1.18 to 1.82 *bit*. The recorded average temperatures had an approximately sinusoidal temporal pattern of very low amplitudes while the entropies had an almost horizontal temporal pattern in all of the three groups of values. Total rainfall for the 7-day values ranged between 17 and 117 mm and that for 14-day values was between 23 and 117 mm, while that for the 21-day values ranged from 26 to 107 mm in a convex temporal pattern in all of the three groups of values (Figure 3.2).

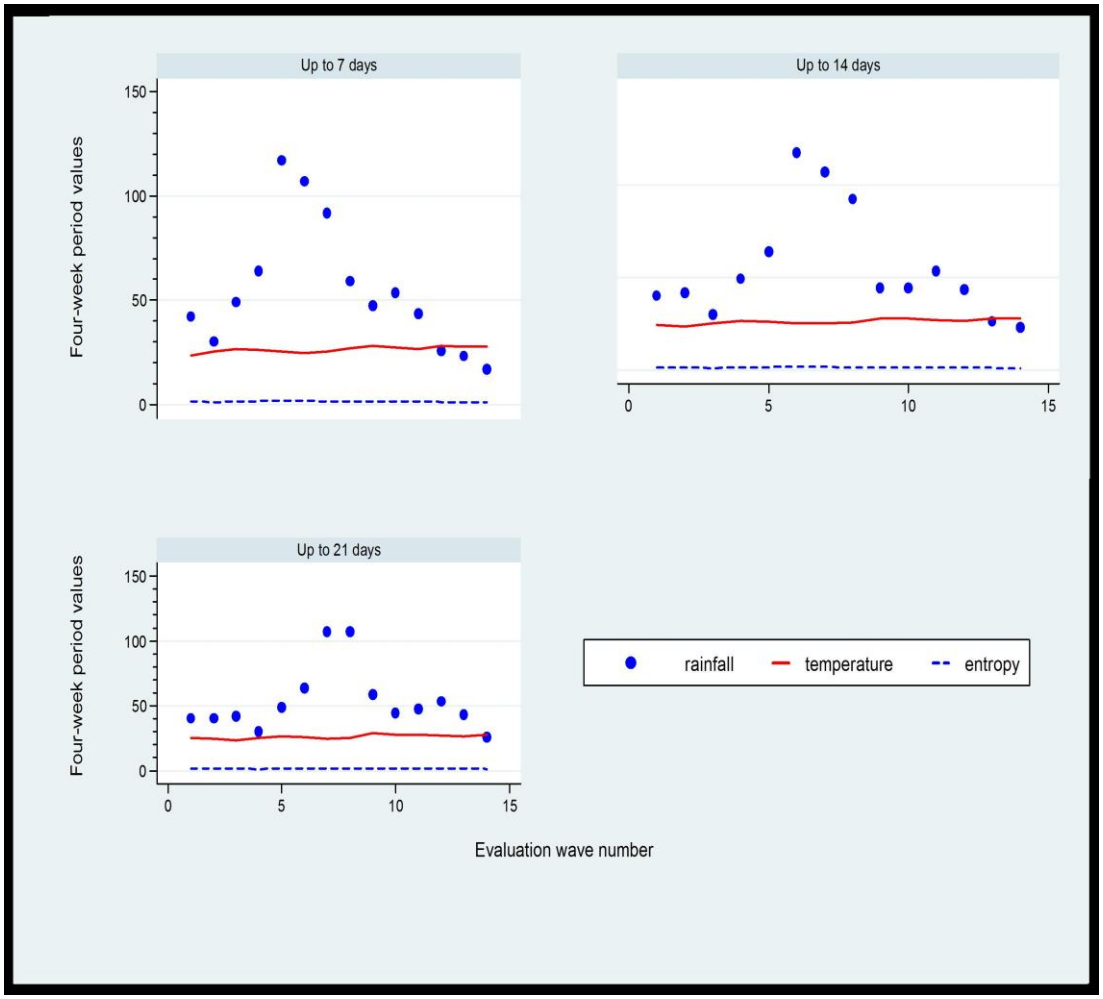


Figure 3.2: On-farm four-week period values for temperature, rainfall and entropy up to 7, 14 and 21 days before the day of each of the 14 weekly FAMACHA© evaluation waves (events) conducted and considered during the period of investigation (see Chapter 2).

The predictors in the random effects (RE) model, with only the entities (cluster groups) included, failed to adequately predict the FOI when using the 14-day and 21-day values. This is so because none of the predictors were significant [Appendix 2C, Table 2C (ii) and (iii), respectively]. In contrast, when using the 7-day values, both temperature and rainfall significantly predicted the interval FOI ($Z = 3.52$, $p < 0.001$ and $Z = 3.12$, $p = 0.002$, respectively) while entropy did not ($Z = -1.06$, $p = 0.289$) at $p < 0.05$ level of significance. Also, the RE model including entropy, average temperature and total rainfall, using 7-day values was highly significant (Wald $Chi^2 = 20.30$, $df = 3$, $p < 0.001$) which means that the three predictors collectively accounted for a statistically significant proportion of the variability in interval FOI [Appendix 2C, Table 2C (i)].

Differences across the three groups (dry, pregnant and lactating), when using the 7-day values, accounted for approximately 90.35% of the variability in the interval FOI over the period of investigation [Appendix 2C, Table 2C (i)]. Non-significant results using the 7-day values were obtained for the Hausman test [$Chi^2 = 0$, $df = 3$, $p = 1.00$], indicating lack of significant evidence against the null hypothesis. Hence the choice for a random effects model was statistically justifiable [Appendix 2C, Table 2C (iv)]. Testing to see if time-random effects are required using the 7-day values also yielded non-significant results ($Chi^2 = 17.39$, $df = 11$, $p = 0.097$), indicating lack of significant evidence against the null hypothesis, thus time-random effects are not needed [Appendix 2C, Table 2C (v)]. The Hausman test and test to see if time-random effects are required were conducted using the 7-day values only, since the 7-day values with only entity-random effects included, gave superior regression analysis results in relation to outputs for the 14-day and 21-day values. The summarized RE regression model output results for the 7-day, 14-day and 21-day values are shown in Table 3.1.

Based on the best predictive RE regression model output coefficients, the regression equation for the estimation of the interval FOI at any considered date, given four-week period values for average temperature and total rainfall up to seven days before that considered date was:

$$E\,[FOI \mid ave.temp., total\ \ rain\,] = 0.98 * ave.temperature + 0.06 * total\ \ rain - 19.67$$

The interpretation of the regression equation is that, after controlling for average temperature over a four-week period up to seven days before the specific date of interest, a unit increase in total rainfall over the same period will result in 0.06 unit increase in the predicted FOI per 100 ewes from one weekly FAMACHA© evaluation event to the next. Also, after controlling for total rainfall over the same period, a unit increase in average temperature over the same period will result in 0.98 unit increase in the predicted FOI per 100 ewes from one weekly FAMACHA© evaluation event to the next. From the normal P-P plot, the residuals are reasonably clustered along the diagonal and hence normally distributed (Figure 3.3), which means that the assumption of residual normality is met. The predicted average FOI across all the three reproduction class groups on the farm for the period of investigation (Chapter 2), as deduced from the model run using the 7-day values, ranged from a minimum of 2.29 to a maximum of 7.78, with a median FOI value of 6.44 per 100 ewes per evaluation event (Figure 3.4).

Table 3.1: Regression analysis results summary table for the dependency of force-of-infection (FOI) on the on-farm average temperature (temp), total rainfall and entropy for the 7, 14 and 21-day values as experienced by the three reproductive status groups (reproclass).

Variables	(1) FOI against 7-day values	(2) FOI against 14-day values	(3) FOI against 21-day values
Entropy	-2.24	-2.34	-4.04
	(2.117)	(2.549)	(3.042)
Temp	0.98***	0.33	-0.16
	(0.278)	(0.300)	(0.287)
Rainfall	0.06***	0.04*	0.04
	(0.019)	(0.021)	(0.022)
Constant	-19.67**	-1.03	14.59
	(9.216)	(10.121)	(10.708)
R-sq	0.063	0.021	0.012
Observations	42	42	42
No. of reproclass	3	3	3

Standard errors in parentheses
*** p<0.01, ** p<0.05, * p<0.1

***** Two-tailed p-value: probability of getting the coefficient obtained or more extreme if there is no true difference between the coefficient and zero under the null hypothesis that each coefficient is not truly different from zero and the alternative hypothesis that each coefficient is truly different from zero.

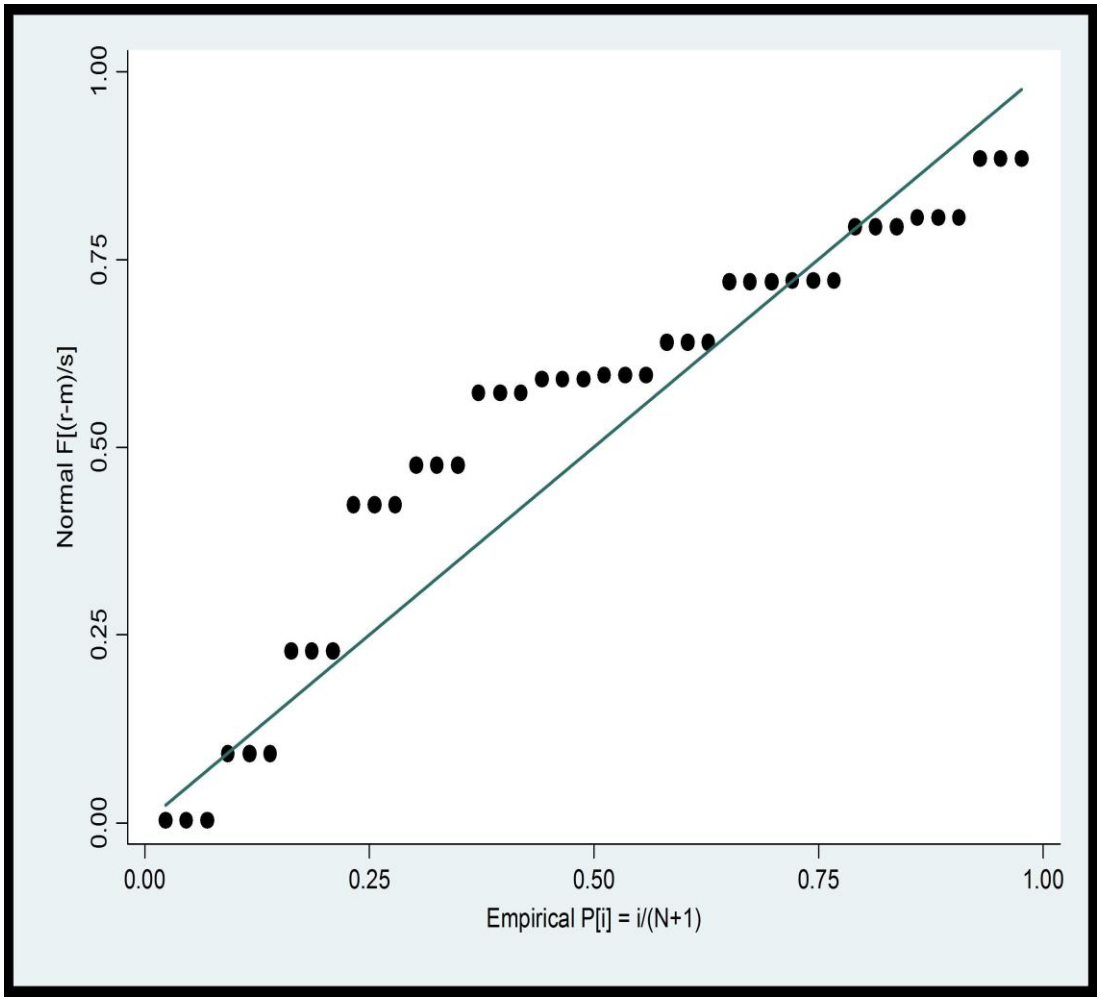


Figure 3.3: Regression standardized residual normal probability (normal P-P) plot to test for the linear model assumption of residual normality, using the deduced significant model based on 7-day values.

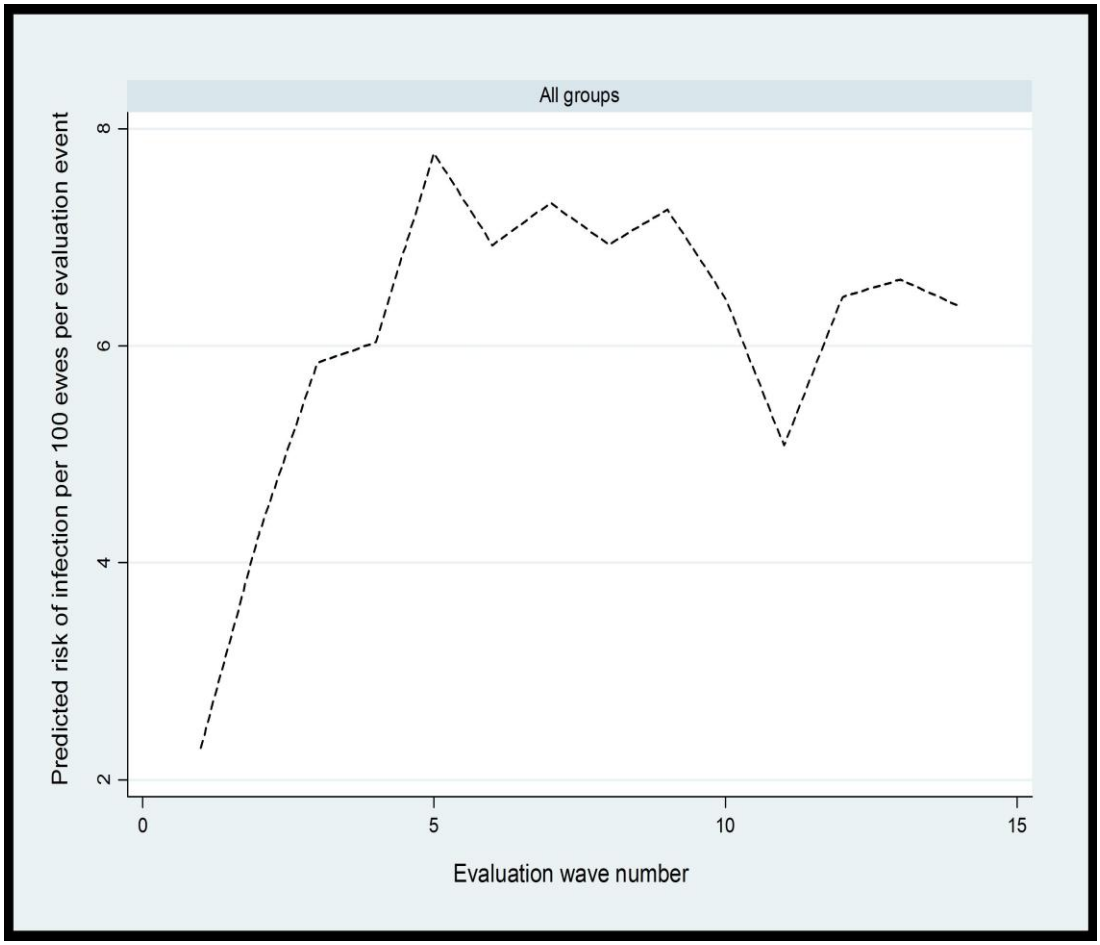


Figure 3.4: Regression model prediction of average force-of-infection (FOI) per 100 ewes per evaluation event across the three reproductive status groups of ewes on the farm at each of the 14 evaluation waves conducted and considered during the period of investigation (Chapter 2), using the significant model based on 7-day values.

3.4 Discussion

The lack of temporal variability in the calculated entropy values for the period of investigation in this study could be explained by the character of the rainfall in South Africa, whereby, on average, rain falls consecutively for a couple of days interspaced with dry spells, hence low consistent entropy values. This is in contrast to a situation where total rain over a certain period falls in: (i) One day, with entropy taking the minimum value of zero; or (ii) Equal proportions each day of the period of interest, in which case entropy takes the maximum value possible (Kawachi et al., 2001).

From the current study on this particular farm, it was deduced that the risk of infection, hence case numbers, realised at each FAMACHA© evaluation event was significantly dependent on variability in total rainfall and average temperatures recorded over a four-week period up to seven days before the day of evaluation. This significant dependency is in agreement with results from other studies conducted by many others elsewhere including, Soulsby (1982), O'Connor et al. (2007a, b) , Van Dijk and Morgan (2011), (McCulloch et al., 1984) and Khadijah et al. (2013), in which temperature and rainfall were found to be the main determining factors behind larval pasture contamination intensities.

Interestingly, a high percentage of total variability (90.35%) in the risk of infection (FOI) over the period of investigation was attributed to the differences across the reproduction-class groups. The notable differences across the three groups (dry, pregnant and lactating ewes) that probably had this profound effect on the predicted interval FOI are: (i) Physiological predisposition to parasite infection (Stoll, 1958; Malan et al., 2001; McClure, 2012); and (ii) Intervening on-farm husbandry practices, including movement of animals to parasitologically “cleaner” pastures and paddock stocking rates (Morley and Donald, 1980; Stromberg and Averbeck, 1999; Waller, 1999). For example, husbandry management differences across the three groups were evident on the farm during the period of investigation, in that lactating ewes were kept on irrigated pastures until weaning, while the other two groups were rotationally grazed on natural grassland-type veldt pastures (Chapter 2). Irrigation is known to increase both larval survival (Bullick and Andersen, 1978) and infection rate by facilitating migration of L3 upwards onto grass, hence making them

accessible to grazing animals (Silangwa and Todd, 1964; Levine and Andersen, 1973; Stromberg, 1997).

The derived FOI formula, with easily obtained predictor variables (temperature and rainfall), could be used on its own to predict future weekly risks of infection on this particular farm or be substituted for the FOI parameter in the novel case prediction model developed in Chapter 2 to provide a practical, readily available decision support tool for the necessary evasive actions to be taken on the farm. These evasive actions could include but are not limited to moving animals to different paddocks, shortening the FAMACHA© evaluation interval and increasing the range of FAMACHA© category scores to treat depending on the risk level predicted (Van Wyk and Bath, 2002). This ought to increase the efficiency of adoption of the FAMACHA© system for targeted selective treatments, especially where labour or time are limiting. Reduced need to handle sheep for evaluation and more robust prediction of infection risk are likely to have welfare benefits for sheep included in this system.

Limitations with this particular modelling framework include the fact that the model was fitted by heuristic variation in parameter values to match observed case incidence on the study farm. Also, the relationship between climate, management and disease incidence is complex and likely to vary between farms. Therefore, although the principle of model-predicted variation in FOI to guide FAMACHA© application has been demonstrated by the current work, the findings may lack external validity (Rothman et al., 2008; Carlson and Morrison, 2009). Adaptation to new farms might require a ‘burning-in’ period, during which case data are collected alongside relevant climate and management factors, so that the model parameters and relationships are built up for that particular farm.

3.5 Conclusion

The regression model used to assess the dependency of FOI on rainfall and temperature was found to provide an adequate fit to climatic data 7 days prior to the start of the relevant infection period (i.e. 4 weeks before FAMACHA© evaluation). The fact that there was a significant correlation between the model-predicted FOI and weather elements (temperature and rainfall) in this study, which is consistent

with the results of many other studies, adds credibility to: (i) The model structure; (ii) Estimated parameter values; and (iii) Overall applicability of the novel case-based prediction model developed and discussed in Chapter 2 as a decision support tool, at least for the study farm.

Targeted frequency of monitoring has the potential to significantly reduce the labour costs of applying **s**IPM. Automated methods for monitoring animal performance would further support the efficient application of **s**IPM, and are considered in the next chapters.

CHAPTER 4

Evaluation of a prototype radio frequency identification (RFID) system for remote monitoring of individual animal activity levels

4.1 Introduction

Targeted selective treatment (TST) as a principle has been accepted globally as a valuable means to arrest onset and propagation of AR (Bath and Van Wyk, 2009; Kenyon et al., 2009; Leathwick, 2013). However, application of TST strategy requires the ability to identify the overly susceptible individuals within a flock for treatment (Kenyon et al., 2009), while the monitoring associated with application of TST decisions currently uses limited (Bath and Van Wyk, 2009) and largely labour intensive (Bath, 2006; Van Wyk et al., 2006) indicators, with high opportunity costs to farmers. For example, scheduled Five Point Check© evaluation (Bath and Van Wyk, 2009), or even only FAMACHA© on its own (Van Wyk and Bath, 2002), is not feasible to undertake in a large flock as frequently as may be required. While the acceptability of clinical evaluation of anaemia with the FAMACHA© system, which was used in the present studies, is relatively high, the labour component severely limits its application in small ruminant flocks or herds larger than a few hundred (Van Wyk and Bath, 2002; Van Wyk et al., 2002; Van Wyk, 2003a; Van Wyk et al., 2006; Besier, 2012). As a result, current high levels of AR are perpetuated by persistence with conventional practices of drenching all the animals when one or two die or when any show signs of heavy endoparasite infection (Malan et al., 2001). It is paramount to develop a method for detection of animals possibly suffering from the anaemia of haemonchosis without having to manhandle and evaluate the conjunctivae of every animal.

In dairy farming, decreased oestrus detection rates in dairy cows (Senger, 1994; Washburn et al., 2002), leading to poor reproductive performance, has also been attributed to high labour inputs associated with visual oestrus detection on farms (Firk et al., 2002; López-Gatiusa et al., 2005; Valenza et al., 2012). In reaction, advances in technology have been adopted and assimilated accordingly to alleviate labour inputs associated with visual oestrus detection, particularly on large farms, by using remote electronic systems that incorporate accelerometers as a means to associate heightened physical activity with oestrus behaviour (bulling) in dairy cows

(Xu et al., 1998; Firk et al., 2002; López-Gatiusa et al., 2005; Løvendahl and Chagunda, 2010; Valenza et al., 2012). Also, remote wireless technology that uses radio frequencies is being explored in the area of automated animal health monitoring by mapping specific aspects of animal behaviour related to specific diseases on sensors. The sensors are then attached (on-cow or in-cow) or non-attached (off-cow sensors monitoring cows as they walk by) to farm animals to remotely capture the mapped physiological and behavioural indicators (Kwong et al., 2009; Helwatkar et al., 2014).

Helwatkar et al. (2014) has described various sensor types available on the market that could be ideally mapped to measure certain behavioural indicators or parameters that are associated with fever, lameness, oestrus, mastitis, ovarian cysts, displaced abomasum, ketosis, milk fever, retained placenta, heifer diarrhoea and heifer pneumonia on dairy farms. These behavioural indicators mainly include activity, lying time and body temperature, hence the dominance of temperature and accelerometer sensors in automated animal health monitoring applications. However, the majority of the mapped indicators cut across a number of physiological and behavioural statuses, hence are nowhere near pathognomonic, making automated animal health monitoring an inexact science. Therefore, interpretation of sensor data at bare minimum should involve use of an established algorithm in combination with non-sensor data about animal history and on-farm husbandry management routine, in a heuristic classification platform, to differentiate between true-positive and false-positive statuses (Steeneveld et al., 2010; Marchioro et al., 2011).

Radio frequency identification (RFID) is the basis for the sensor technology most commonly used for automated animal health monitoring. It is a wireless, non-contact system that uses radio-frequency waves to transmit data from an electronic RFID tag or label, through a reader (interrogator) for the purpose of automatically identifying and tracking animate or inanimate objects to which it is attached (Anon., 2012a). The technology can be traced as far back as 1945 (Stockman, 1948; Hatch, 2008), when first put to practical use during World War II by deploying transponders in allied aircraft that would acknowledge radar interrogations from friendly aircraft. At that stage the cost to acquire the technology was out of reach for routine use but the size and cost of RFID tags have since followed the progression of Moore's law (Moore,

1965) to the extent that it has been adopted in many applications such as logistics, retail, asset management, access control, animal husbandry and health care (Palmer, 2004; Yang et al., 2010).

In South Africa there are existing RFID-based activity level monitoring systems which were originally used mainly to monitor high value game animals on private properties, but are progressively being expanded to commercial flocks of small ruminants and cattle as an alarm system in case of predator attack and stock theft. However, as the credentials of such a system have never been extensively and rigorously interrogated and evaluated, there is limited, if any, measurement reference procedure (Dos Santos Silva, 1999) in existence for this or similar systems. While RFID for remote monitoring of animal activity has the potential to serve as indicators of health status in support of TT and TST decisions, an essential step in its development for this purpose is thorough evaluation of performance and limitations in the livestock farming environment and context.

The objectives of this part of the current study were to: (i) Set up a prototype RFID-based system capable of remote monitoring and communication of individual animal activity levels, hence behaviour at pasture in a typical small ruminant commercial enterprise; and (ii) Subsequently evaluate the performance of such a system against various animal behaviours and disease states, with the emphasis on debilitating helminth infection. The evaluation was first done through a series of hand simulated movement trials within the reader's interrogation zone (Engels and Sarma, 2002; Zhou et al., 2007) and analysis of the resultant data transfer rates (DTR) and actual values of the transmitted data. Hand simulation was opted for during this preliminary work because, in addition to being timely in delivering the necessary data needed, it also allows for easily controlled trial designs compared to when real animals are tagged. The hypotheses tested during the series of trials were that DTR, as an indicator of the prototype system set-up optimization, is affected by: (i) Distance between tags and reader; (ii) Movement of tags or lack thereof; (iii) Various physical obstacles; (iv) Background noise; and (v) Numbers and disposition of tags within the reader's interrogation zone. If it can be shown that RFID technology can reliably work as a remote tool for monitoring flock or individual animal behaviour at pasture, this would be a step in the intended direction of devising an automated on-farm

decision support system in line with Weiser (1991)'s vision of ubiquitous computing with seamless integration of useful technology into everyday human life activities.

4.2 Materials and methods

4.2.1 Prototype system set-up

The prototype RFID system set-up for the following trials involved a single solar-panel-charged reader operating at an ultra-high frequency band of 868 MHz, and mounted above ground at the top of a five metre wooden pole, and a number of active tags operating at the same frequency. The active tags contain both a radio transceiver and an on-board battery to power the transceiver (Ni et al., 2004; Egea-López et al., 2007; Sample, 2008; Bueno-Delgado and Vales-Alonso, 2010). An A1-type sensor (accelerometer) inbuilt into the tags was used to measure activity levels of various simulated hand movements. The accelerometer had a set acceleration threshold of twice the gravitational acceleration (2 g), such that whenever the accelerometer needle displacement against the transponder casing due to the simulated movements was equivalent to at least 2g, a digit one was registered. In contrast a zero was registered when movements failed to equal or exceed the 2g threshold. The tag then sums the accelerations (ones and zeros) over the set reader's 'collection' command signalling interval of one minute, to give an activity score representing the overall activity level over that period. When the signal to noise ratio is below 10 dB the tag reader cannot demodulate the signal and the data will be lost.

An active tag is 'woken up' when it receives a 'collection' command signal from a reader during an open window and then transmits its unique identity plus any other data from incorporated sensors to the reader which subsequently sends back a 'sleep' command signal to the successfully read tag. In this way an active tag conserves its energy (battery life) by only broadcasting its signal when it is challenged to do so, within range of a reader (Bueno-Delgado and Vales-Alonso, 2010). The activity score ranges from zero, indicating no activity or activity levels below 2g to a maximum average of 124 indicating activity levels above 2g within the one minute interval. Data from the reader are relayed via general packet radio services (GPRS) to a web based server, which processes and logs the data with outputs in the form of alarms and reports, and user interface is via a web site (Figure 4.1).

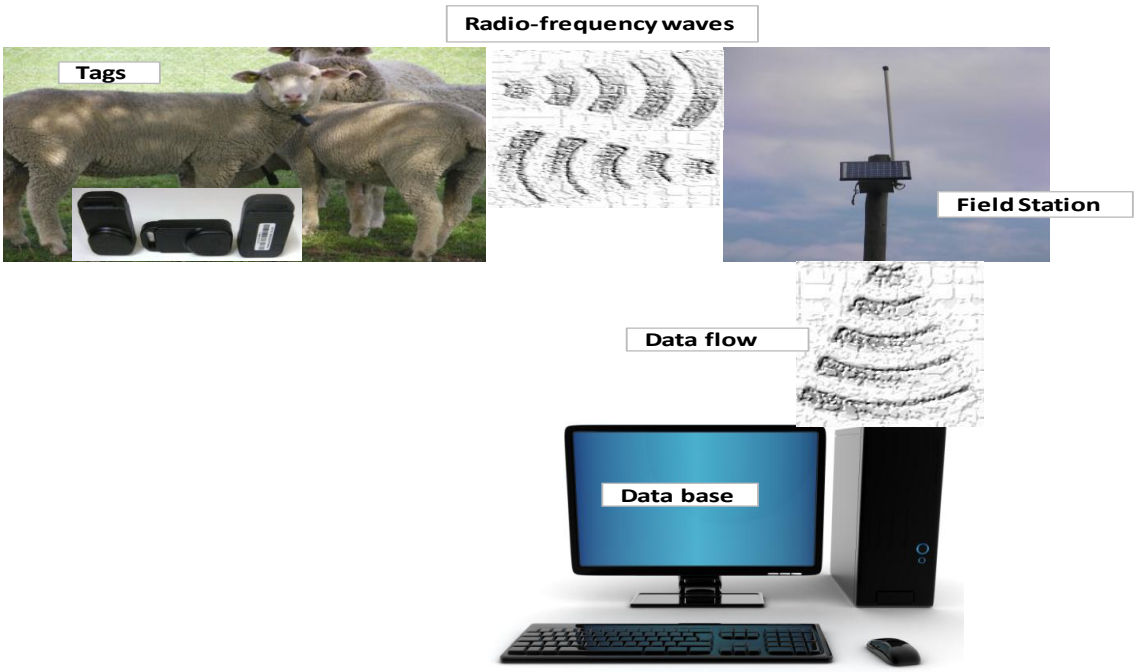


Figure 4.1: Prototype radio frequency identification (RFID) system set-up to measure intensity of simulated hand movements per unit time (activity level). Tags consist of an accelerometer and RFID transponder.

In the present set-up a non-persistent (no system handshake) carrier sense multiple access (CSMA) protocol, with an energy-based clear channel assessment physical layer activity as a medium access control (MAC), was adopted to reduce the occurrence probability of tag-to-tag collisions. This employed binary exponential backoff (BEB) technique to resolve collisions and increase tag read rates (Kleinrock and Tobagi, 1975; Tay et al., 2004; Jamieson et al., 2006; Ramachandran and Roy, 2006; Egea-López et al., 2007; Bueno-Delgado and Vales-Alonso, 2010).

4.2.2 Experimental design

The following four controlled experiments were designed and conducted to determine the effects of various factors on DTR of the prototype activity level monitoring system as well as the magnitude of the transmitted scores: (i) The combined effects of distance (between tags and reader) and tag movement or lack of it, on DTR and magnitude of values transmitted; (ii) The effect of various physical obstacles within the reader's interrogation zone on DTR; (iii) The effect of background noise on DTR by proxy, through comparison of day and night time DTR; and (iv) The combined effects of numbers and disposition (clustered or spread-out) of tags within the reader's interrogation zone on DTR. Transmission rates of the activity level scores (DTR) and the actual values of the activity level scores resulting from the hand simulated movement experiments were captured and used for analysis. These investigations were preliminary to attempts at deploying the tags onto sheep, on the principle that thorough evaluation of performance under controlled conditions would underpin interpretation in a field setting, and provide a gold standard against which added variation and error could be measured. Analysis of data from *Experiments (i)-(iii)* was undertaken using distribution-free or non-parametric methods because of the small sample sizes, while for *Experiment four* adequate sample sizes justified use of parametric methods.

4.2.2.1 Experiment one: tag movement and distance

Extensive hand simulations of 10 randomly selected, uniquely identifiable and functionality-tested tags were undertaken at various pace-measured linear distances between 25 and 100m from the reader along a clear route free from physical obstacles like trees and buildings. The simulations were done by tapping the tags individually against the surface of a clip board held vertically, at each selected

distance. In order to standardise the tapping rhythm and intensity, each tag was suspended in turn by the same length of rope tied to the handle of the clip at the tip of the board held vertically, with its tapping surface facing the reader. Keeping the elbow joint of the arm holding the tag tightly apposed to the ribcage to simultaneously fix the shoulder and elbow joint movements, the wrist joint of the arm holding the tag was used at non-strenuous extension-flexion angles to hold the tag at practically the same distance from the clipboard before releasing it to swing and knock against the board. This was repeated for each tag for roughly two minutes before resting the tag on the ground for at least another two minutes. Data from a period of one minute was randomly identified and selected for each tag for the period it was being tapped and when rested on the ground. From the randomly selected data, the potential signal attenuation effect of distance on: (i) Data transferred in one minute (data transfer rate, DTR), (ii) Total activity level score (sum) and (iii) Mean activity level score, were investigated for each tag while it was being tapped (with movement) and rested (without movement). Also, the effect of movement (all or none) on the magnitude of the activity level scores transmitted was investigated.

Mean data transmission rates and their 95% confidence intervals were calculated using bootstrap resampling (Efron, 1979) in Microsoft Excel (2010), with 1000 Monte-Carlo simulations (Anderson, 1986) for all the tags which functioned properly out of the 10 tags used and for all the test distances, 25 to 100 m from the reader, to ascertain significant differences in mean DTR between the tested distances.

4.2.2.2 Experiment two: physical obstacles

The potential detuning effects caused by various common farm obstacles were investigated by tapping each of the 10 uniquely identifiable and randomly selected tags in turn for two minutes, as above, in a with-and-without fashion: (i) Behind and beside tree shrubs; (ii) Inside and outside a sheep shed with corrugated iron roof; (iii) Inside and in front of a brick-built room with rhino-board ceiling; (iv) Behind and in front of a brick-built multi-room building; and lastly, (v) Closely underneath the belly line and above the body line of the furthest sheep in a tightly packed flock of 18 ewes. All the above-mentioned obstacles were selected within a 50 metre radius from the reader to ensure that tapping was done within the reader's read range. The

with-and-without tapping ground positions for each identified physical obstacle were chosen such that the distances to the reader were equivalent, thus as far as possible to ensure that the with-and-without signal routes beyond the identified obstacle of interest towards the reader were similar in terms of other available obstacles. This would automatically control for the effect of other physical obstacles along the signal route to the reader, beside the physical obstacle of interest to be able to attribute the with-and-without differences in DTR to the particular obstacle of interest. As before, a full minute of data was randomly identified and selected for each tag from the web-based server for both periods it was being tapped with-and-without each obstacle of interest.

A detuning factor for each of the 10 tags per all the identified obstacles was calculated by dividing the DTR without each obstacle by DTR with the obstacle of interest. The mean of the detuning factors for all the tags for each identified obstacle and its 95% CI was calculated using bootstrapping with 1000 Monte-Carlo simulations in Microsoft Excel (2010). Also, for comparative purposes the Wilcoxon signed rank test (Kirkwood and Sterne, 2003) was applied to the differences between the individual tag's without-obstacle DTR and the with-obstacle DTR for each obstacle of interest, under the null hypothesis that the median of the paired differences is zero.

4.2.2.3 Experiment 3: background noise

Ten uniquely identifiable tags, each tightly secured to a metal fence dropper at a randomly selected height of ~ 80 cm above ground were placed randomly within a 70 X 70 m^2 paddock, for 6 days from 0600hrs on day one up to 0600hrs on day six. The near corner of the paddock was approximately 25 m from the reader. The time between days one and six was then divided into day-time (0600hrs – 1800hrs) and night-time (1800hrs – 0600hrs) hours and data transferred during the day and night times for each tag for the six days' duration were used to assess the potential impact of background noise on the functionality of this particular prototype RFID system set-up at this particular locality.

Mean day-time and night-time DTR point estimates and their respective signal detuning factors (SDF) were calculated for each tag. SDF was calculated for each tag

by dividing tag' mean night-time DTR with its mean day-time DTR. The mean SDF for all the tags and its 95% CI were then calculated using bootstrap method with 1000 times Monte-Carlo simulations in Microsoft Excel (2010). A significant test two-tailed p-value was also manually calculated from the individual tag's differences between day-time and night-time DTR using the Wilcoxon signed rank test method under the null hypothesis that the median of the paired differences is zero, i.e. that both distributions are the same.

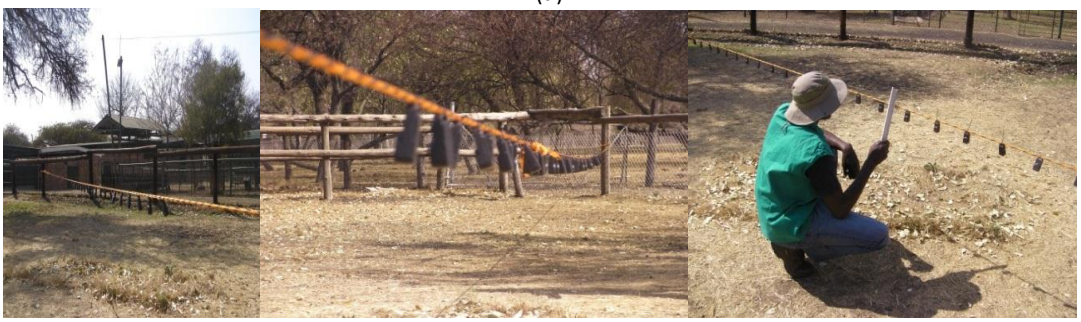
4.2.2.4 Experiment four: clustering of tags

At first, 10 functionality-tested and uniquely identifiable tags were attached ~20 cm apart on a long rope held taut, but stationary ~ 80 cm above ground for a duration of 10 minutes within the reader's interrogation zone and with no obvious obstacles between the spread-out tags and the reader. Thereupon the taut rope with the tags was swung continuously for a similar period by tapping uniformly on the rope at the centre of the suspended tags. The tapping was standardised as far as possible through only moving the forearm while pivoted at the elbow. Then the above routine was repeated in turn with increments of 10 up to 100 tags. Tags were spaced such that there was little or no likelihood at any time of tag-to-tag physical obstruction from the reader, as could occur with tags in close proximity or clustered, so that any effect on DTR that could be detected could be attributed to tag-to-tag air interface collisions but not to physical obstruction [Figure 4.2 (a)].

The experiment described above was repeated with the same set of transponders in a similar sequence of transponder numbers and testing periods but this time with tags clustered instead of being spread-out. The clustering was achieved by suspending the tags at ~ 80 cm from the ground in a plastic netting bag, and to keep the tags compact particularly during swinging, a rope was tied around the bag, immediately above the tags [Figure 4.2 (b)]. Movement was elicited by swinging the bag from side to side. This was done to investigate the potential effect, if any, of both tag-to-tag detuning caused by physical signal obstruction due to clustering, i.e. tag out of line of sight of the reader and tag-to-tag air interface collisions on DTR. Tapping as described above for the spread out tags and swinging of the bag for the clustered tags was opted for as it represents the most probable tag movement once hung on animals. The relevance of tag disposition (clustered or spread-out) on DTR in real

practice can be associated with the gregarious behaviour of small ruminants and the tendency for them to cluster when resting and spread out when grazing.

(a)



(b)

Figure 4.2: Experimental set-up to investigate the effect of numbers and disposition of tags on data transfer rates (DTR) and tag read rates showing: (a) clustered tags; and (b) spread-out tags, and the rope tapping protocol adopted.

Data transferred by each tag in 10 minutes, for each combination of tag numbers and movements, was captured using Epidata (version 3.1) software (Lauritsen and Bruus, 2004). For each clustered and spread-out combination of tag numbers and movements, descriptive histograms of DTR against the categorical levels of tag numbers were plotted. Also, the effect of tag numbers on DTR adjusted for movement, disposition and distance was exploited by plotting the mean DTR against categorical levels of tag numbers, i.e. mean data transferred in 10 minutes for all combinations of tag numbers and movements against the tag batches of 10 - 100 tags. Linear prediction curves with 95% confidence intervals, based on the standard error of the mean, were fitted to the mean DTR plots as a way of graphically exploring for any discernible trends. A test for trend was carried out across the categorical levels of tag numbers according to the method of Cuzick (1985) to check if DTR systematically increases or decreases with increase in categorical levels of tag numbers.

Since tags from the lower category formed part of the following higher category, it means that repeated DTR measurements were made on individual tags under different experimental conditions, being categorical levels of tag numbers, hence dependent or correlated data. A bivariate multilevel mixed-effects linear regression model was fitted in STATA®/IC 10 statistical software (StataCorp, 2007) via maximum restricted likelihood (reml) to determine: (i) Dependency of DTR on categorical levels of tag numbers within the reader's range, adjusted for movement, disposition and tag' distance from the reader; and (ii) If there is a significant performance difference across the 100 tags used in the trial. Each individual tag was taken as a cluster at level two with repeated DTR measurements on each tag at level one in the two-level mixed-effects regression model. The coefficient of determination, i.e. goodness of fit measure (R-squared) was calculated to determine how much of the variance in DTR is explained by the categorical levels of tag numbers within the reader's interrogation zone, using the formulae described by Steenbergen and Bradford (2002).

The linear regression assumption of normality of DTR residuals in the regression models was checked by conducting diagnostic plots of: (i) Histogram of residuals; (ii) Kernel density of residuals with a normal density plot; (iii) Standardized normal

probability plot (normal P-P); and (iv) Inverse normal plot of residuals. Based on the diagnostic plot results, estimating equations for the expected average DTR per 10 minutes per individual tag operating at different categorical levels of tag numbers (10 up to 100), in different dispositions (clustered and spread-out) and experiencing different movements (stationary and swinging) were reported. The analysis commands carried out are shown in Appendix 3.

4.3 Results

4.3.1 Experiment 1: tag movement and distance

Out of the initial 10 tags used during the first simulation trial, four suffered malfunctions at different tapping distances as indicated by a cross (x) in Table 4.1 (a). Data from these tags were excluded from further analysis. Data transfer rates (DTR) during a minute of tapping for each transponder were much higher than the expected value of one reading per minute, as per the set reader's 'collection' command signalling interval of one minute, while for the same tags when stationary (resting on the ground) the rates are close enough to the expected value of one reading per minute. The magnitude of the activity scores was consistently higher during tapping than when tags were stationary. Also, the mean activity score registered by each functional tag during tapping appeared consistent between the considered distances, which means that distance had no significant effect on individual tag DTR during tapping between 25 and 100 m from the reader, but the mean activity score registered by each tag did not appear consistent between individual tags at each considered distance [Table 4. 1 (a)].

All the functional tags transmitted up to 50 metres from the reader both during tapping and when rested on the ground but from upwards of 65 metres up to the furthest tested distance of 100 metres from the reader, transmission was realised only from tags when being tapped [Table 4.1 (b) and (c)]. Also, during tapping there was no significant difference in mean DTR of all tags between the 25 metre distance and the furthest tested distance of 100 metres which again suggests that tapping tags between the distances of 25 and 100 metres from the reader had no significant effect on DTR of the individual tags, hence the consistent DTR [Table 4.1 (b)].

Table 4.1 (a): Summary table showing data transfer rates (DTR), sum (∑) and mean ($\bar{X}$) activity scores for each of the 10 randomly selected tags, both when stationary (S) and being tapped (T). Some tags malfunctioned during the tapping exercise, resulting in no readings (x).

Tag numbers	Testing Distances																							
	25 m					50 m					65 m					75 m					100 m				
	T			S		T			S		T			S		T			S		T			S	
	∑	*DTR*	$\bar{X}$	∑	*DTR*	∑	*DTR*	$\bar{X}$	∑	*DTR*	∑	*DTR*	$\bar{X}$	∑	*DTR*	∑	*DTR*	$\bar{X}$	∑	*DTR*	∑	*DTR*	$\bar{X}$	∑	*DTR*
4012100126	1508	12	126	0	2	1300	11	118	0	2	613	5	123	0	0	745	6	124	0	0	870	7	124	0	0
4012100811	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
4012100805	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
4012100447	564	5	113	0	3	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
4012100075	1358	11	123	0	2	863	7	123	0	1	1019	8	127	0	0	1250	10	125	0	0	1118	9	124	0	0
4012100225	2527	19	133	0	2	2143	16	134	0	2	1497	12	125	0	0	2517	20	126	0	0	2131	17	125	0	0
4012100614	2091	17	123	0	1	1094	9	122	0	1	1228	10	123	0	0	1593	13	123	0	0	1590	13	122	0	0
4012100101	2040	16	128	0	2	3025	24	126	0	2	1383	11	126	0	0	1375	11	125	0	0	1884	15	126	0	0
4012100464	1354	11	123	0	2	254	3	85	0	2	234	3	78	0	0	233	3	78	0	0	x	x	x	x	x
4012100712	1986	16	124	0	2	716	6	119	0	2	731	6	122	0	0	1371	11	125	0	0	974	8	122	0	0

Table 4.1 (b): Tapped tags' mean data transmission per minute (DTR) with confidence intervals (CI) at various distances from the reader.

Testing distance (metres)	Mean DTR	95% CI
25	15	13 – 17
50	12	8 – 17
65	9	7 – 11
75	12	9 – 16
100	12	9 - 15

Table 4.1 (c): Stationary tags' mean data transmission per minute (DTR) with confidence intervals (CI) at various distances from the reader.

Testing distance (metres)	Mean DTR	95% CI
25	2	2
50	2	1 - 2
65	0	0
75	0	0
100	0	0

4.3.2 Experiment 2: physical obstacles

From the 10 transponders that were initially involved in this testing, two malfunctioned and were omitted from the follow-up analysis. As shown in Table 4.2, the data are consistent with there being no significant signal detuning by: (i) Being behind a tree shrub (mean SDF 2.5, 95% CI 1.1 – 4.5 and T -score = 5.5, n = 8, p > 0.05); (ii) Inside a sheep shed (mean SDF 1.8, 95% CI 1.1 – 2.6 and T -score = 2.5, n = 7 p > 0.05); and (iii) Inside a single brick-built room (mean SDF 0.9, 95% CI 0.9 – 1.0 and T -score = 2, n = 6, p = 0.10). This is evidenced by confidence intervals which span or almost span the null value of one as well as corresponding p-values higher than one in 20 at p < 0.05 significance level, indicating lack of significant evidence against the null hypothesis that the median of the paired differences is zero. However, being behind a brick-built multi-room building (mean SDF 4.4, 95% CI 2.2 – 7.1 and T -score = 0, n = 6, p < 0.05) and behind a flock of sheep (means SDF 2.0, 95% CI 1.2 – 3.3 and T -score = 1.5, n = 7, p < 0.05) had a significant signal detuning effect.

Table 4.2: A minute data transmission (DT) from a number of randomly selected tags during tapping exercises for investigating the effect of various obstacles on DTR, with calculated signal detuning factor (SDF) for the indicated obstacles.

	Tree Shrub			Sheep shed			Brick-built single room building			Brick-built multi-room building			Sheep flock		
Tag number	behind DT	beside DT	SDF	inside DT	outside DT	SDF	inside DT	in-front DT	SDF	behind DT	in-front DT	SDF	below belly-line DT	above back-line DT	SDF
40121100614	2	10	5.0	12	10	0.8	15	12	0.8	2	12	6.0	3	18	6.0
40121100101	4	3	0.8	3	13	4.3	12	12	1.0	1	12	12.0	13	17	1.3
40121100464	8	10	1.3	4	11	2.8	10	9	0.9	9	9	1.0	4	11	2.8
40121100712	10	13	1.3	9	14	1.6	13	10	0.8	9	10	1.1	10	13	1.3
40121100225	2	17	8.5	17	17	1.0	21	19	0.9	3	19	6.3	17	16	0.9
40121100126	6	8	1.3	8	11	1.4	10	9	0.9	3	9	3.0	10	10	1.0
40121100447	9	7	0.8	7	8	1.1	9	9	1.0	2	9	4.5	6	7	1.2
40121100075	8	9	1.1	10	12	1.2	10	11	1.1	11	11	1.0	8	13	1.6
Mean SDF (95% CI)*	-		2.5 (1.1 - 4.5)	-		1.8 (1.1 - 2.6)	-		0.9 (0.9 - 1.0)	-		4.4 (2.2 - 7.1)	-		2.0 (1.2 - 3.3)
Wilcoxon signed rank test ‡	T = 5.5, n = 8, p > 0.05		-	T = 2.5, n = 7, p > 0.05		-	T = 2, n = 6, p = 0.10		-	T = 0, n = 6, p < 0.05		-	T = 1.5, n = 7, p < 0.05		-

***** Mean SDF was calculated using Monte-Carlo method with 1000 simulations.

‡ p-value: calculated using Wilcoxon signed rank test under the null hypothesis that the median of the paired differences is zero.

4.3.3 Experiment 3: background noise

From a total of 10, functionality-tested tags which were randomly placed within the camp (paddock), three transferred very low numbers of readings, probably due to the combined effects of distance and canopy cover between the randomly placed tags and the tag reader. Hence, the three tags were excluded from the analysis shown in Table 4.3. There was no significant difference in DTR between day-times and night-times (mean SDF 1.0, 95% CI 0.98 – 1.05 and T-score = 10, n = 7, p > 0.10), as indicated by the registered SDF 95% confidence intervals which spans the null value of one as well as by a p-value above the 0.05 significance level.

Table 4.3: Day-time (0600 - 1800hrs) and night-time (1800 - 0600hrs) data transfer rates (DTR) over six consecutive days, showing signal detuning factor (SDF) per individual tag, as well as the mean SDF with its confidence intervals (95 % CI) and the Wilcoxon signed rank test results.

Tag number	Mean DTR		SDF
	day-time	night-time	
40121100603	579	609	1.1
40121100633	614	589	1.0
40121100642	626	635	1.0
40121100654	580	595	1.0
40121100688	634	595	0.9
40121100706	501	540	1.1
40121100751	627	639	1.0
Mean SDF (95 % CI)	-		1.0 (0.98 – 1.05)
Wilcoxon signed rant test*	T = 10, n = 7, p > 0.10		-

***** p-value: represent the probability of obtaining the T-score of 10 or higher if the median of the paired differences is zero.

4.3.4 Experiment 4: clustering of tags

The simulated data transmissions per 10 minutes (DTR) for: (i) Clustered, stationary; (ii) Clustered, swinging; (iii) Spread-out, stationary; and (iv) Spread-out, swinging categorical levels of tag numbers appeared normally distributed within the categorical levels of tag numbers (Figure 4.3). Exploratory linear fits to mean DTR values approximated the dependency trends of mean DTR values upon categorical levels of tag numbers as follows: (i) Decrease with increase in clustered, stationary; (ii) Increase with increase in clustered, swinging; (iii) Decrease with increase in spread-out, stationary; and (iv) Increase with increase in spread-out, swinging categorical levels of tag numbers (Figure 4.4).

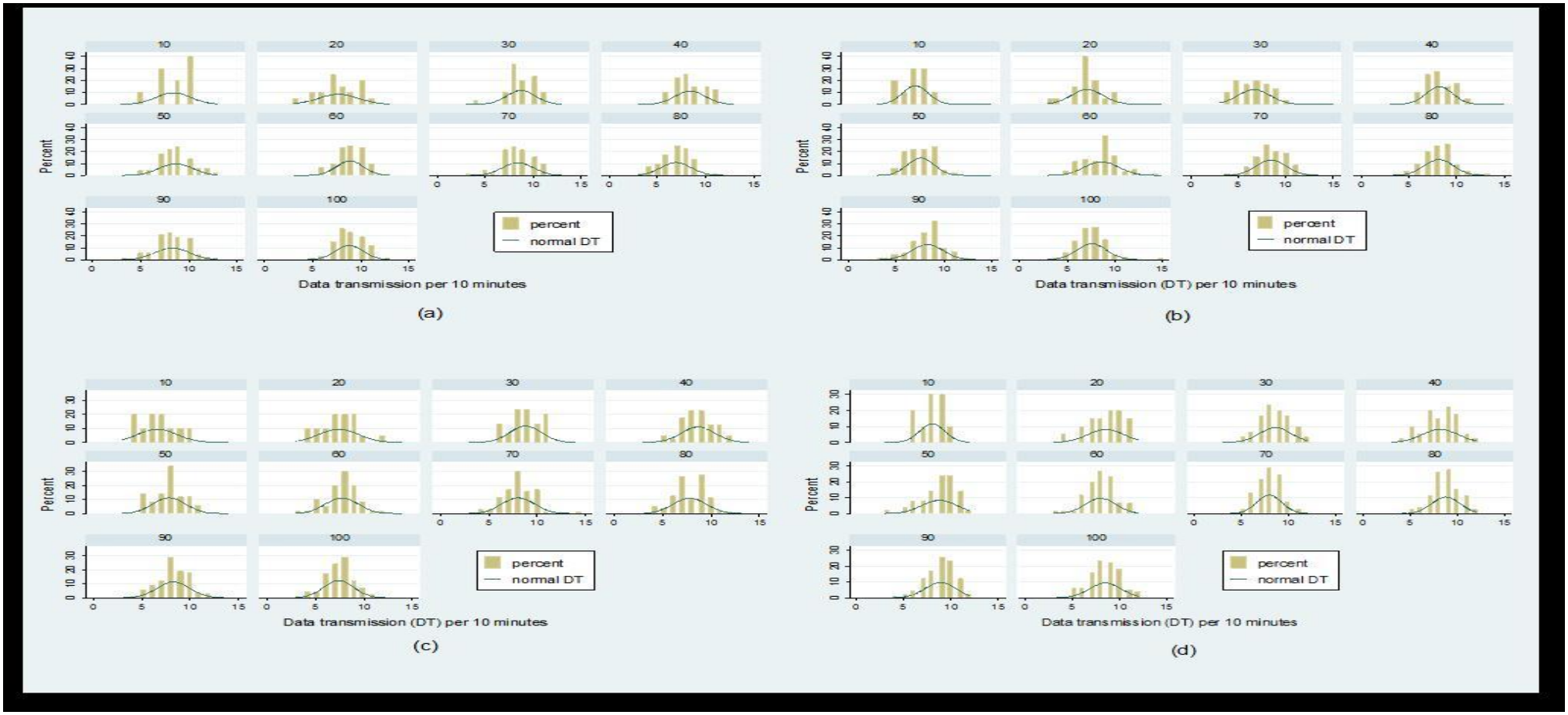


Figure 4.3: Histograms of tag data transmission per 10 minutes (DTR) as a percentage of total tags in that particular group, with exploratory normal distribution fittings for: (a) clustered, stationary; (b) clustered, swinging; (c) spread-out, stationary; and (d) spread-out, swinging categorical levels of tag numbers.

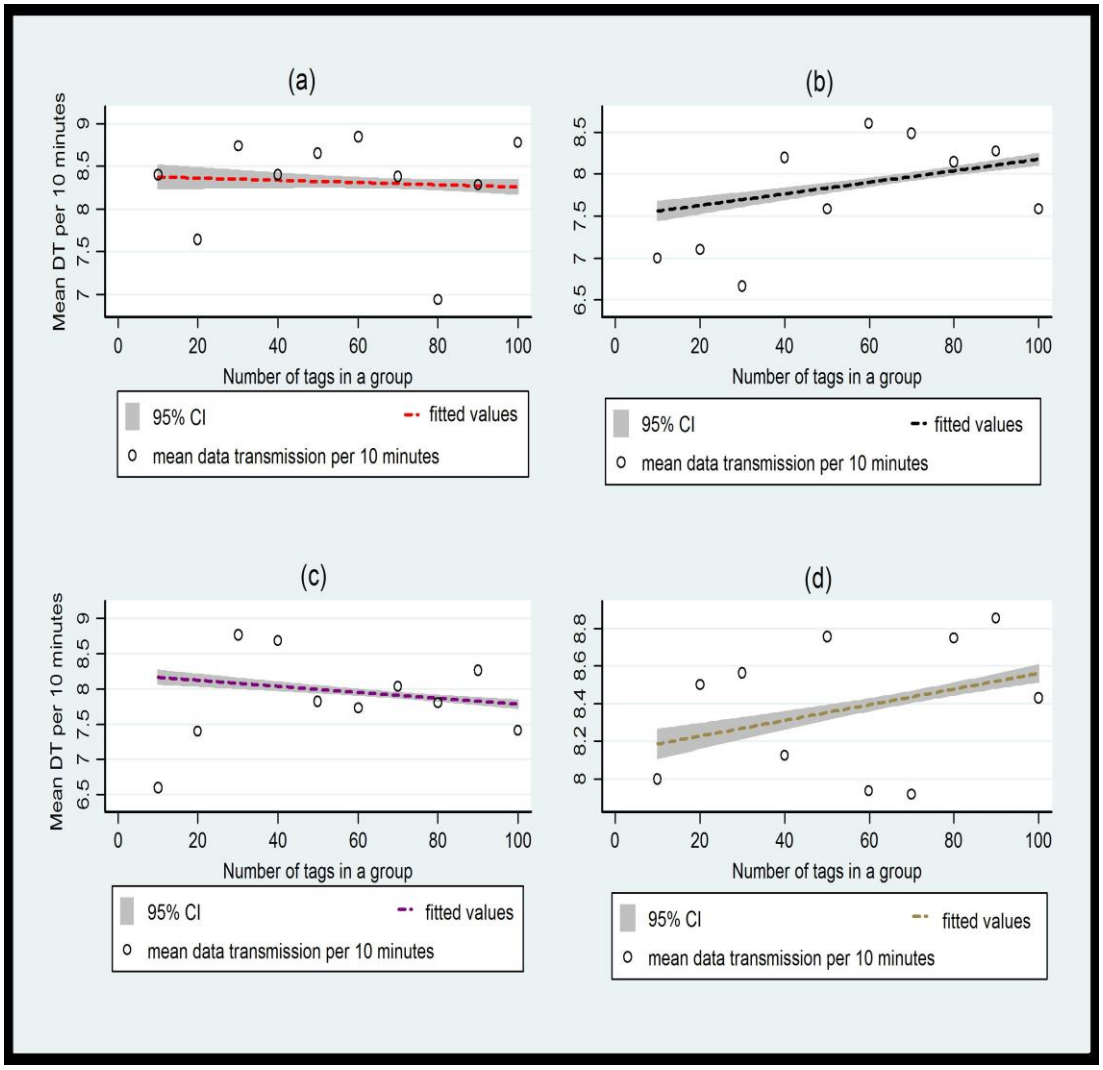


Figure 4.4: Scatter plots of mean data transmission per 10 minutes (DTR) and their exploratory linear fittings against: (a) clustered, stationary; (b) clustered, swinging; (c) spread-out, stationary; and (d) spread-out, swinging categorical levels of tag numbers.

In addition, the non-parametric test for trend based on the arithmetic mean DTR values for the categorical levels of tag numbers, to see if mean DTR systematically increases or decreases with increase in categorical levels of tag numbers, showed sufficient evidence against the null hypothesis, for there being a trend in mean DTR and across: (i) clustered, swinging ($Z = 4.14$; $p < 0.001$); (ii) spread-out, stationary ($Z = -4.96$; $p < 0.001$); and (iii) spread-out, swinging ($Z = 5.07$; $p < 0.001$) ordered categorical levels of tag numbers [Table 4.4 (b)]. This provides more convincing evidence of a causal effect of exposure, i.e. ordered categorical levels of tag numbers, than a simple comparison of other categorical levels of tag numbers (exposed levels) with the baseline group of 10 tags (unexposed level). However, even though a decreasing general trend in mean DTR values across the ordered categorical levels of clustered, stationary tags was predicted, it was shown not to be systematic ($Z = -0.96$; $p = 0.336$) and this could be due to the existence of an influential outlier on this particular data, which is highly probable as the trend tests were conducted on a relatively small dataset of 10 arithmetic mean DTR values.

From the two-level mixed-effects linear regression model analysis results of the normally distributed DTR values against the categorical levels of tag numbers, a significant difference in mean DTR values was shown between the baseline categorical level of 10 tags and other categorical levels of tag numbers depending on the tag's disposition and movement status [Table 4.4 (a)].

Table 4.4 (a): Regression analysis summary table for the dependency of data transfer rate (DTR) per 10 minutes on numbers of tags (group total) within the reader's interrogation zone, adjusted for disposition (clustered or spread-out), movement (stationary or swinging) and distance (same distance) from the reader.

Variables	(1) DTR (clustered, stationary)	(2) DTR (clustered, swinging)	(3) DTR (spread-out, stationary)	(4) DTR (spread-out, swinging)
_Igrouptotal_20	-0.939	0.103	0.705	0.526
	(0.592)	(0.584)	(0.641)	(0.595)
_Igrouptotal_30	0.128	-0.271	2.081***	0.528
	(0.561)	(0.553)	(0.607)	(0.564)
_Igrouptotal_40	-0.253	1.217**	1.996***	0.136
	(0.545)	(0.537)	(0.589)	(0.548)
_Igrouptotal_50	-0.00804	0.569	1.144**	0.774
	(0.535)	(0.528)	(0.577)	(0.539)
_Igrouptotal_60	0.170	1.595***	1.077*	-0.0267
	(0.529)	(0.521)	(0.570)	(0.532)
_Igrouptotal_70	-0.287	1.486***	1.366**	-0.0340
	(0.524)	(0.516)	(0.565)	(0.527)
_Igrouptotal_80	-1.757***	1.157**	1.126**	0.764
	(0.520)	(0.513)	(0.561)	(0.524)
_Igrouptotal_90	-0.427	1.278**	1.591***	0.847
	(0.518)	(0.510)	(0.557)	(0.521)
_Igrouptotal_100	0.0750	0.590	0.731	0.410
	(0.516)	(0.508)	(0.555)	(0.519)
Constant	8.705***	6.990***	6.679***	8.020***
	(0.496)	(0.489)	(0.532)	(0.500)
Observations	550	550	550	550
Number of groups	100	100	100	100

Standard errors in parentheses
*** p<0.01, ** p<0.05, * p<0.1

***** Two-tailed p-value: probability of getting the coefficient obtained or more extreme if there is no true difference in coefficients between that categorical level and the baseline category of 10 tags and test the homogeneity (Ho) hypothesis that the coefficients across the categorical levels of tag numbers are equal and Heterogeneity (Ha) hypothesis that the coefficients across the categorical levels of tag numbers are not equal. For the baseline category, Ho is that the coefficient (constant) is equal to 0.

A highly significant proportion of the overall variability in DTR values was accounted for by: (i) Clustered, stationary (Wald = 92.53; df = 9 ; $p < 0.001$); (ii) Clustered, swinging (Wald = 64.17; df = 9; $p < 0.001$); (iii) Spread-out, stationary (Wald = 38 ; df = 9; $p < 0.001$); and (iv) Spread-out, swinging (Wald = 27.15; df = 9; $p = 0.001$) categorical levels of tag numbers [Table 4.4 (b)].

The estimates of between-tag DTR variance, i.e. the variance of the mean DTR value deviations of each tag from the grand mean of all tags were: (i) 0.33 (0.16 - 0.67) for clustered, stationary; (ii) 0.30 (0.16 – 0.56) for clustered, swinging; (ii) 0.18 (0.06 - 0.51) for spread-out, stationary; and (iv) 0.37 (0.19 - 0.68) for spread-out, swinging categorical levels of tag numbers [Table 4.4 (b)]. There was no significant difference between the estimated between-tag DTR variances as evidenced by the overlapping confidence intervals. These estimates imply that the between-tag variance was not significantly affected by movement and disposition of the tags. However, the between-tag estimated variances were truly different from zero as shown by the likelihood ratio test results for: (i) Clustered, stationary ($\bar{\chi}^2 = 15.79$; df = 1; $p < 0.001$); (ii) Clustered, swinging ($\bar{\chi}^2 = 18.75$; df = 1; $p < 0.001$); (iii) Spread-out, stationary ($\bar{\chi}^2 = 4.98$; df = 1; $p = 0.013$); and (iv) Spread-out, swinging ($\bar{\chi}^2 = 20.79$; df = 1; $p < 0.001$) categorical levels of tag numbers under the null hypothesis that there is no significant cross-tag variation in DTR, i.e. between-tag variance equals 0 [Table 4.4 (b)]. Therefore, there was significant DTR variation across tags and grouping DTR by entities (tags) was useful as it would not have been statistically proper to run a simple linear regression model for this analysis.

The intra-class correlation (ICC), i.e. the proportion of total DTR variance that is due to performance differences across tags, or the correlation between the measurements on different occasions for the same tag after accounting for the effect of categorical levels of tag numbers was: (i) 13% for clustered, stationary; (ii) 12% for clustered, swinging; (iii) 6% for spread-out, stationary; and (iv) 14% for spread-out, swinging tags [Table 4.4 (b)]. Estimates of within-tag DTR variance, i.e. the variance of the repeated DTR measurements deviations of each tag from the mean DTR of that specific tag were: (i) 2.29 (2.00 – 2.61) for clustered, stationary; (ii) 2.23 (1.96 –

2.53) for clustered, swinging; (iii) 2.70 (2.37 – 3.08) spread-out, stationary; and (iv) 2.31 (2.03 – 2.63) for spread-out, swinging categorical levels of tag numbers [Table 4.4 (b)]. There was no significant difference between the estimated within-tag DTR variances as shown by the overlapping confidence intervals, hence the within-tag DTR variance was also not significantly affected by movement and disposition of the tags. The proportion of total DTR variance that was accounted for by the predictor variable, in this case categorical levels of tag numbers, was: (i) 9% for clustered, stationary; (ii) 11% for clustered, swinging; (iii) 5% for spread-out, stationary; and (iv) 3% for spread-out, swinging categorical levels of tags numbers [Table 4.4 (b)].

Table 4.4 (b): Dependency of data transmission per 10 minutes (DTR) on disposition, movement and numbers of tags adjusted for distance from the reader.

Number of tags in a group	Clustered, swinging tags	Clustered, stationary tags
	mean DTR (95 % CI)	mean DTR (95 % CI)
10	6.99 (6.03 - 7.95)	8.71 (7.73 - 9.68)
20	7.09 (6.41 - 7.77)	7.77 (7.07 - 8.46)
30	6.72 (6.16 - 7.28)	8.83 (8.26 - 9.40)
40	8.21 (7.72 - 8.69)	8.45 (7.96 - 8.95)
50	7.56 (7.12 - 8.00)	8.70 (8.25 - 9.14)
60	8.58 (8.19 - 8.98)	8.88 (8.47 - 9.28)
70	8.48 (8.11 - 8.85)	8.42 (8.04 - 8.80)
80	8.15 (7.80 - 8.49)	6.95 (6.59 - 7.30)
90	8.27 (7.94 - 8.60)	8.28 (7.94 - 8.61)
100	7.58 (7.27 - 7.89)	8.78 (8.46 - 9.10)
‡Wald test	W = 64.17; df = 9; p < 0.001	W = 92.53; df = 9 ; p < 0.001
δVar (_cons)	0.30 (0.16 – 0.56)	0.33 (0.16 - 0.67)
†Var (residual)	2.23 (1.96 – 2.53)	2.29 (2.00 – 2.61)
ψICC (*rho*)	0.12	0.13
*R^2	0.088	0.11
χLR test	$\overline{\chi}^2$ = 18.75; df = 1; p < 0.001	$\overline{\chi}^2$ = 15.79 ; df = 1; p < 0.001
ξTrendtest	Z = 4.14; p < 0.001	Z = - 0.96; p = 0.336

‡ Wald test: gives the probability of getting the Wald Chi^2- value obtained or more extreme if there is no association between mean DTR and all the ten categorical levels of tag numbers unde

† Var (residual): variance of the repeated DTR measurements deviations of each tag from the mean DTR of that specific tag, i.e. within-tag variance.

δ Var (_cons): variance of the mean DTR deviations of each tag from the grand mean of all tags, i.e. between-tag variance.

ψICC (*rho*): Intra-class correlation (ICC) which is the proportion of the total variance that is between tags or due to tags.

* R^2: measure of how much of the variance in DTR is explained by number of tags in a group.

χ LR test: test statistic for the Ho hypothesis that *Var (_cons)* = 0, i.e., that there is no significant cross-tag variation in DTR.

ξ Trendtest: same as Wald test but under Ha hypothesis that mean DTR systematically increases or decreases over the categorical levels of tag numbers.

Model diagnostic test results showed that the DTR residuals are normally distributed for: (i) Clustered, stationary [Figure 4.5 (i)]; (ii) Clustered, swinging [Figure 4.5 (ii]; (iii) Spread-out, stationary [Figure 4.5 (iv)]; and (iv) Spread-out, swinging [Figure 4.5 (iv)] categorical levels of tag numbers. For the normal probability (normal P-P) and inverse normal plots, normality of residuals is evidenced by the clustering of residuals along the diagonal line. Therefore, the assumption of a linear relationship between DTR and categorical levels of tag numbers was satisfied for all tag movements and dispositions. The resulting regression equations for the estimation of mean DTR values from categorical levels of tag numbers in multiples of 10 up to 100 are as shown below the relevant Figure 4.5 plots.

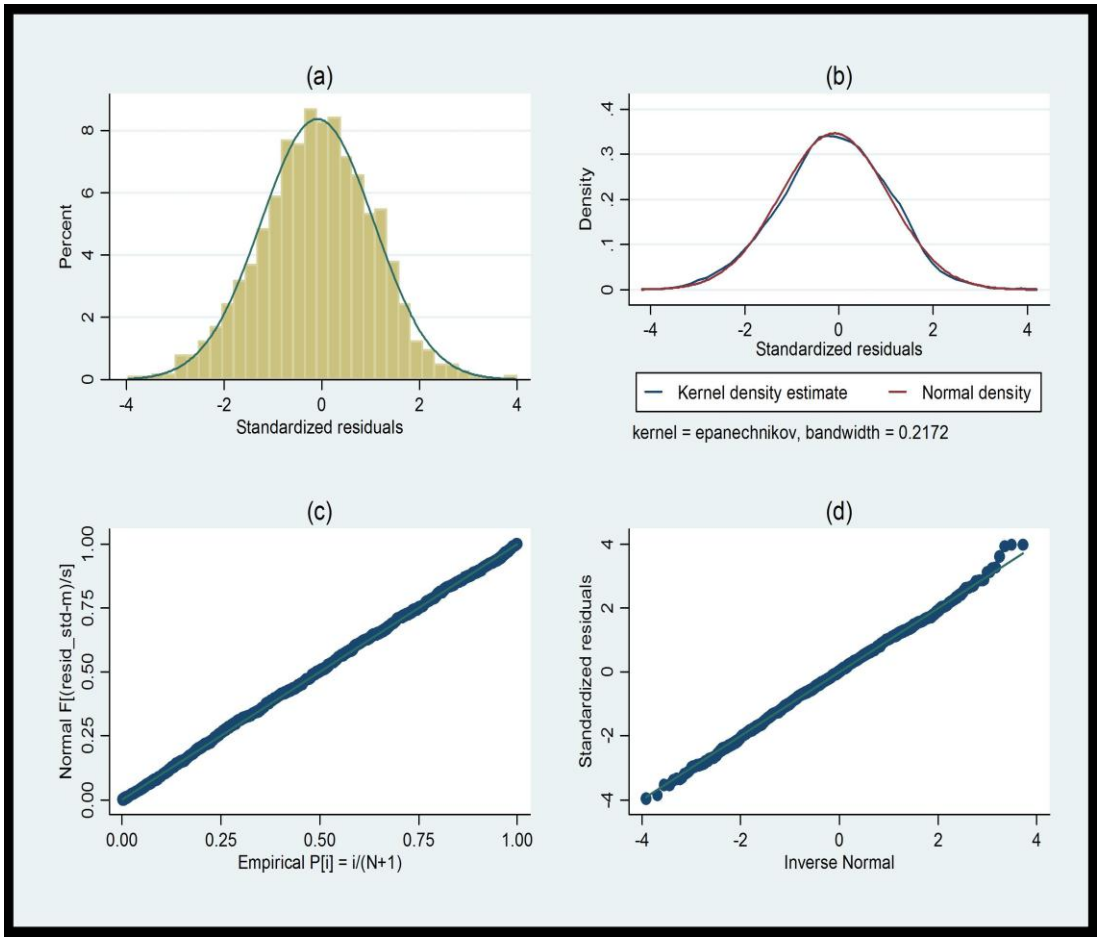


Figure 4.5 (i): Clustered, stationary tags: regression analysis diagnostics plots of assumptions for normality of residuals showing: (a) Histogram of residuals; (b) Kernel density estimate of residuals with normal density plots; (c) Standardized normal probability plot; and (d) Inverse normal plot of residuals for mean data transmission per 10 minutes against categorical levels of tag numbers. The resulting regression model is;

*E [dtr|grouptotal] = 8.71 – 0.94*Igrouptotal20 + 0.13*Igrouptotal30 – 0.25*Igrouptotal40 – 0.01*Igrouptotal50 + 0.17*Igrouptotal60 – 0.27*Igrouptotal70 – 1.76*Igrouptotal80 – 0.43*Igrouptotal90 + 0.08*Igrouptotal100*

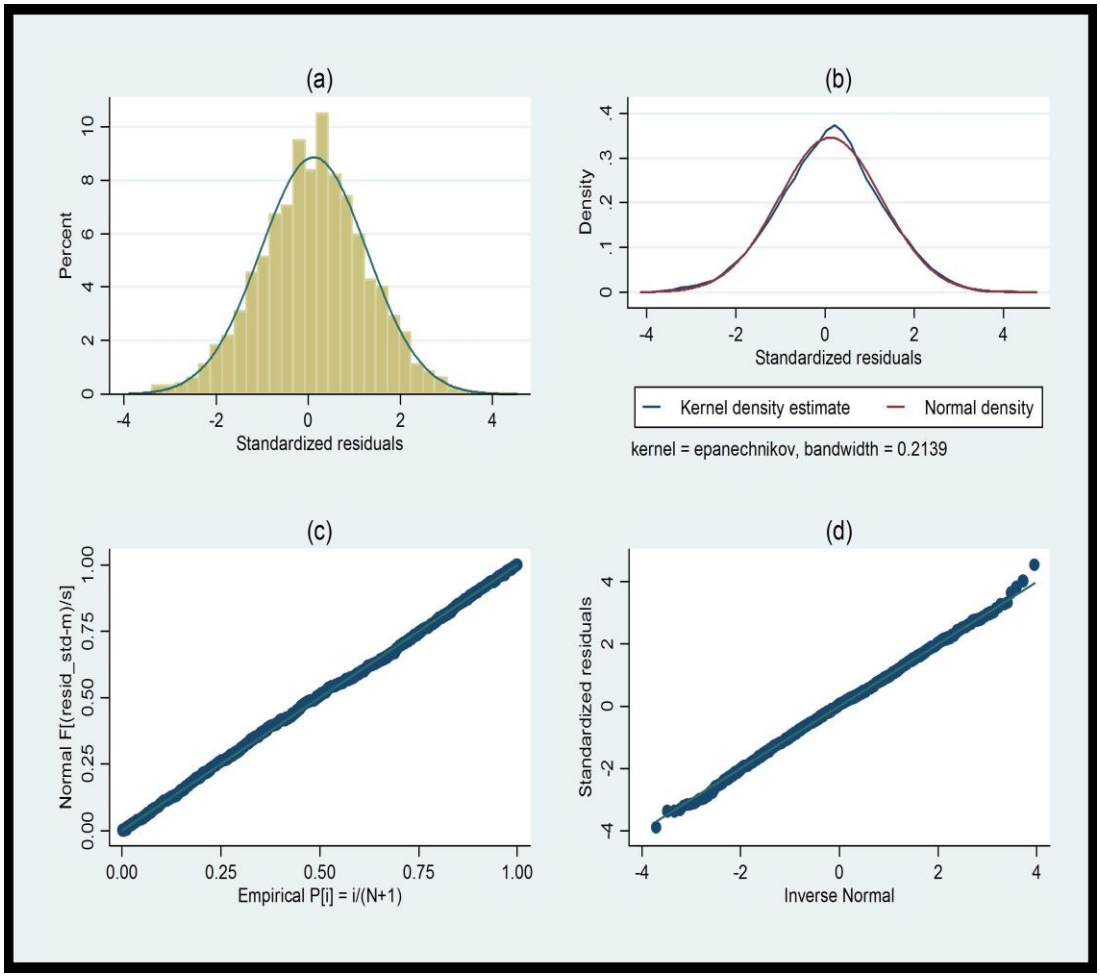


Figure 4.5 (ii): Clustered, swinging tags: regression analysis diagnostics plots of assumptions for normality of residuals showing; (a) Histogram of residuals; (b) Kernel density estimate of residuals with normal density plots; (c) Standardized normal probability plot; and (d) Inverse normal plot of residuals for mean data transmission per 10 minutes against categorical levels of tag numbers. The resulting regression model is;

*E [dtr|grouptotal] = 6.99 + 0.10*Igrouptotal20 – 0.27*Igrouptotal30 + 1.22 *Igrouptotal40 + 0.57*Igrouptotal50 + 1.59*Igrouptotal60 + 1.49*Igrouptotal70 + 1.16*Igrouptotal80 + 1.28*Igrouptotal90 + 0.59*Igrouptotal100*

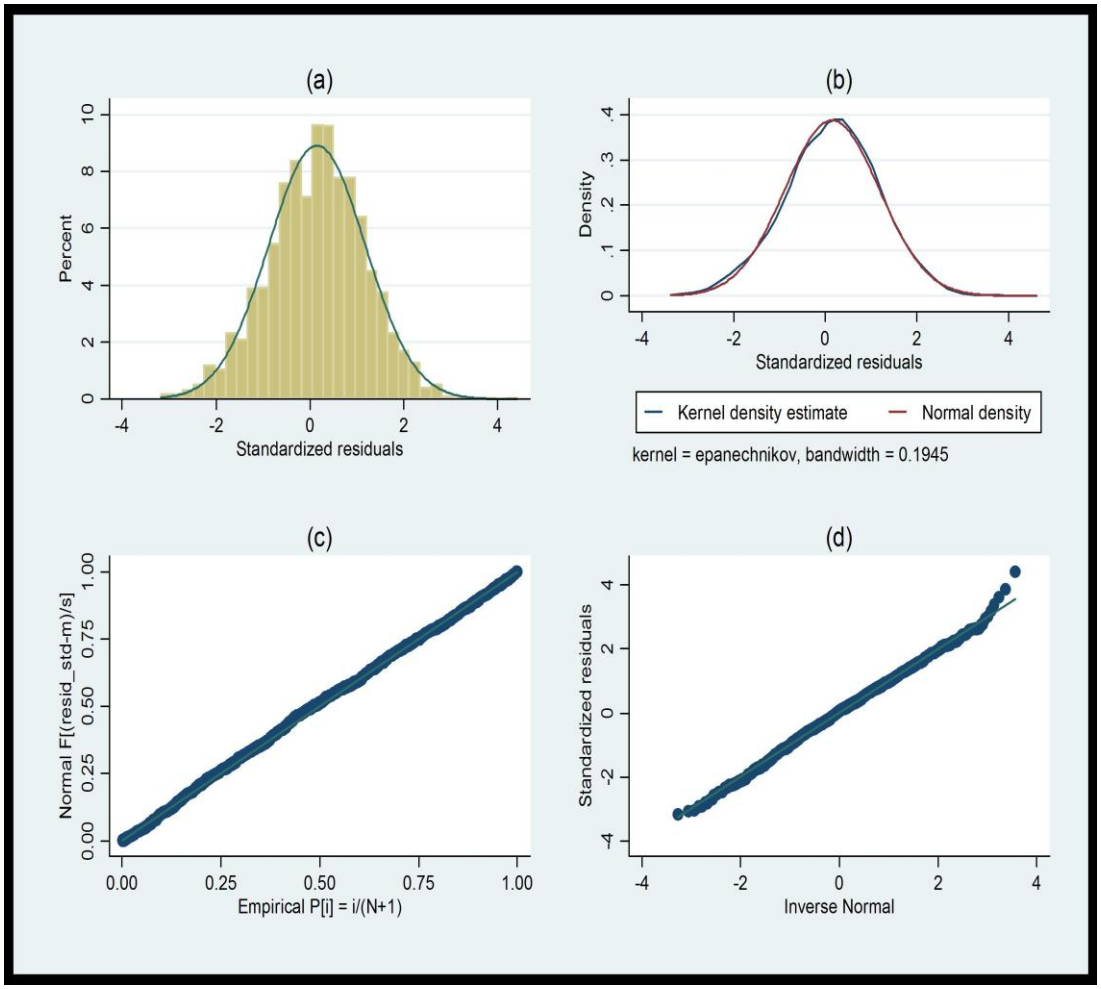


Figure 4.5 (iii): Spread-out, stationary tags: regression analysis diagnostics plots of assumptions for normality of residuals showing: (a) Histogram of residuals; (b) Kernel density estimate of residuals with normal density plots; (c) Standardized normal probability plot; and (d) Inverse normal plot of residuals for mean data transmission per 10 minutes against categorical levels of tag numbers. The resulting regression model is;

*E [dtr|grouptotal] = 6.68 + 0.71*Igrouptotal20 + 2.08*Igrouptotal30 + 2.00*Igrouptotal40 + 1.14*Igrouptotal50 + 1.08*Igrouptotal60 + 1.37*Igrouptotal70 + 1.13*Igrouptotal80 + 1.59*Igrouptotal90 + 0.73*Igrouptotal100*

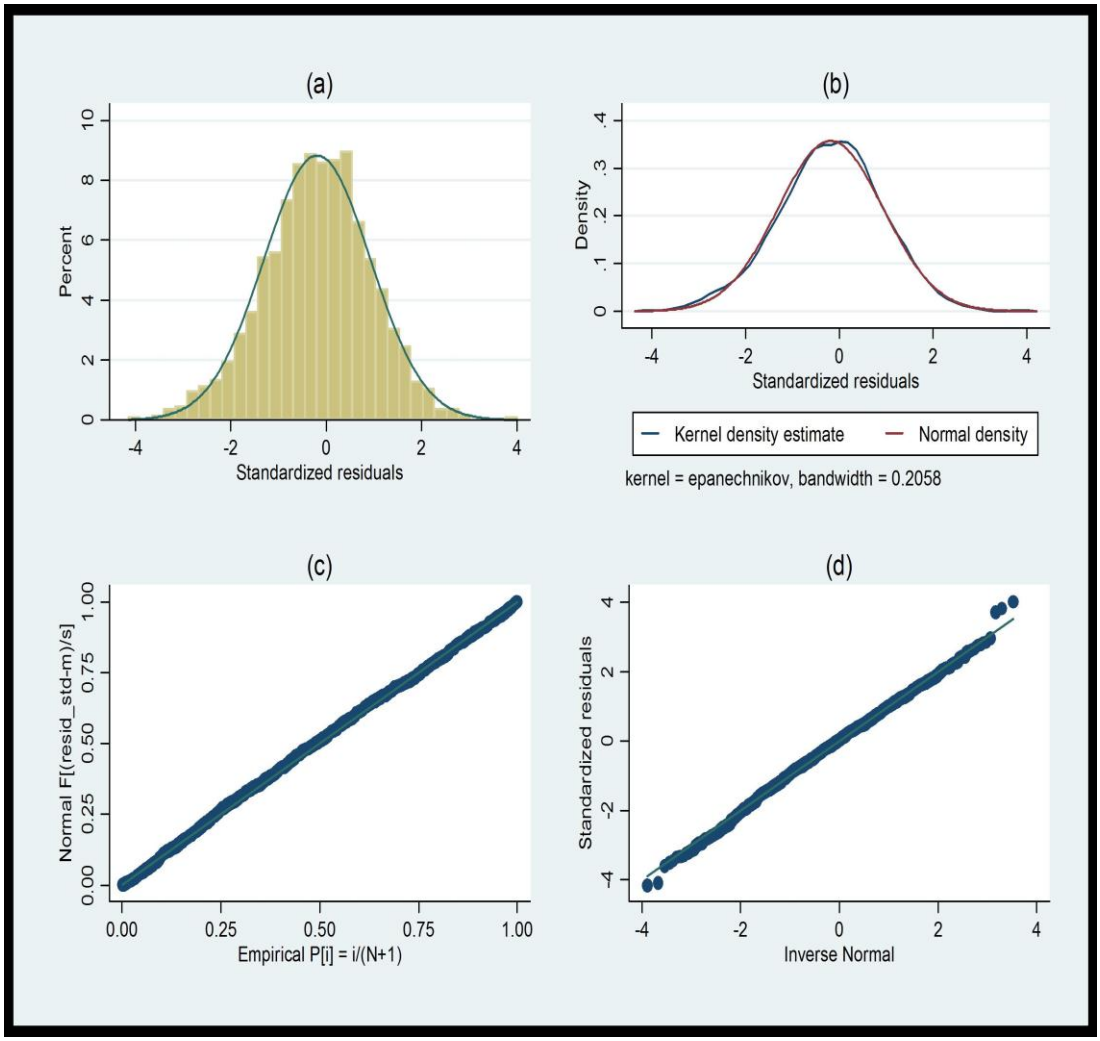


Figure 4.5 (iv): Spread-out, swinging tags: regression analysis diagnostics plots of assumptions for normality of residuals showing: (a) Histogram of residuals; (b) Kernel density estimate of residuals with normal density plots; (c) Standardized normal probability plot; and (d) Inverse normal plot of residuals for mean data transmission per 10 minutes against categorical levels of tag numbers. The resulting regression equation is;

*E [dtr|grouptotal] = 8.02 + 0.53*Igrouptotal20 + 0.53*Igrouptotal30 + 0.14*Igrouptotal40 + 0.77*Igrouptotal50 - 0.03*Igrouptotal60 – 0.03*Igrouptotal70 + 0.76*Igrouptotal80 + 0.85*Igrouptotal90 + 0.41*Igrouptotal100*

4.4 Discussion

From the earlier direct tag tapping trials, high tag fatality rates of up to 40% were realised which seems to suggest limited tag robustness. This limitation might need to be improved if the tags are to withstand harsh farm conditions. However, during the later trials when swinging movement was substituted for direct tag tapping, the fatality rate dropped to zero and this is encouraging since swinging movement is expected to be the dominant movement most likely to be experienced by the tags once hung on animals.

It appears that tag movement elicited through tapping led to perfect demodulation of the signal by the reader and 100% read rates (identification) of all the tags at all the tested distances up to 100 metres. Therefore, tag movement seems to elicit a compensatory effect to the negative signal attenuation effect of tag distance from the reader through signal strength enhancement in relation to background noise as described before by Wieselthier et al. (1989). This has a potential to differentiate between sick and healthy animals in that sick animals are expected to move less voluntarily when compared to healthy animals. Therefore, if the flock happens to be beyond the 65 metre mark from the reader, little or no identification of tags hung on sick (less active) animals may be realised when compared to identification of tags hung on healthy (moving) animals. However, this stands to be investigated.

The more than expected one reading per minute registered with tapping of tags is hypothesised to be due to the Ethernet capture effect in which the tag being tapped acquires a higher signal strength due to movement and then captures the channel to transmit a large sequence of data often equal to the complete window size, while the other tags are experiencing a long collision back-off. The reader would then only send an acknowledgement at the end of the transmission of the large sequence and the Ethernet effect is thought to have a short-term unfairness and is long-term fair as every tag has a fair opportunity to capture the medium once one tag is done transmitting (Ramakrishnan and Yang, 1994).

As frequency of radio waves increases, they start to behave more like light and cannot penetrate materials as well and tend to bounce off many objects (Anon., 2005), which is highly likely in this set-up as ultra-high frequency (UHF) waves at

868 MHz band were used. The lack of significant signal detuning associated with being behind a tree shrub, inside a sheep shed and inside a brick-built room could be due to possible air flow through these obstacles as air is the wireless transmission medium through which radio waves are propagated in this prototype RFID system set-up. On the other hand, multiple walls and metal structural components of a brick-built multi-room building probably led to the realised significant detuning associated with it in this study as previously reported by Floerkemeier and Lampe (2004). Also, the absorption of UHF radio waves by abdominal fluids particularly water (2005) could have led to the realised significant detuning with tapping underneath a flock of sheep. Only the effect of a single obstacle was investigated during these trials, which could mean a linear multiple overlap of the same obstacles between tags (e.g. tree shrub thicket instead of a single tree shrub), and the reader could give different results with different conclusions from what has been concluded here. Therefore, there is potential for variability in data transmissions between farms on account of differences in available obstacles such as those investigated here.

The contrast between day and night time DTR was meant to capture the differential effect of various background noise generators if present in the particular environment in which the activity monitoring system was set-up. These noise generators include devices like walkie-talkies, electric motors and some machines which can cause unwanted ambient electrical signals or electromagnetic energy (noise) in the operating environment of the RFID equipment, with a potential detuning effect on the RF signal of interest (2005). Other potential background noise generators that have been documented and can have an operational effect on such a system as set up here either during the day or night, includes the sun's effect on the environmental temperature and air moisture which affects the performance of tag components like chips resulting in poor propagation of radio waves through attenuation, hence poor read rates (Potdar et al., 2007; Bolić et al., 2010; Anon., 2012b). The potential impact of background noise at this particular locality due to noise generators would have been taken care of by proxy through comparison of DTR between night and day times. This is expected to be so because the use and availability of most potential noise generators is supposed to differ between day and night, just as temperature and air moisture are expected to differ between day and night times. Since no significant difference in DTR was realised between night and

day at this particular urban locality, the prototype system seems ideal for use on most farms where background noise is expected to be even minimal.

From the multi-tag environment studies, the greater than zero within-tag DTR variance consists of two components, being the differences between experimental conditions (categorical levels of tag numbers) under which the repeated measurements were made, and error or residual variation (Sullivan, 2008). Since a significant association between DTR and categorical levels of tag numbers was shown, it can be assumed that the larger contribution to the within-tag DTR variance was due to the different experimental conditions under which the repeated measurements were made. Hence, individual tag performance consistency, i.e. individual tag measurement reliability under similar conditions can be concluded to be adequate as the implication of the observation that the variance of the repeated DTR measurements was largely due to the differences in experimental conditions under which the repeated measurements were made. This is also supported by findings from individual tag tapping trials which showed consistent individual tag DTR values at each considered tapping distance between 25 and 100 metres from the reader. Individual tag activity level score measurements reliability makes the tags ideal for longitudinal study data capture. This is where time series activity level data of an individual animal is measured using the same tag to remotely monitor the individual animal's temporal or longitudinal behaviour at pasture, with the same tag acting as its own control. Therefore, any variation in activity level scores with time can be reasonably attributed to temporal change in behaviour of the tagged animal, but not to inherent poor measurement reliability (measurement error) of the tag.

In contrast to the above, the significant between-tag DTR variation indicates significant performance differences between individual tags, i.e. poor performance consistency, hence poor measurement reliability between individual tags (Steenbergen and Bradford, 2002; Sullivan, 2008) and this could indicate an inherent factory quality control problem between tag batches along the production line. This is also supported by findings from individual tag tapping trials which showed inconsistence between tag DTR values at each considered tapping distance between 25 and 100 metres from the reader. The significant between-tag variation has a potential to lead to activity level random measurement error where the tags have

been randomly allocated to animals, due to poor measurement reliability across tags. This will render the tags unsuitable for use in data capture in cross-sectional studies (Kirkwood and Sterne, 2003; Carlson and Morrison, 2009). Random measurement error of activity level scores as an exposure of interest will lead to regression dilution bias, i.e. will bias the regression coefficient (slope) towards the null. On the other hand, random measurement error of activity level scores as an outcome of interest will have minimal effect on the regression coefficient but will increase the standard errors resulting in an association being overlooked because of lack of statistical significance (Hutcheon et al., 2010).

The dependency of DTR on the number of tags within the reader's interrogation zones was explored further to come up with mean DTR estimating regression equations from tag numbers between 10 and 100 operating within the reader's range. The estimating equations can be used to optimise the initial set-up of the prototype RFID system on farms, as well as in continued monitoring of individual tags for faults, by comparing the mean DTR of the particular tag to that of the expected mean DTR for a tag operating within a particular categorical level of tag numbers. On-farm activity level monitoring system set-up optimisation using these equations currently seems to be the only option available, as there is not yet a developed measurement reference procedure or 'gold standard' in existence for this prototype activity level monitoring system.

4.5 Conclusion

A comprehensive description and characterisation of a prototype RFID system set-up with potential to remotely monitor individual animal activity levels in a typical small ruminant enterprise has been attempted by conducting various hand simulation trials. However, these mechanical simulation trials were conducted under controlled semi-artificial conditions devoid of the biological influence from animals, such that, once hung on animals, different values of DTR, including their distributions, could prevail instead. Nevertheless, knowledge of such basics as the effects of: (i) Tag distance from reader; (ii) Potential obstacles within a farm setting; (iii) Numbers of tags within the reader's interrogation zone; (iv) Tag disposition; and (v) Quality control status of the tags is indispensable to the future development and better understanding of this particular prototype activity level monitoring system. As such, these system

characterisation trials constituted a vital contribution towards basic understanding of the likely behaviour of the prototype RFID system once set up on real small ruminant farms. This has also contributed to better anticipation of the potential capabilities of the system as well as probable missing data mechanisms (Carpenter and Kenward, 2007) likely to be encountered with the use of this system on farms. The next intuitive step is to assess the performance of the system on real animals on real farms under both longitudinal and cross-sectional study designs. These steps form the basis of the following chapters.

CHAPTER 5

Remote longitudinal individual animal activity level monitoring at pasture to deduce changes in sheep and goat behaviour

5.1 Introduction

Most livestock farming enterprises have a routine husbandry management protocol which the animals undergo almost daily. In livestock farming regions affected by significant levels of predation, animals are normally kraaled or yarded overnight and let out for grazing during the day in the presence of herders. Where practised, all-day physical monitoring of animals by stockmen as a way of guarding against predators has a measurable opportunity cost particularly in this modern era of high labour shortage and costs. A remote monitoring system with the potential to profile the expected daily husbandry routine of animals at individual or flock level as well as being able to remotely alert the stockmen of any unexpected significant deviations from the norm could reduce the opportunity costs associated with continuous direct daily monitoring of animals at pasture. As well as detecting disturbance due to predator attack, such a system could in principle detect more subtle alterations in behaviour, for example due to ill health, including parasitism (Forbes et al., 2007; Forbes, 2010).

Apart from technical obstacles relating to collection and collation of animal activity levels (see Chapter 4), a robust activity monitoring system should be able from a rich and noisy data set to detect deviation from normal behaviour patterns. Change-point analysis is a candidate method for this purpose. First described by Page (1955), change-point analysis is the process of detecting distributional changes within time-ordered observations (Matteson and James, 2014). It has applications in a wide variety of fields, including but not limited to: (i) Bioinformatics to identify specific disease-associated genes (Muggeo and Adelfio, 2011); (ii) Financial sector to detect credit card fraud (Bolton and Hand, 2002); (iii) Data classification in data mining (Mampaey and Vreeken, 2011); (iv) Signal processing to detect significant changes within a stream of images (Kim et al., 2009); (v) Statistical process control as a quality assurance method to ensure that processes operate at full potential and produce conforming products (Barlow and Irony, 1992); and (vi) Health sector to evaluate benefit from prescribed therapy (Cassidy et al., 2002; Cram et al., 2003;

Foffani et al., 2003). Change-point analysis could be usefully applied to detect changes in animal activity levels, hence behaviour, because it aims to detect any change in the mean of a process in time-ordered historical data. The fundamental questions addressed by this approach are: (i) Did a change occur? (ii) Did more than one change occur? (iii) When did the change(s) occur? and (iv) How confident are we that the detected changes are real? (Taylor, 2000).

The objectives of this chapter were, firstly, to evaluate the potential of the prototype activity level monitoring system described in Chapter 4, to continuously capture and relay sequential or time series (longitudinal) activity level data from sheep during routine on-farm behaviour. Secondly, to apply change-point analysis to differentiate the interfaces between: (i) Resting and running, under the hypothesis that activity level score significantly increases with transition from resting to running; (ii) Onset of lameness and recovery from lameness, under the hypothesis that both daily mean activity level score and activity level score count decrease with onset of lameness and subsequently increase to previous levels with recovery from lameness; and (iii) A particular daily husbandry management routine in free grazing sheep on a farm, under the hypotheses that hourly activity level scores will decrease with increase in distance of tagged ewe from the reader and vice-versa, while hourly mean activity score will either increase or decrease, depending on which of the activities performed between grazing at pasture and yarding at night are more exerting.

5.2 Materials and methods

5.2.1 Trial design

The activity monitoring system described in Chapter 4 was set up to site-specific optimum settings on two farms, one with goats and another with sheep. The system was set to capture and transmit one-minute interval activity level data from each tagged animal prior to, during and after undertaking a particular husbandry routine or experiencing a particular health condition of interest. The particular health condition experienced or husbandry routines undertaken were timed and manually annotated for reference purposes, in order to enable selection of transmitted activity data from the relevant period.

5.2.1.1 Trial one: transition between resting and running

Four tagged ewes were monitored at rest, with Ewe 40061201002 and 40061200992 lying down and Ewe 40061200933 and 40061201138 standing up, from 0819 to 0830hrs. Then the ewes were individually chased as follows: (i) Ewe 40061201002 was chased continuously from 0832 to 0835hrs; (ii) Ewe 40061201138 continuously from 0835 to 0838hrs, whereupon she suddenly lay down during the chase; (iii) Ewe 40061200992 was chased continuously from 0840 to 0842 hrs, when she suddenly lay down, as above. She then stood up at 0843hrs and was again chased continuously until she similarly lay down again at 0844hrs; and (iv) Ewe 40061200933 chased continuously from 0849 to 0852hrs. To enable data continuity between resting and running events, the respective captured activity level scores were directly apposed by deleting any data transmissions which occurred between the respective manually annotated resting and running times for each ewe. The resulting respective sequential data was then used to test if the relayed scores showed any significant difference at the point of transition from resting to running for each respective ewe as per the working hypothesis.

5.2.2.2 Trial two: transition between sound and lame gait

Goats on the farm involved underwent a routine fortnightly Five Point Check© evaluation (Bath and Van Wyk, 2009) during which most clinical health conditions were diagnosed and recorded. Perchance one tagged doe, Goat 40061201024, became lame. She had a history of having been evaluated on three consecutive fortnightly occasions: (i) 15th January 2013, she was not lame; (ii) 29th January 2013, she was recorded as lame, presenting with an interdigital abscess which was lanced and a topical wound spray applied; and (iii) 14th February 2013, by which time she had recovered from the lameness. Daily mean activity level score and activity level score count were calculated from the transmitted one-minute interval activity level data for the goat for the duration, spanning 16th January 2013 to 18th February 2013. The daily activity mean score, i.e. daily average activity score, and daily score count, i.e. daily number of activity scores transmitted, for the entire duration were used separately to assess if there was or were any detectable significant change(s) in their distribution, temporally associated with the manually recorded times of onset of lameness and recovery from it, as per the working hypothesis.

5.2.2.3 Trial three: transition between housing and grazing

On the sheep farm involved, a flock of 34 tagged pregnant ewes collectively underwent the following husbandry management routine for four consecutive days: (i) Spending night time in an open kraal next to the reader, which was mounted on top of an adjacent shed; (ii) Flock let out at ~ 0730hrs and trekked a distance of ~ 2.5 km along the road to the furthest paddock for grazing in a right-angled triangle shape between the reader and the grazing paddock. The direct distance between the reader and the nearest side of the paddock was ~ 2.1 km; and (iii) The flock was rounded up at ~ 1630hrs and was herded back along the same route, and fed concentrates next to the kraal (pen) before being closed in again for the night. Hourly mean activity level score (calculated by averaging the transmitted minute-interval activity level scores for each hour) and hourly activity level score count (calculated by counting the transmitted minute-interval activity level scores for each hour) for the flock were calculated from the data that was transmitted, supposedly at intervals of one minute, from all the tagged ewes. The serial hourly mean score and hourly score count were analysed separately for each of the four days of monitoring to assess if there was or were any detectable significant change(s) in their distribution, temporally associated with the manually recorded daily husbandry routine as per the working hypothesis.

5.2.2 Statistical analysis

Analysis of data from the conducted trials was done using Change-Point Analyzer® software (Taylor, 2000), which shows both control charting and change-point analysis results of individual animals in its output. For the individual control charting results, upper (U) and lower (L) control limits (CL) are plotted as lines, which identify the maximum range over which the values are expected to vary, assuming no significant change has occurred. Activity level score values outside the control limits indicate that a significant change has occurred. The control limits assume that the scores come from a normal distribution. Scatterplots of the activity level scores were also constructed.

Change-point analysis, which is data distribution-free, iteratively uses a combination of cumulative sum charts (CUSUM) and bootstrapping to detect changes and assumes independent error structure, i.e. that the data points are not correlated (Taylor, 2000). The results are shown in the shaded background (blue in colour

prints) with significant changes in the particular data series denoted by a shift upwards or downwards in the shaded background area, while a change in the height of the shaded background indicates a significant change in the standard deviation (variation) of the data. Also, below each graphical presentation of the change-point analysis results, are additional important statistical test details showing: (i) Point time estimate of the detected change in serial activity level scores and its 95 % confidence interval (CI); (ii) Level of confidence that the detected change in serial activity level scores did indeed occur at that point in time; (iii) Detected change range, i.e. from–to; and (iv) Analysis iteration number during which the particular change was detected (level). During the analysis, ranks of the transmitted activity level scores, instead of the actual scores, were used for the respective trials to control for the potential existence and effects of outliers.

5.3 Results

5.3.1 Trial one: transition between resting and running

Results showed a significant difference in activity level scores between resting and running for all four ewes, both with individual control charting and change-point analysis [Figure 5.1 (a) to (d)]. Change-point analysis times coincided precisely with the relevant manually annotated resting and running times during the trials. The changes were significant enough to be detected in a single iteration. Also, the direction of change for all the detected significant changes in activity level scores of the four tagged trial ewes occurred as hypothesised. Interestingly, the shortterm manoeuvres by Ewe 40061200992 subsequent to the initial start of running were only captured in the individual's control charting and not in the change-point analysis results as shown in Figure 5.1 (d).

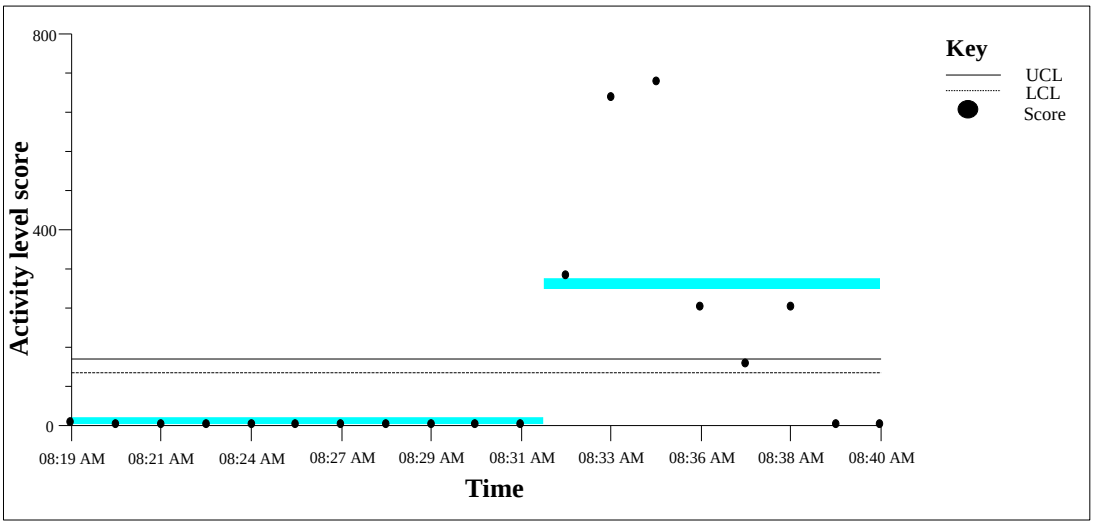


Table of significant changes for activity level score

Confidence level for Candidate Changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
08:32:58 AM	(08:31:38 AM, 08:36:07 AM)	100%	0.36	284.62	1

Figure 5.1 (a): Plot of change-point analysis results of the transmitted activity level score ranks for Ewe 40061201002. She was resting in recumbency from 0819 to 0830 hrs and was then chased continuously from 0832 to 0835 hrs.

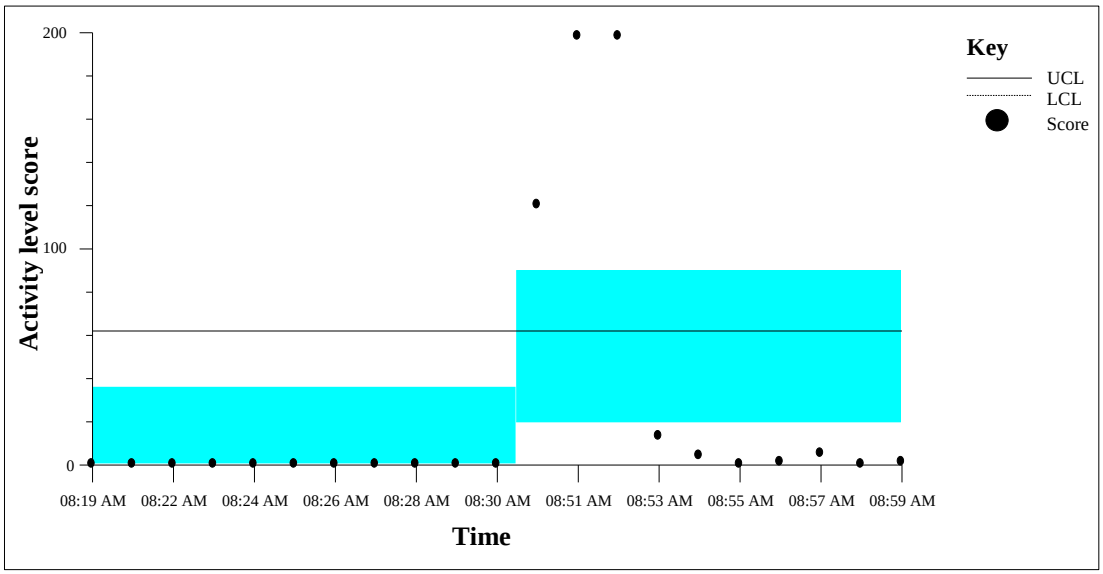


Table of significant changes for activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
08:49:59 AM	(08:49:59 AM, 08:53:13 AM)	100%	0.00	54.00	1

Figure 5.1 (b): Plot of change-point analysis results of the transmitted activity level score ranks for Ewe 40061200933. She was standing at rest from 0819 to 0830 hrs and was chased continuously from 0849 to 0852 hrs.

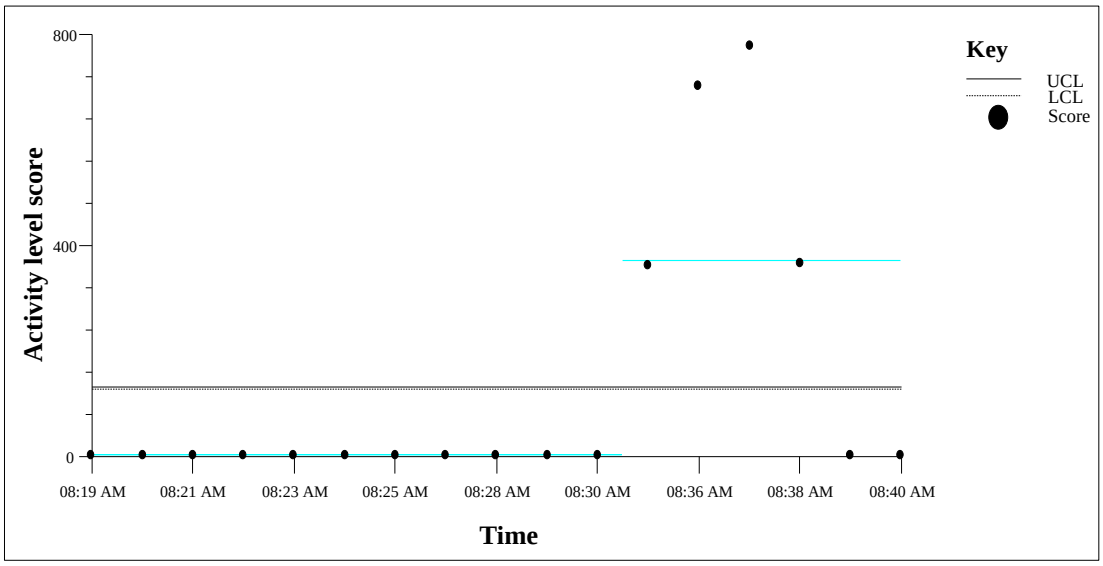


Table of significant changes for activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
08:35:30 AM	(08:29:08 AM, 08:38:40 AM)	98%	0.09	366.67	1

Figure 5.1 (c): Plot of change-point analysis results of the transmitted activity level score ranks for Ewe 40061201138. She was standing at rest from 0819 to 0830 hrs and was chased continuously from 0835 to 0838 hrs, whereupon she suddenly lay down during the chase.

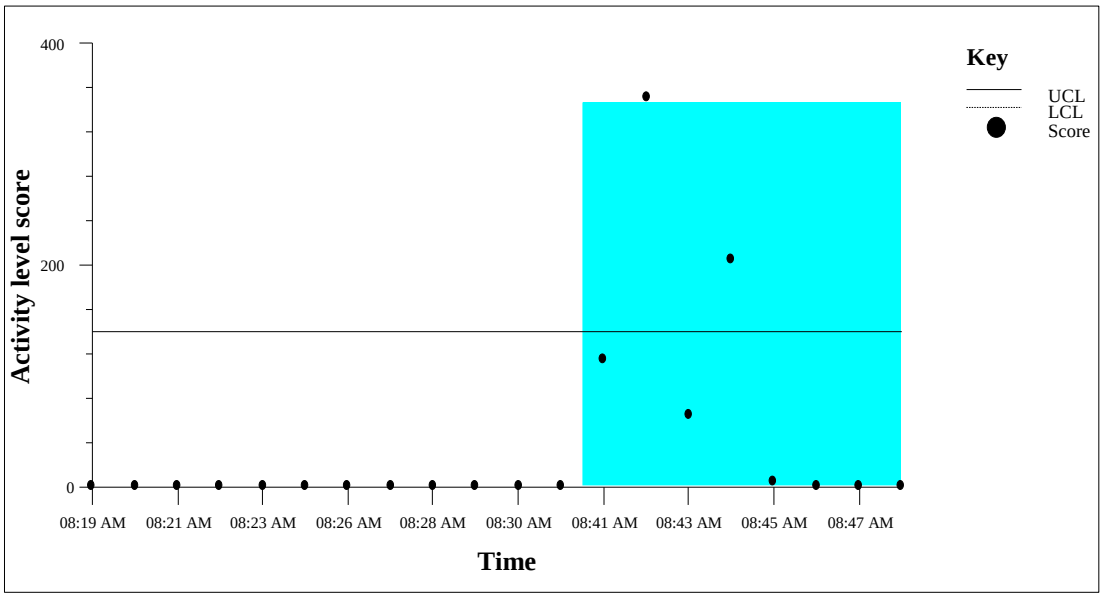


Table of significant changes for activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
08:41:32 AM	(08:41:32 AM, 08:46:33 AM)	100%	0.00	92.50	1

Figure 5.1 (d): Plot of change-point analysis results of the transmitted activity level score ranks for Ewe 40061200992. She was lying down, resting, from 0819 to 0830 hrs and was chased continuously from 0840 to 0842 hrs, whereupon she suddenly lay down during the chase. She then stood up at 0843 hrs and was again chased continuously until 0844 hrs, whereupon she once more suddenly lay down during the chase.

5.3.2 Trial two: transition between sound and lame gait

The earliest detected significant drop in activity level score count from the change-point analysis results that might be associated with the diagnosed lameness of the goat involved was on the 22nd January 2013 (95% CI 20th – 22nd January 2013 with 98% confidence level). This initial drop predates the date when the lameness was diagnosed, which was on the 29th January 2013 during a routine Five Point Check© evaluation. Another significant drop in activity score count was on the 30th January 2013 (95% CI 24th – 30th January 2013) with 94% confidence level before the beginning of a significant rise in activity level score count, firstly on the 04th February 2013 (95% CI 04th - 11th February 2013) and lastly on the 17th February 2013 (95% CI 09th – 20th February 2013) to initial pre-drop levels with 99% and 93% confidence level respectively. Also, the 95% confidence interval for the last significant rise in daily activity level score count (from the 9th to the 20th February 2013) includes the 14th of February 2013, which is the date the goat was noted as recovered during the following routine fortnightly flock evaluation. The noted date of recovery from lameness, 14th February 2013, also coincides with spiking in activity level score count data outside of the control limits in the control charting results of the individual, 17th February 2013, indicating a significant increase in daily activity level score count [Figure 5.2 (a)].

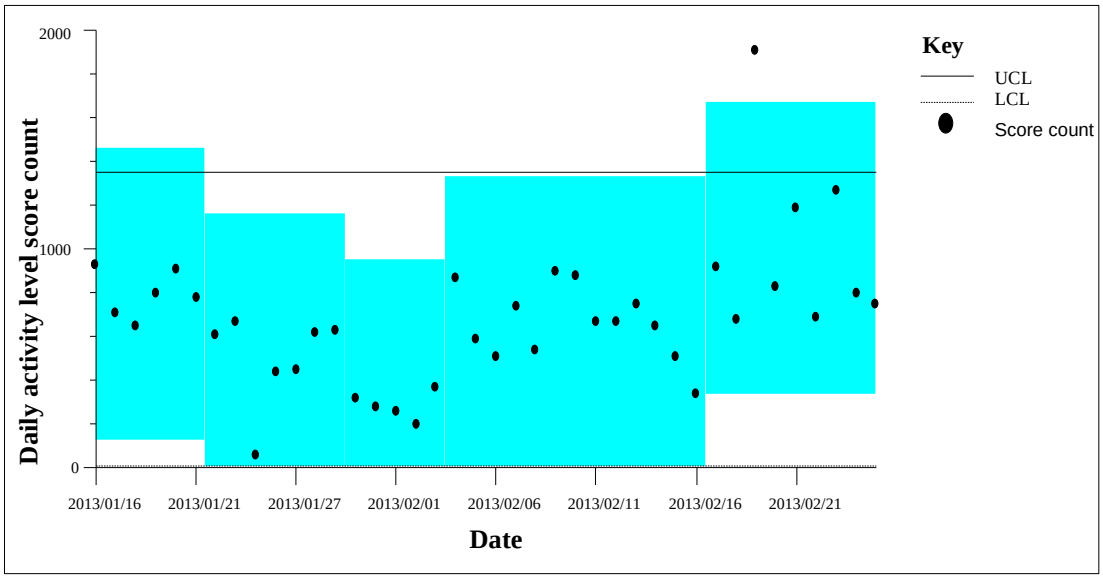


Table of significant changes for daily activity level score count

Confidence level for candidate Changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Date	Confidence interval	Confidence level	From	To	Level
2013/01/22	(2013/01/20, 2013/01/22)	98%	787.33	487.14	5
2013/01/30	(2013/01/24, 2013/01/30)	94%	487.14	275.60	4
2013/02/04	(2013/02/04, 2013/02/11)	99%	275.60	654.08	5
2013/02/17	(2013/02/09, 2013/02/20)	93%	654.08	995.11	1

Figure 5.2 (a): Daily activity level score count: change-point analysis rank-based results plot for Goat 40061201024. She had a history of having been evaluated on three consecutive fortnightly occasions: (i) 15th January 2013, she was not lame; (ii) 29th January 2013, she was recorded as lame with an interdigital abscess, which was treated; and (iii) 14th February 2013, she had recovered from the lameness.

In contrast to the above, change-point analysis results, based on daily mean activity level score ranks, showed only a single significant change point on the 05th February 2013 (95% CI 04th – 09th February 2013with 100% confidence level), which is a date between the 29th of February 2013, the date the goat was noted as lame, and the 14th of February 2013, the date the goat was noted as having recovered from the lameness [Figure 5.2 (b)]. Both the profiles of the change point analysis results based on daily activity score count and daily mean activity score ranks are in agreement with the hypothesised profiles of changes with onset of lameness and recovery from it.

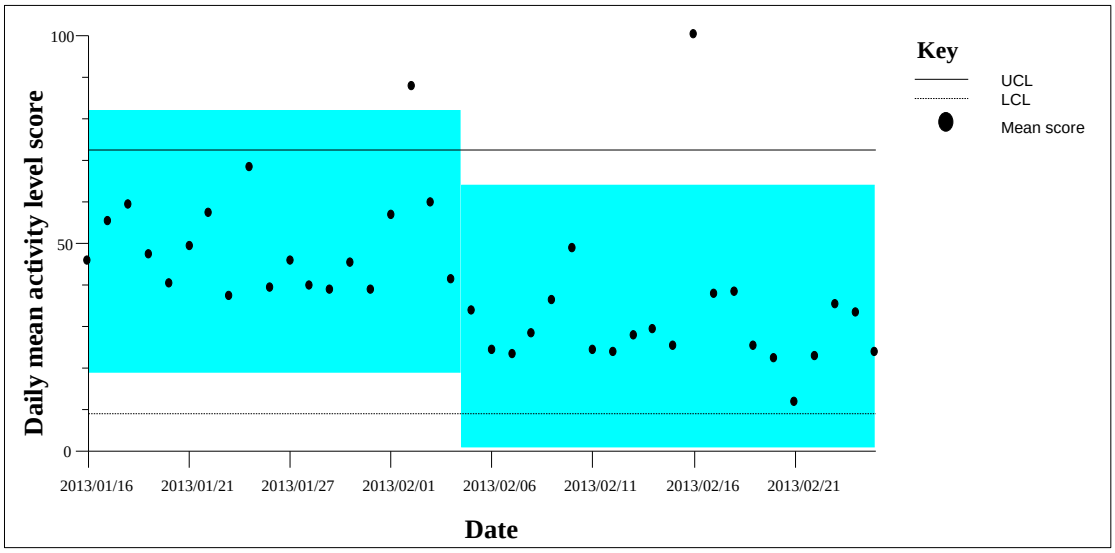


Table of significant changes for daily mean activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Date	Confidence interval	Confidence level	From	To	Level
2013/02/05	(2013/02/04, 2013/02/09)	100%	49.87	31.86	2

Figure 5.2 (b): Daily mean activity level score: change-point analysis rank-based results plot for Goat 40061201024. She had a history of having been evaluated on three consecutive fortnightly occasions: (i) 15th January 2013, she was not lame; (ii) 29th January 2013, she was recorded as lame with an interdigital abscess, which was treated; and (iii) 14th February 2013, by which time she had recovered from the lameness.

5.3.3 Trial three: transition between housing and grazing

For the total of four days of monitoring, change point analysis results based on the ranks of the hourly activity level score count showed a significant change as a shift downward in hourly activity level score count in the morning when the flock was let out from the kraal (pen) [09 AM, 95% CI 09 – 09 AM; 07 AM, 95% CI 07 – 07 AM; 06 AM, 95% CI 06 – 07 AM; and 07 AM 95% CI 07 – 07 AM for day 1, 2, 3 and 4, respectively] with 100% confidence level, staying low for the duration of walking to pasture and during the day when the flock was at pasture. A shift upward was realised in the evenings when the flock was back in the kraal for the night [06 PM, 95% CI 06 – 06 PM; 05 PM, 95 % CI 05 – 05 PM; 06 PM, 95% CI 06 – 07 PM and 05 PM, 95% CI 05 – 05 PM for day 1, 2, 3 and 4, respectively) also with 100% confidence level. These matched well with the control charting results of the individuals, with the plot of the actual data points correspondingly going below the minimum control limit for the downward shift and above the maximum control limit for the upward shift in hourly activity level score count. Also, the hourly activity score count data points for the four days of monitoring fell within the shaded background area representing the change analysis results [Figure 5.3 a (i), 5.3.b (i), 5.3 c (i) and 5.3 d (i)].

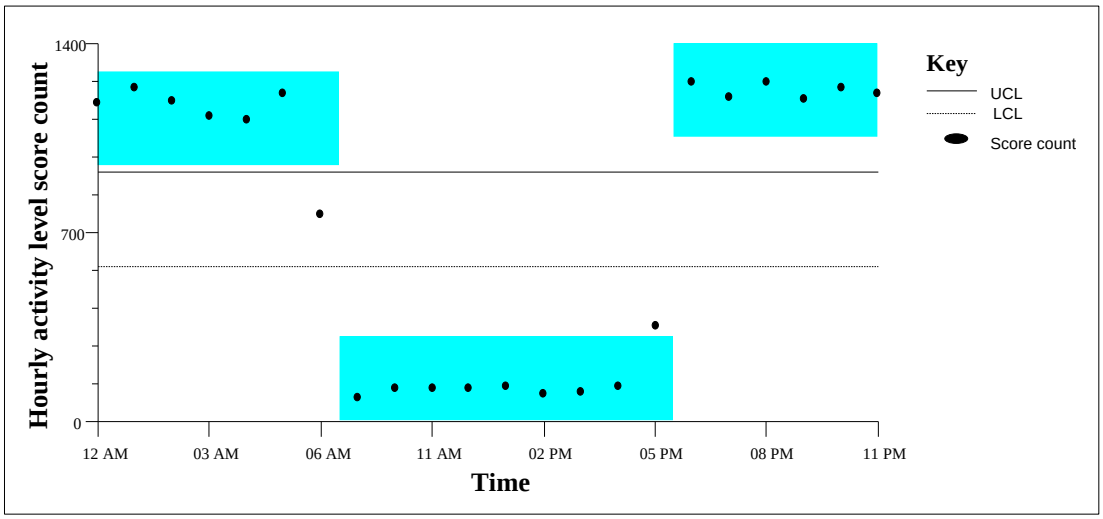


Table of significant changes for hourly activity level score count

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
09 AM	(09 AM, 09 AM)	100%	1114.40	138.22	2
06 PM	(06 PM, 06 PM)	100%	138.22	1222.00	1

Figure 5.3 a (i): Hourly activity level score **count** on day 1: – Results of a rank-based change-point analysis for a flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open (pen) (kraal) next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

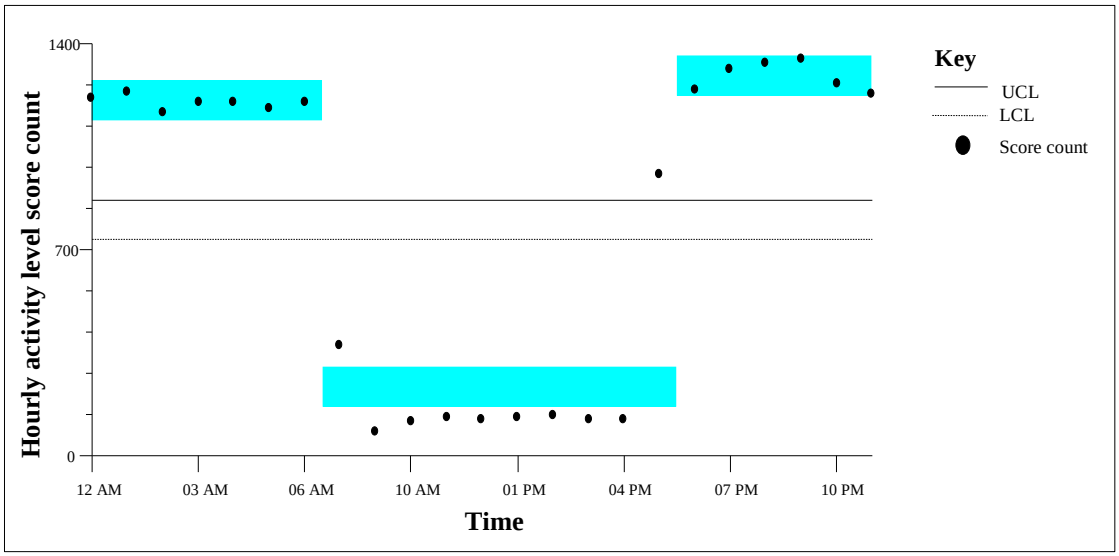


Table of significant changes for hourly activity level score count

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
07 AM	(07 AM, 09 AM)	100%	1198.00	225.80	2
06 PM	(06 PM, 06 PM)	100%	225.80	1283.80	1

Figure 5.3 b (i): Hourly activity level score **count** on day 2: – Results of a rank-based change-point analysis for a flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open pen (kraal) next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

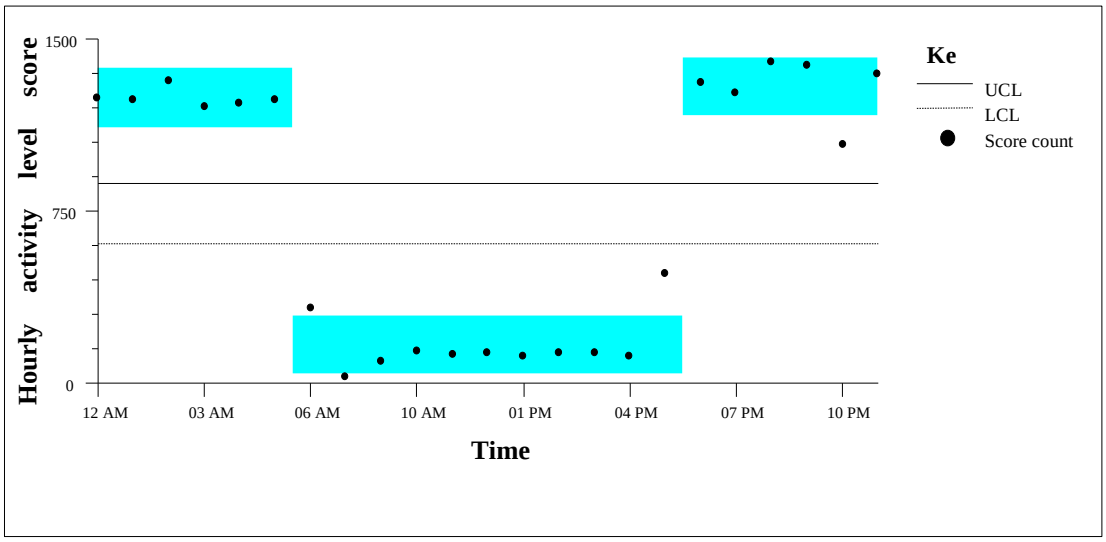


Table of significant changes for hourly activity level score count

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
06 AM	(06 AM, 07 AM)	100%	1237.70	160.73	2
06 PM	(06 PM, 07 PM)	100%	160.73	1287.00	1

Figure 5.3 c (i): Hourly activity level score **count** on day 3: – Results of a rank-based change-point analysis for a flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open kraal next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

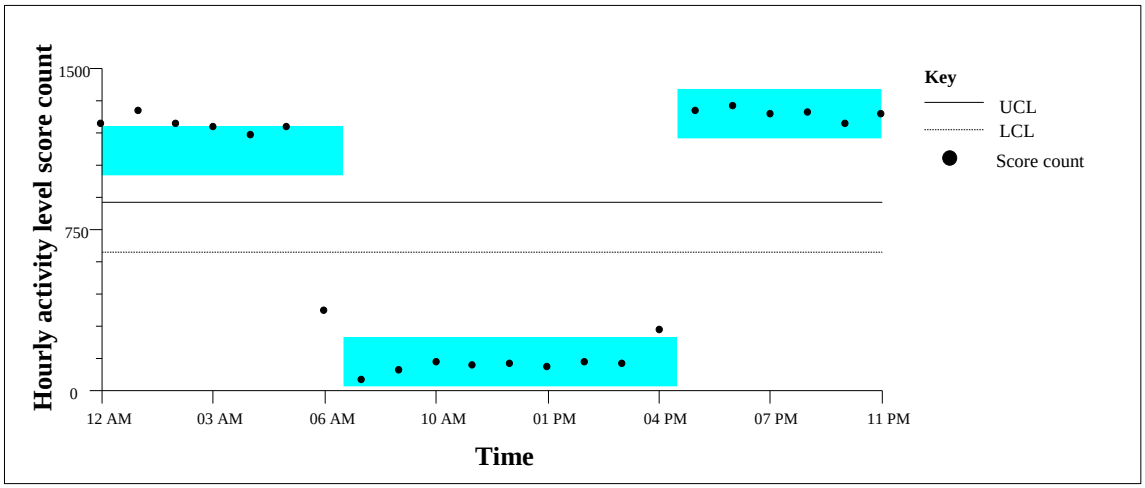


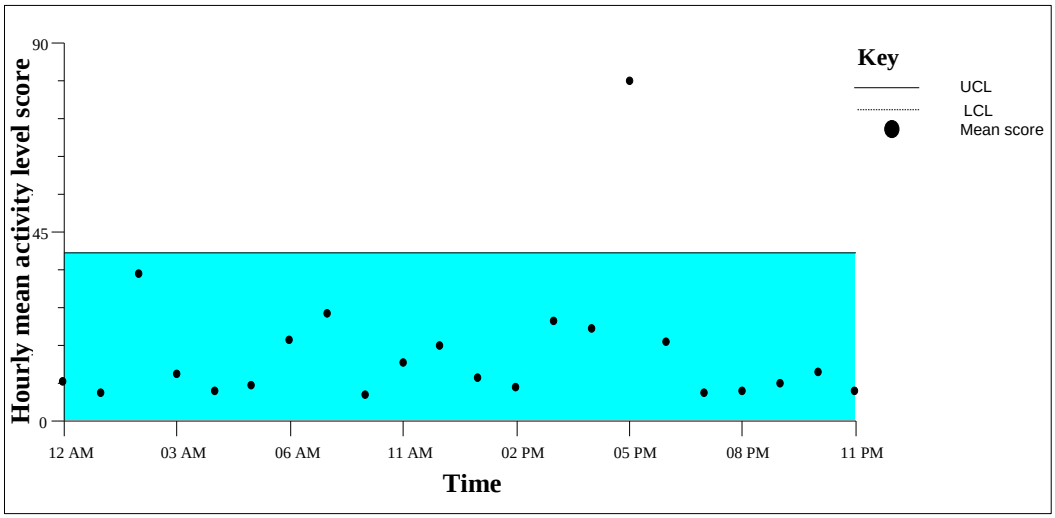
Table of significant changes for hourly activity level score count

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
07 AM	(07 AM, 07 AM)	100%	1110.90	124.89	2
05 PM	(05 PM, 05 PM)	100%	124.89	1284.00	1

Figure 5.3 d (i): Hourly activity level score **count** day 4: – Results of a rank-based change-point analysis for a the flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open kraal next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

In contrast to the above, change point analysis based on the ranks of the hourly mean activity score for day 1 showed no significant changes from time of let-out in the morning to period of kraaling in the evening with an estimated average hourly mean score of 16.23 [Figure 5.3 a (ii)]. For day 2, not only a shift upwards in hourly mean activity score in the morning was realised (05 AM, 95% CI 05 – 05 AM) with 97% confidence level but also a significant change in the hourly mean activity score standard deviation or variation was realised (06 AM, 95% CI 04 – 05 AM) with 97% confidence level [Figure 5.3 b (ii)] and both were sustained throughout the day including period of kraaling in the evening.



No significant changes for hourly mean activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Estimated average = 16.23

Figure 5.3 a (ii): Hourly **mean** activity level score on day 1: – Results of a rank-based change-point analysis for a flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open kraal next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

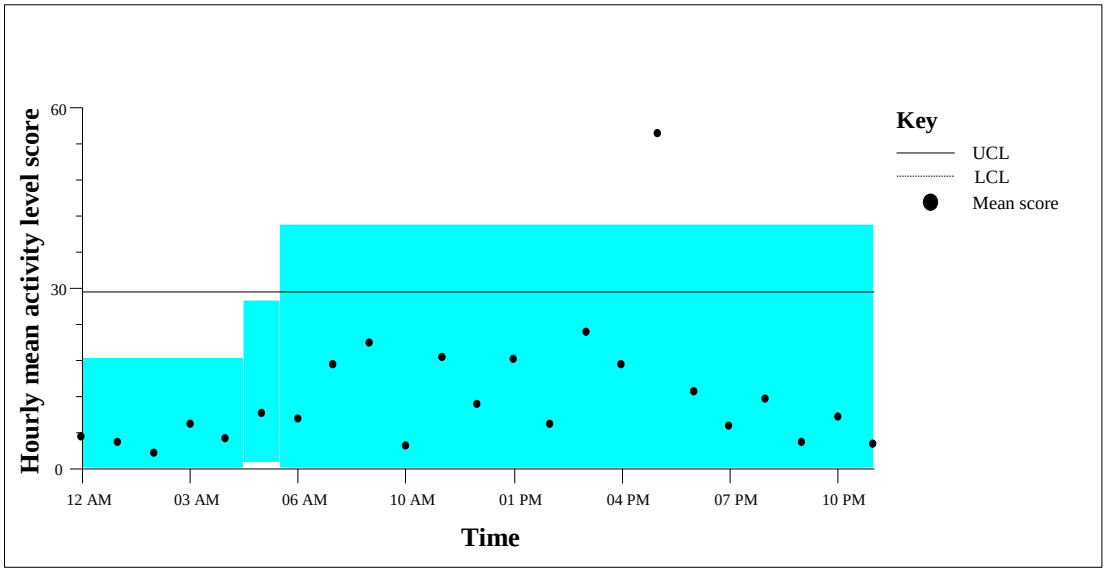


Table of significant changes for hourly mean activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence interval	Confidence level	From	To	Level
05 AM	(05 AM, 05 PM)	97%	4.71	14.17	1

Table of significant changes for hourly mean activity score standard deviation

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 100, without replacement, MSE estimates, analyze ranks

Time	Confidence Interval	Confidence Level	From	To	Level
06 AM	(04 AM, 05 PM)	97%	4.4	8.68	1

Figure 5.3 b (ii): Hourly **mean** activity level score on day 2: – Results of a rank-based change-point analysis for a flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open kraal next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

For day 3 [Figure 5.3 c (ii)] and 4 [Figure 5.3 d (ii)] there was a shift upwards in hourly mean activity score at approximate times of let-out from the kraal (05 AM, 95% CI 05 – 06 AM and 10 AM, 95% CI 06 – 10 AM, respectively) with 96% and 98% confidence level respectively. Also there was a shift downwards in hourly mean activity level score in the evenings for day 3 and 4 (07 PM, 95% CI 04 – 07 PM and 06 PM, 95% CI 04 – 10 PM, respectively) at times of kraaling in the evening with 91% and 96% confidence level respectively.

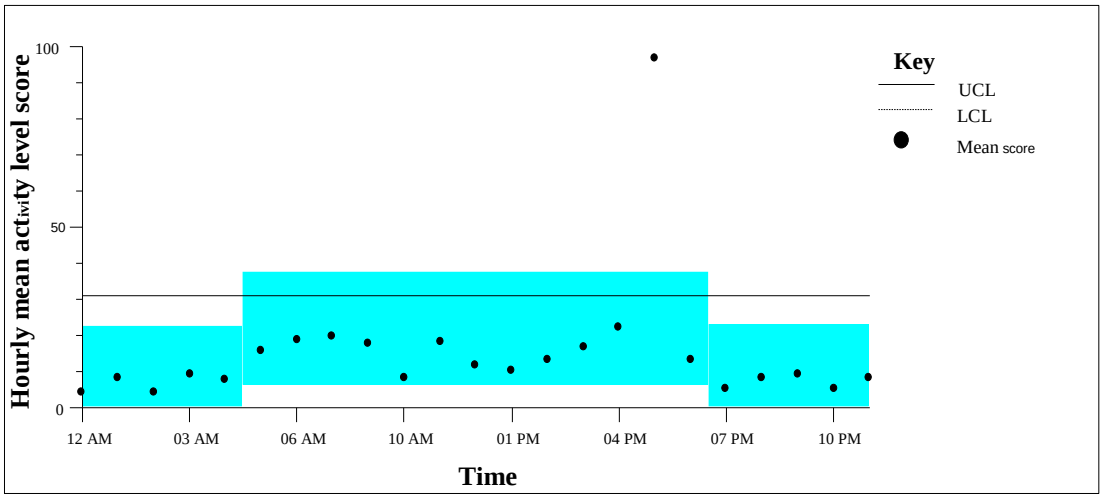


Table of significant changes for hourly mean activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence Interval	Confidence Level	From	To	Level
05 AM	(05 AM, 07 AM)	98%	6.39	21.47	1
07 PM	(04 PM, 07 PM)	91%	21.47	7.02	2

Figure 5.3 c (ii): Hourly **mean** activity level score on day 3: – Results of a rank-based change-point analysis for a flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open kraal next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

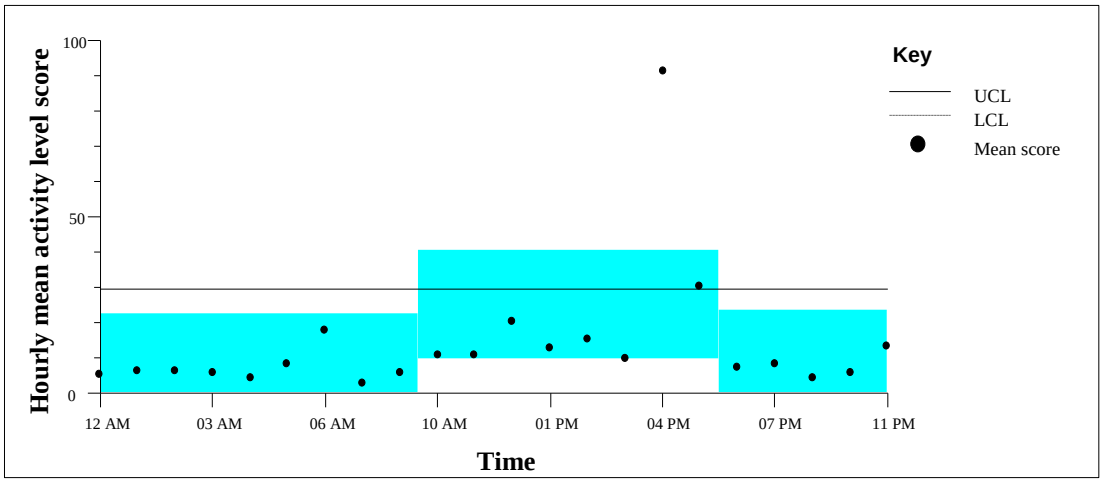


Table of significant changes for hourly mean activity level score

Confidence level for candidate changes = 50%, confidence level for inclusion in table = 90%, confidence interval = 95%, bootstraps = 1000, without replacement, MSE estimates, analyze ranks

Time	Confidence Interval	Confidence Level	From	To	Level
10 AM	(06 AM, 10 AM)	97%	6.70	24.79	1
06 PM	(04 PM, 10 PM)	95%	24.79	7.46	2

Figure 5.3 d (ii): Hourly **mean** activity level score day 4: – Results of a rank-based change-point analysis for a flock of 34 tagged ewes, which underwent a husbandry routine of: (i) Spending night time in an open kraal next to the reader; (ii) Let out at ~ 0730 hrs and walked a distance of ~ 2.5 km along the road to the furthest paddock for grazing; (iii) Rounded up at ~ 1630 hrs and walked back along the same route; and (iv) Fed concentrates next to the kraal before kraaled again for the night.

For the four days of monitoring, individuals' control charting results based on the hourly mean activity score ranks also picked up the significant change in activity associated with the routine feeding of concentrate pellets in the evening as a spike in data points above the upper control limit. Based on the average hourly activity score count predicted for this particular flock while at pasture for the four days of monitoring, the mean hourly activity score count value and its 95% confidence interval, as calculated using bootstrap method with 10 000 times Monte-Carlo simulations, was 162, 95% CI 132 – 204.

5.4 Discussion

The prototype system demonstrated adequate functionality to remotely and precisely detect the transition in activity level score from resting to running by individually tagged ewes at pasture. Also, the system managed to detect significant changes in activity levels temporally compatible with the documented approximate times of lameness for a single goat that came down with lameness during the trials.

Although lameness scoring (Seaman and Evers, 2006) was not undertaken for this particular lame goat, it is plausible that the severity of lameness experienced by the goat led to the difference in the analysis result profiles between daily activity level score count and daily mean activity level score. It is reasoned that, depending on severity, lameness can either affect just the gait of the animal but not its overall mobility, or both, such that when the lameness is so severe that it affects the overall mobility of the animal it would be better profiled by change-point analysis based on daily activity level score count. Conversely, when lameness affects just the gait of the animal but not necessarily its overall mobility, it will be better profiled by change-point analysis based on daily mean activity score. This is so because it was shown in Chapter 4 that increased overall mobility of tags leads to increased signal strength (Wieselthier et al., 1989), subsequently resulting in increased data transmission rates, hence more influence on the hourly activity level score count than on hourly mean activity level score. On the other hand, gait is expected to have more influence on the values of the transmitted activity level scores, hence more influence on the hourly mean activity level score than on hourly activity level score count.

It seems from the results obtained that the lameness experienced by this goat might have affected its overall mobility more markedly than its gait, hence the limited profiling observed with analysis based on daily mean activity level score, when compared to that observed with analysis based on daily activity level score count. The multi-stepped downward shifts in the daily activity level score count with time may indicate the insidious worsening of the lameness, resulting in gradual reduction in mobility, hence a decrease in the activity level score count. On the other hand, the multi-stepped upwards shifts in the daily activity level score count with time, after the goat had been treated for the condition, to initial levels before the downward shift commenced, can conceivably denote gradual recovery from lameness, with reciprocal increase in both the mobility of the goat and in the activity level score count.

The daily routine of the flock for the four days of close monitoring was well characterised by the change-point analysis results based on hourly activity level score count. This was evidenced by all hourly activity score count data points falling within the shaded background region, which is the region expected to contain all the data points if the current model that two changes occurred, thus fully explaining the variation in the data (Taylor, 2000). The initial shift downward in hourly activity level score count in the mornings at times of let-out to pasture is thought to be due to the signal attenuation effect of distance on tag read rates such that only the tags experiencing certain levels of movement would transmit (Chapter 4). On the other hand, the upward shift in hourly activity level score count seen on change-point analysis results and plotted data points appearing above the UCL on control charting results of individuals in the evenings, to similar levels indicated in the mornings before the flock was let out, could be due to the closeness of the tagged flock to the system reader. Mounting distance of tags from the reader has been shown in Chapter 4 progressively to affect tag read rates, such that, the further the tags are from the reader, the less the read rates. Therefore systematic changes in flock distance from the reader should be taken into account when scanning for deviation from normal activity patterns. The relative activity of different members of the flock should not be affected by this artefact.

Activity level changes associated with concentrate feeding in the evenings were well profiled in all the control charting results but not on change-point analysis results for the four days of monitoring. This could be attributable to the fact that concentrate feeding took place within an hour, hence appearing as a single isolated point, which change point analysis is known to be poor at detecting, unless the event is sustained over time (Taylor, 2000). Since the daily routine of the flock was better profiled by change-point analysis based on hourly activity score count than on hourly mean activity score, it may be that activity score count is a more sensitive measure than mean activity score and this could be plausible because the mean of ten numbers of the same value will be the same as that of 1, 2, and 3 up to infinite numbers of the same value. Therefore, seeking a change point in a serial data of similar values would not detect anything, while basing it on a count of similar values in a serial data set could detect a change point if it exists. Ignoring a single outlier point could have the advantage for disease detection that short transient events do not trigger a false detection in underlying state of the animal or flock.

Using bootstrap method with 10 000 times Monte-Carlo simulations (Efron, 1979; Anderson, 1986), it was possible to estimate the maximum threshold score count to be 204 transmissions per hour. Since this animal activity monitoring system incorporates an alarm as part of its output, it would have been possible to remotely guard this particular flock against predator attacks during the day while at pasture by setting the alarm to go off at the above score count. Predator attack from jackals, which was reported to be common and frequent on the farm, is expected to cause mass heightened movements of the tagged ewes. The increased movements would then lead to increased signal strength from the active tags (Wieselthier et al., 1989), hence increased tag read rates resulting in increased hourly activity score count well above the expected maximum threshold.

Change point analysis has shown in this study to be a more sensitive test when compared to control charting of individuals, as it detected most significant changes otherwise missed on individuals' control charting. In addition, it also better characterised the detected changes, in that it gave additional information like confidence intervals and confidence levels as part of its standard output. However, change point analysis has some downfalls when compared to control charting of

individuals. It cannot detect short-lived or non-sustained events, as seen with short-timed concentrate feeding of ewes in the evenings (although this might carry advantages, see above). Also, since change point analysis uses a bootstrapping approach, it does not produce identical results each time it is performed. Therefore, change point analysis and control charting complement each other and the fact that Change-Point Analyzer© automatically combines the two in its analysis and output does make the software a more robust tool for analysing longitudinal (time series) data for true changes in data distribution. Also, because the software comes with an excel spreadsheet add-in interface, it is better placed for the often required manipulations of the output series data from the prototype activity monitoring system web-based server at individual or flock level after downloading and before data analysis can ensue.

5.5 Conclusion

There were limited natural changes out of the ordinary routine observed during this study and that limited the extent to which it could be evaluated with the activity monitoring system used, for detection of deviations from normal behaviour. However, the flexibility of this monitoring system and analysis thereof to be done at individual animal level or at group animal level, does suggest its potential as an indicator of clinical parasite infection. It is easy to see how this could help in the implementation of **s**IPM strategies like targeted selective treatment (TST) and targeted treatment (TT). This would be most helpful in the implementation of TST and TT against clinical infection by non-haematophagous parasites, since currently there is no validated practical 'crush-side' indicator for that on the market. The system could equally be combined with systems such as FAMACHA©, to trigger animal handling and assessment for anaemia when activity levels become sub-normal.

Although the system is not yet able to specify exactly the event being undertaken or health condition being experienced by the concerned animal or flock as a whole, it is able to alert the stockmen of the need to have an urgent look at the concerned individual animal or flock when the activity level score readings go outside of the set normal range. Hence, currently the system can work as a temporal non-specific early warning system based on serial data, needing a skilled human interface to interpret

the nature and cause of any alerts given by the system. This still has a huge potential to reduce the opportunity cost associated with continuous physical monitoring of animals at pasture, as it allows the stockmen to redirect the time that would have been spent monitoring animals at pasture to other necessary routines on the farm. To this end, the nature of the data relayed by this prototype system is such that over time the data can be subjected to novel analysis techniques like artificial intelligence with an aim to deducing identifiable algorithms denoting composite routines at pasture, like grazing, which is a vital animal production index. Such an achievement would enable the system in the future not only to give alerts to the stockmen of an urgent need to have a look at the concerned individual animal or flock, but also the ability to remotely relay to the stockmen, with established confidence levels, the probable cause of the alert in addition to being able to routinely and remotely keep the stockmen informed of what each individual tagged animal or flock is most probably engaged in at pasture at a given point in time as envisaged by (Helwatkar et al., 2014).

The potential of this system to detect behaviour change in sheep flocks affected by *H. contortus* infection is examined in the next chapter.

CHAPTER 6

Activity level as an indicator of clinical infection of individual goats with *Haemonchus contortus*

6.1 Introduction

Targeted selective treatment (TST) implementation requires the ability to identify for treatment, individual animals within a flock that are unable to cope unaided with current disease or parasite challenge (Kenyon et al., 2009). Currently there is no validated and readily available field-based indicator for clinical infection by non-haematophagous parasites of economic importance in small ruminants. Although the FAMACHA© system (Bath et al., 1996; Malan et al., 2001) has been validated and found to be a sensitive diagnostic tool for clinical infection with *H. contortus*, its labour intensiveness has limited its extensive use, particularly in large farming enterprises, as each individual animal has to be manually evaluated at relatively short intervals, especially during periods of high parasite challenge, to establish its health status before a decision to treat or not to treat can be reached (Van Wyk and Bath, 2002; Van Wyk and Reynecke, 2011).

Longitudinal individual animal activity level monitoring has a theoretical potential to act as a generic risk indicator for clinical infection with both haematophagous and non-haematophagous parasites, as lethargy, a common non-specific clinical sign of disease, can be expected to have an effect on the activity level of a clinical case (Helwatkar et al., 2014). For example, both anaemia and anorexia, which are the respective clinical manifestations of haematophagous and non-haematophagous parasite infection, can lead to lethargy and resultant decrease in movement of affected animals. As a potential 'universal' risk indicator for clinical parasite infection, longitudinal individual animal activity level monitoring has a potential to facilitate the undertaking of risk-based evaluations of animals collectively in a flock. Under risk-based animal evaluations, only the animals that are singled out by the indicator, as possibly suffering from haemonchosis, will be handled for assessment with the chosen system for clinical infection, ideally at the time of presenting. Therefore, risk-based Five Point Check© (Bath and Van Wyk, 2009) evaluation of animals when carried out instantly as and when the need for it is indicated by a sufficiently sensitive and effective, automated indicator, presents the potential

advantage of limiting cumulative production losses and labour cost over time in relation to scheduled whole-flock evaluations at set intervals, due to reduced duration of the pathological or animal management condition involved. Just like random selection of a proportion of the flock to evaluate, risk-based evaluation holds the advantage of evaluation of only a portion of the given flock. However, since risk-based evaluation employs a sufficiently sensitive risk indicator for clinical infection to select the animals to be evaluated, it has an added advantage of being unlikely to miss currently existing cases in the flock, when compared to evaluation of a randomly selected proportion of the flock (Chapter 2).

The objectives of this chapter are to: (i) Determine the dependency of individual animal activity level in goats exposed on pasture to natural *H. contortus* challenge, under the hypothesis that an increase in *H. contortus* infection burden, estimated through FAMACHA© scores, leads to a decrease in individual animal activity level; and (ii) Determine the most appropriate activity score cut-off for clinical haemonchosis. Realisation of the intended objectives from the current study is anticipated to lead to the use of the cut-off point activity level score as a risk indicator for clinical infection with *H. contortus*, such that only those animals registering an average mean activity level score below the cut-off point score will have to undergo an instantaneous Five Point Check© evaluation. Hence the anticipation is to develop a risk-based evaluation with potential to reduce TST labour intensiveness and associated costs.

6.2 Materials and methods

6.2.1 Trial design

The remote activity monitoring system described in Chapter 4 was set up on a goat farm and optimized in relation to the grazing paddocks used and the on-farm obstacles like trees and buildings so as to allow for optimum data transmission from the tagged goats. Initially, 100 individually identifiable female native South African breed of goats, with an age range of 3 – 7 years, were tagged. All of the goats shared the same grazing pasture as part of the same flock and underwent fortnightly Five Point Check© evaluation. At inception of the trials, 20 goats, randomly selected by weight (Sanderson, 2006) from the total of 100 and designated the suppressive treatment group (STG), were subjected to four-weekly blanket drenching with

effective anthelmintics. The remaining 80 were all allocated to the other, targeted selective treatment group (TSTG), and were subjected to drenching, based on individual FAMACHA© score at time of evaluation, with only those goats registering a score ≥3 considered as cases and drenched. Also, at inception of the trials, another 20 out of the 80 goats in the TSTG were similarly randomised and allocated to a monitor group (MG) that was sampled every four weeks for faecal egg count profiling, as an additional way of monitoring that the adopted FAMACHA©-based TST protocol was not compromising animal welfare.

For this study, two cross-sectional FAMACHA© evaluation waves, a fortnight apart, were considered. The two study waves, one on the 23rd March 2013 and the other on the 09th April 2013, respectively, were at late pregnancy and kidding stages, at the height of the *H. contortus* season, the time when the range of the FAMACHA© scores within the flock is expected to be at its maximum. Mean activity level score for the two study waves was calculated for each tagged goat from the transmitted data for the seven days prior to the day of evaluation, up to 0600hrs on the day of evaluation. As per the programmed data transmission rate (every minute), each tag was expected to have transmitted 10080 records during the seven days. An inclusion criteria for each tag was set at a minimum total of 2000 (20%) transmissions in seven days and that the records should not be all zeros. The above criteria were hypothetically deemed to be adequately representative of a functional tag. Additional data from each trial goat was collected on the day of evaluation for the two study waves, being; (i) FAMACHA© scores; (ii) Age in years; (iii) Presence or absence of other diseases (parasitic and non-parasitic) besides haemonchosis; and (iv) Reproductive status (dry, pregnant or lactating). The data were captured using Epidata (version 3.1) software (Lauritsen and Bruus, 2004) and analysed using STATA®/IC 10 statistical software (StataCorp, 2007).

6.2.2 Statistical analysis

A baseline summary table of the tagged trial goats' initial enrolment and the tags that relayed satisfactory data was constructed as per the set inclusion criteria for the considered two study waves. Also, histograms of the mean activity level scores transmitted (outcome of interest) and the assigned FAMACHA© scores (exposure of interest) were constructed to enable graphical appreciation of the data distribution

and its ranges. Average activity level scores for animals in the TSTG and STG were compared, as well as that for cases (≥3 FAMACHA© score) and non-cases (≤2 FAMACHA© score).

A random effects model was used to assess the dependency of mean activity level score on FAMACHA© score. The mixed effects model was chosen so as to enable explicit modelling of the time-invariant a priori confounders like age which became time-invariant in this study because it was entered in years and the two study waves were done in a fortnight time interval within one year. A Hausman test was conducted to ascertain whether the choice of a random effects model over a fixed effects model was statistically justifiable under the null hypothesis that the preferred model is random effects, i.e. that the unique errors (u_i) are not correlated with the predictors (regressors) against the alternative hypothesis that the fixed effects model is the preferred model (Torres-Reyna, 2013). The random effects model was first run without the inclusion of the a priori confounders and subsequently with their inclusion, being age, presence or absence of other field diagnosable diseases and reproductive status of each goat. Lastly a nonparametric receiver operating characteristic (ROC) analysis was performed to determine the most sensitive risk indicator mean activity level score cut-off point for clinical *H. contortus* infection, by using categorical levels of FAMACHA© scores as reference for the true haemonchosis (disease) status of the goats with FAMACHA© score ≥3, assumed to represent truly diseased status. The commands used during the analysis in STATA®/IC 10 statistical software (StataCorp, 2007) are shown in Appendix 4A.

6.3 Results

Almost 42% of the goats initially enrolled in the study were excluded from the subsequent two cross-sectional study waves because their tags did not satisfy the set inclusion criteria, indicating tag malfunctions of some sort. However, the number of goats included in the two study waves considered is similar, most probably owing to the relatively short time interval between the two studies, hence limited dropout between assessments due to off-take or tag malfunctions (Table 6.1).

Table 6.1: Baseline numbers of goats which were enrolled in the study at the start and the numbers included in the two cross-sectional study waves according to the set inclusion criteria.

	Totals	Suppressive treatment (ST) group size	Targeted selective treatment (TST) group size	Monitor group size*
Initial enrolment	112	18	94	23
1st study wave	66	18	48	8
2nd study wave	65	16	49	8

* Animals in the monitor group are part of the TST group.

Mean activity level scores across the two evaluation waves ranged from a minimum of 0.09 to a maximum of 61.95, while the total number of activity level scores transmitted during the seven-day period prior to the day of evaluation, by the tags that satisfied the set inclusion criteria, ranged from a minimum of 2463 to a maximum of 8838 records. Both the mean activity level and FAMACHA© scores across the two study waves were within the expected ranges, with the frequency distribution of registered FAMACHA© score displaying a right skew [Figure 6.1 (a)]. Right skew is also evident in the frequency distribution of mean activity level score [Figure 6.1 (b)]. Scatter plot of mean activity level score against FAMACHA© shows that: (i) Few animals were scored highly at 4 and 5; and (ii) Mean activity level score decreased with increasing FAMACHA© score (Figure 6.2).

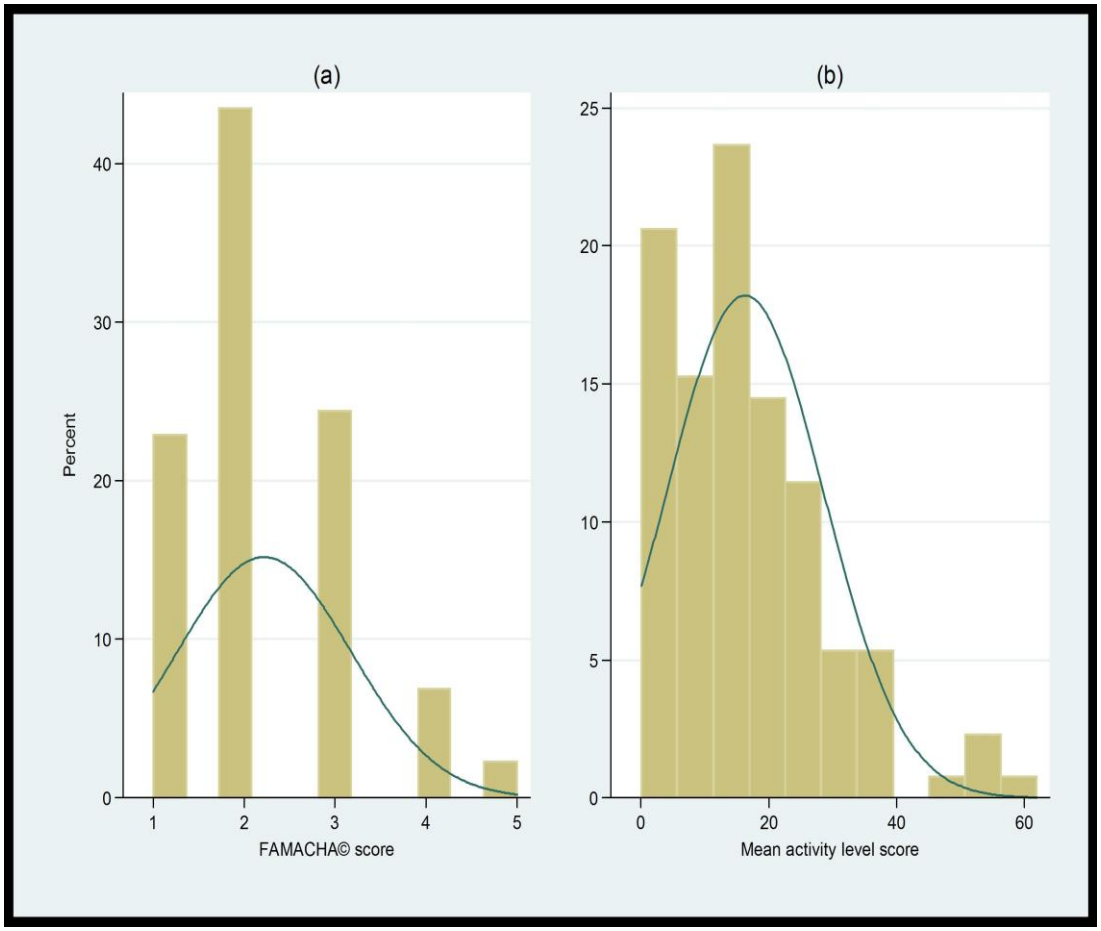


Figure 6.1: Combined histogram plot of FAMACHA© score (a) and mean activity score (b), represented as percent with superimposed normal distribution plots.

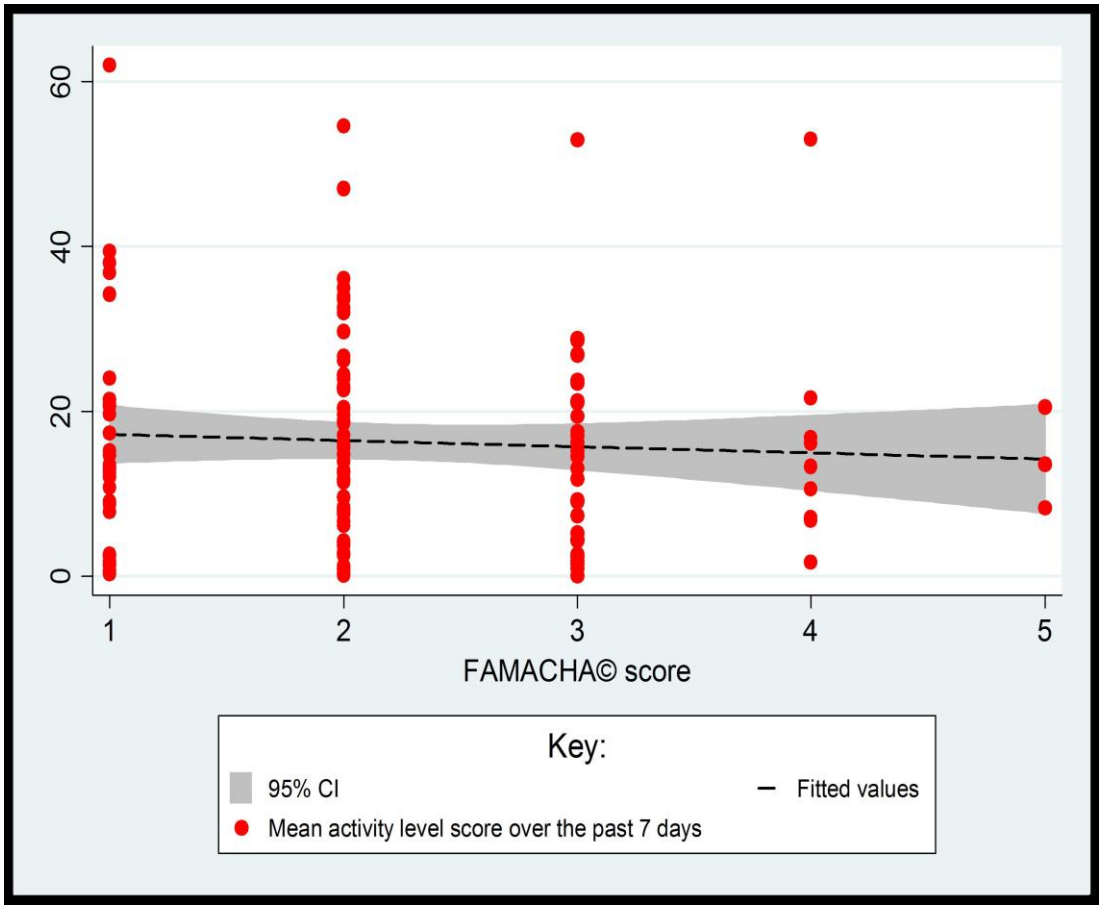


Figure 6.2: Scatter plot of mean activity score and its exploratory linear fit against categorical levels of FAMACHA© score across the two evaluation waves conducted.

The mean of mean activity level scores across the two evaluation waves was not different between STG (17.78, 95% CI 14.93 – 20.63) or TSTG (15.76, 95% CI 13.05 – 18.48) groups. Mean activity score across the two evaluation waves was slightly lower for cases of haemonchosis (≥3 FAMACHA© score) at 15.03, 95% CI (11.40 – 18.67) than for non-cases (≤2 FAMACHA© score) at 16.92 95% CI (14.25 – 19.59), but the difference was similarly not significant, as seen from overlapping 95% confidence intervals.

The Hausman test results showed insufficient evidence against the null hypothesis that the preferred model is random effects vis-à-vis the alternative that the fixed effects is the preferred ($\chi^2 = 3.24$; df = 4; p = 0.52), hence the choice of the random effects model, for this regression analysis is statistically supported.

For the crude analysis of the dependency of mean activity level on natural *H. contortus* infection burden, there was poor prediction of mean activity level by the ordered categorical levels of FAMACHA© scores representing *H. contortus* infection burden. This was seen by the lack of significant difference between the baseline FAMACHA© category (unexposed FAMACHA© score 1) and the exposed ordered FAMACHA© score categories, being: (i) FAMACHA© score 2 (Z = -0.40, p = 0.69); (ii) FAMACHA© score 3 (Z = -0.89, p = 0.37); (iii) FAMACHA© score 4 (Z = 0.49, p = 0.63); and (iv) FAMACHA© score 5 (Z = -0.98, p = 0.33) at $p < 0.05$ significance level. Also, the model with only the ordered categorical levels of FAMACHA© scores included was inadequate as the probability of getting the Wald Chi^2- value obtained or more extreme if there is no association between mean activity level and *H. contortus* infection burden was not significant evidence (Wald $\chi^2 = 1.50$; df = 4; p = 0.83), at $p < 0.05$ significance level, against the null hypothesis that all the coefficients in the model are not different than zero. The crude regression analysis results are summarised in Table 6.2 (1) and Appendix 4, Table 4B (a).

For the model including a priori confounders there was also poor prediction of mean activity level by FAMACHA© scores as evidenced by the lack of significant difference between the baseline FAMACHA© category (unexposed FAMACHA©

score 1) and the exposed ordered FAMACHA© score categories, being: (i) FAMACHA© score 2 (Z = -0.65, p = 0.52); (ii) FAMACHA© score 3 (Z= -0.85, p = 0.39); (iii) FAMACHA© score 4 (Z = -0.51, p = 0.61); and (iv) FAMACHA© score 5 (Z = -0.45, p = 0.65). Despite there being a lack of significant statistical difference between the mean activity level score for the baseline FAMACHA© score category and the exposed ordered categorical levels of FAMACHA© scores at $p < 0.05$ significance level, an evident decrease in mean activity level score with increase, i.e. change from lower to higher FAMACHA© scores, in natural *H. contortus* infection burden was discernible, as hypothesised. The two-tailed p-value for FAMACHA© score represents the probability of getting the coefficient obtained, or more extreme, if there is no true difference in the mean activity level score of a particular exposed FAMACHA© category score (2, 3, 4 or 5) and the baseline level FAMACHA© category score 1. It tests the homogeneity (Ho) hypothesis that the coefficients across the FAMACHA© scores are equal and the heterogeneity (Ha) hypothesis that the coefficients across the FAMACHA© scores are not equal.

Presence of diseases other than haemonchosis diagnosed on the trial goats during the two evaluation, as well as age of the individual goats were not associated with the registered mean activity level scores across the same study waves (respectively Z = 0.75, p = 0.46; and Z = 0.63, p = 0.53). The two-tailed p-value for diagnosed diseases other than haemonchosis represents the probability of getting the coefficient obtained, or more extreme, if there is no true difference in the mean activity level score of goats that were positive for diseases, other than haemonchosis, from that of goats that were negative for diseases other than haemonchosis. For age, the two-tailed p-value represents the probability of getting the coefficient obtained, or more extreme, if there is no true difference between the coefficient obtained and zero under the null hypothesis that the coefficient is not truly different from zero and the alternative hypothesis that the coefficient is truly different from zero. Only reproductive status of the goats was significantly correlated with the registered mean activity levels across the two evaluation waves with a transition from pregnant to lactation status leading to a seven unit increase in mean activity level (Z = 4.84, $p < 0.001$). For reproductive status, the two-tailed p-value represents the probability of getting the coefficient obtained, or more extreme, if there is no true difference in the mean activity level score of pregnant goats from that of lactating goats.

The model including the predictor of interest, natural *H. contortus* infection burden as indicated by the ordered categorical FAMACHA© scores, and all the considered a priori confounders, being: (i) Other diseases other than haemonchosis; (ii) Age in years; and (iii) Reproductive status, was highly significant (Wald $\chi^2 = 27.13$; df = 7; $p < 0.001$) under the null hypothesis that none of the coefficients in the model are different from zero. This indicates that the included predictors in the model collectively accounted for a statistically significant proportion of the variance in the mean activity level score. The a priori confounder adjusted regression analysis results are summarized in Table 6.2 (2) and Appendix 4, Table 4B (b).

The area under the Receiver Operating Characteristic (ROC) curve, using FAMACHA© scores as reference for true disease status with FAMACHA© score ≥3 assumed to represent truly diseased status, was predicted to be 0.458, 95% CI 0.354 – 0.563 (Figure 6.3), which is a poor fit for any purported indicator of clinical infection as it includes 0.5 in its 95% confidence interval.

Table 6.2: Regression analysis summary table for the dependency of mean activity level score (MAL) on *H. contortus* natural infection burden, determined by FAMACHA© score (famascore), of 67 goats (animalid), with FAMACHA© score 1 taken as the baseline category (unexposed). Results of the crude analysis (1) and the a priori confounder adjusted analysis (2) results are shown. The a priori confounders considered are: (i) age; (ii) other diseases other than haemonchosis (otherdx); and (iii) reproduction class (reprostat).

Variables	(1) MAL excluding a priori confounders	(2) MAL including a priori confounders
_Ifamascore_2	-1.11	-1.62
	(2.739)	(2.508)
_Ifamascore_3	-2.70	-2.40
	(3.022)	(2.814)
_Ifamascore_4	-2.17	-2.04
	(4.455)	(4.026)
_Ifamascore_5	-6.91	-2.93
	(7.072)	(6.507)
_Iotherdx_2		4.02
		(5.386)
Age		0.80
		(1.278)
_Ireprostat_3		7.68***
		(1.587)
Constant	17.70***	6.56
	(2.347)	(8.649)
R-sq	0.005	0.105
Observations	131	131
Number of animalid	67	67

Standard errors in parentheses
*** p<0.01, ** p<0.05, * p<0.1

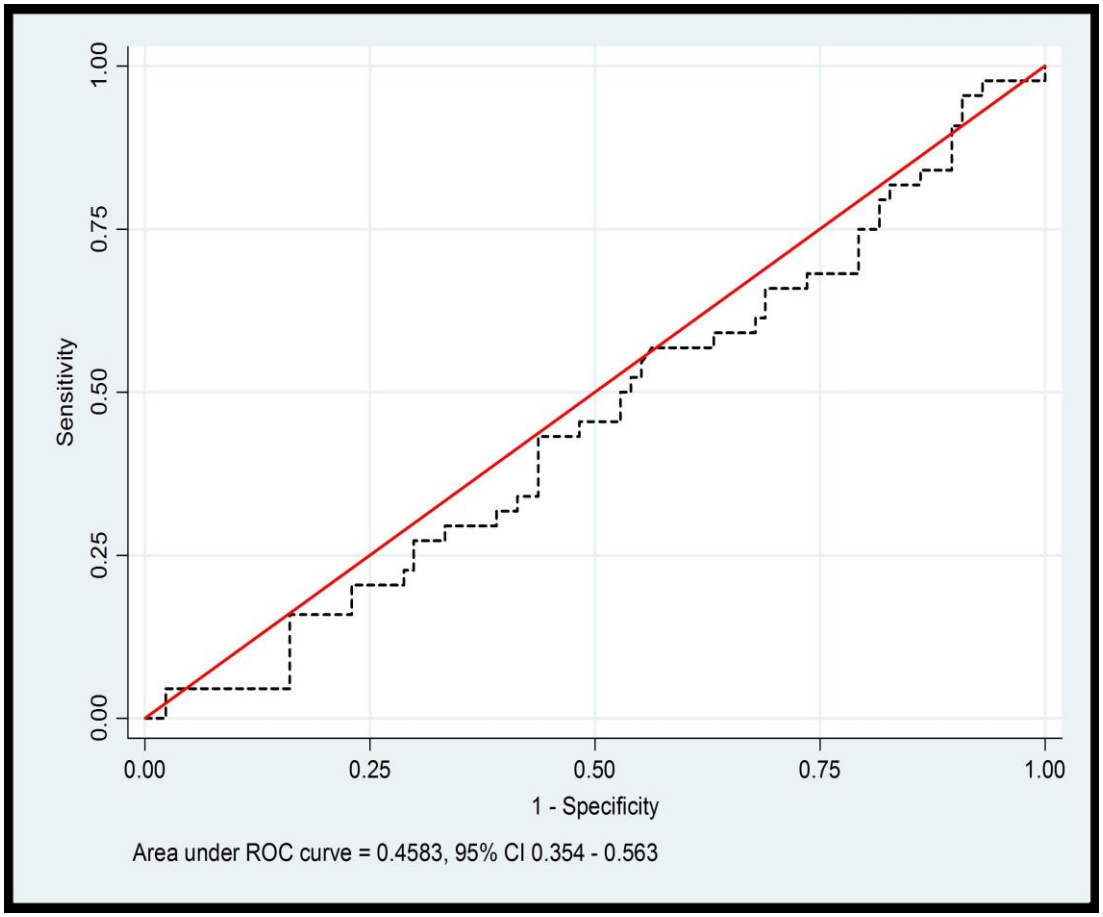


Figure 6.3: Receiver operating characteristic (ROC) curve for mean activity level score by using categorical levels of FAMACHA© scores as reference for the true haemonchosis (disease) status of the goats, with FAMACHA© score ≥3 assumed to represent truly diseased status.

6.4 Discussion

The observed skew to the right in both FAMACHA© scores and activity level scores may indicate the effect of parasite overdispersion (Barger, 1985; Sreter et al., 1994; Hoste et al., 2001), resulting in haemonchosis only on the few animals harbouring the majority of worms in the flock and those few cases having a correspondingly lower activity level scores compared to non-cases. Lower mean activity level recorded for animals in the STG, though not significant when compared to that for animals in the TST group, may indicate the effect of parasites in refugia (Van Wyk, 2001), slowing the activity levels of infected animals as a result of tiring from lower energy levels (lethargy). Also, this could explain the lower mean activity levels recorded for cases when compared to that of non-cases as well as the observed lowering of activity level with increase in infection burden, although neither the difference nor the gradient were statistically significant. This finding appears consistent with previous findings from short-term field studies by Leathwick et al. (2006), Van Wyk (2008), Gaba et al. (2010), and Besier (2012), in which an increase in nematode burden as a result of maintaining a reservoir of susceptible nematodes in refugia, through TST application, showed limited negative effects on production indices which in this study are indicated indirectly by lowered activity levels.

The area under the ROC curve measures quantitatively the rapidity with which the curve of mean activity level rises to the upper left corner of the plot and it included 0.5 in its 95% confidence intervals. For a test with 100% sensitivity and specificity, i.e. a perfect test, the area under the curve is 1. On the other hand, if the area under the curve includes 0.5, then the test has the same discriminatory power as that obtained by fairly tossing a fair coin, i.e. binomial probability, to determine the infection status of an animal. Useful diagnostic tests have an area between these two extremes (Greiner et al., 2000; Reynecke et al., 2011a) hence activity level score measured using the current generation of tags is concluded to be a non-discriminatory risk indicator for clinical infection with *H. contortus*.

Differences in activity levels between TSTG and STG, between cases and non-cases as well as the negative gradient between activity level and infection burden were realised as hypothesised in this study. However, the observed differences and

gradient were not statistically significant, making activity level a non-discriminatory risk indicator for clinical infection with *H. contortus*, hence currently unable to facilitate a risk-based flock evaluation as envisaged. Field trial differences in selected production indices between TSTG and STG that have no statistical significance but are judged to be of biological importance to sustainable control of helminths, particularly *H. contortus*, have been reported and discussed before by Van Wyk (2008).

In the current study, absolute effect of size (Sullivan and Feinn, 2012) seems to be the main reason for the observed lack of significant difference and the calculated absolute effect size may have been affected by: **(i) Well implemented TST on this particular farm**, as evidenced by few cases in the upper FAMACHA© score categories four and five, where impact of infection burden on activity level is expected to be more pronounced; **(ii) Significant performance difference between the tags** as established in Chapter 4, which depending on motion (stationary or swinging) and disposition (spread-out or clustered) statuses, accounted for 18 to 37% of the measured difference in activity levels between animals. This difference in tag performance was most probably due to unstandardized procedures along the production assembly line. However, as neither the standard model tags of the manufacturer, nor of others that were available at the time, catered for the pre-determined needs for the present series of investigations, the existing tags were specifically modified for use in the investigations, and this made them more prone to being damaged by physical shock during daily activity of the goats. Since the tags were randomly allocated to the goats during the trial to measure their activity level, an outcome of interest, random measurement error (Hutcheon et al., 2010) occurred. Random measurement error of activity level scores as an outcome of interest increased the standard errors which resulted in lack of statistical significance. Had activity level been the exposure of interest, measurement error would have led to regression dilution bias, i.e. would have biased the regression coefficient (slope) towards the null (Hutcheon et al., 2010); **(iii) Residual confounding** (Flanders et al., 2011) through a multiplicity of potential confounding factors that could influence activity level of freely grazing goats at pasture. Goats were tracked for yarding in the evenings and out to pasture in the mornings. Level of activity during these periods is largely involuntary and dependent on the demeanour of the goat minders, husbandry

practices and social hierarchy amongst the flock. If tracking to and from pasture or yarding was differentially applied to individual tagged goats, it would also have a differential effect on their activity levels. For example, confinement or slow tracking of goats with kids at foot would have a differential effect between activity levels of goats with and those without kids at foot, just as bullying of young or weak animals by dominant ones during yarding would. In this study involuntary movement was not captured for lack of means to do it and its probable differential effect between goats was not adjusted for in the final analysis such that only the effect of infection burden on voluntary animal movement could be considered; and **(iv) Sample sizes of the compared groups**. The number of animals in the TSTG and STG, just as the number in the cases and non-cases groups, were not statistically determined, based on any estimated absolute effect size of *H. contortus* on activity level between the groups. For TSTG and STG, the number of animals in each group was decided based on available animals on the farm, while for cases and non-cases, the numbers in each group were left to chance of clinical infection with *H. contortus*. Therefore, the number of animals in the compared groups could have been too small, resulting in a low-powered study with a low probability of finding a statistically significant difference in activity levels between the compared groups even if an actual difference did exist (Kirkwood and Sterne, 2003; Sullivan and Feinn, 2012).

6.5 Conclusion

Even though the realised difference in activity level between TSTG and STG, between cases (≥3 FAMACHA© score) and non-cases (≤2 FAMACHA© score) was not significant, it was nonetheless evident and most importantly in the direction predicted. Also, there was a decrease in activity level with increase in FAMACHA© score (*H. contortus* infection burden) gradient, even though it was not significant at $p \leq 0.05$ level of significance. The lack of significant p-value could be attributed to the absolute effect of size, which seems to have been affected by: (i) Limited extent of outbreak, (ii) Varying tag performance; (iii) Residual confounding, especially involuntary animal activity; and (iv) Sample sizes of the compared groups. It seems there are promising leads to using automated animal activity level monitoring as an indicator for clinical parasite infection, just as it has been shown to be a reasonable indicator in other animal diseases and behaviours by Helwatkar et al. (2014).

Further exploration of the observed promising leads with respect to automated animal health monitoring in the field of parasitology is recommended and may require certain aspects of the current research trials to be changed or improved in order to better the odds of success, as follows: (i) Better standardisation of tags to limit random measurement error during activity level measurements, so that what is captured is mainly variation in animal activity, but not tag performance differences, in relation to infection burden; and (ii) Conduct a randomised controlled infection trial (Sibbald and Roland, 1998; Kirkwood and Sterne, 2003; Stolberg et al., 2004) by infecting a randomly selected and controlled cohort of animals and monitor activity level in both groups, instead of an observational study (Carlson and Morrison, 2009), which in the present study did not guarantee enough clinical cases. In this way, the effect of haemonchosis on activity level could be tested directly. Also, a randomised controlled study would ensure that: (a) Confounding through involuntary animal activities is limited; and (b) Sample size of the groups to be compared is based on estimated effect size, resulting in enough powered trials being conducted. This seems particularly important for parasite infection in general, as animal resilience and resistance can be expected to lower the effect of size of infection on activity level. Therefore, it is critical to have a representative sample in order to access the 'true' statistical significance of any effect size obtained.

Because of limited funding and time constraints it was not possible to repeat the trial during the current study, so as to incorporate the above suggested shortfalls. However, future trials in automated animal health monitoring (Helwatkar et al., 2014) should consider incorporating the above in order to improve the odds of finding a significant association between activity level and natural *H. contortus* infection burden, if it exists. That would subsequently allow for the determination of the most sensitive mean activity level score cut-off point to be associated with clinical infection with *H. contortus*, whereupon the determined score can be mapped to the tags and used as a risk indicator (early warning system) for haemonchosis. Granted success with the above, only animals with mean activity level scores below the established risk indicator activity level score cut-off point will need to be evaluated as and when that need arises, hence risk-based evaluation. And, if achievable, it is anticipated that it would drastically cut on the labour intensiveness and associated costs with TST application.

CHAPTER 7

General Discussion

In this thesis, novel on-farm decision support tools involving case-based SEIDPRS modelling and activity level monitoring were pursued. Positive outcomes in line with the intended aims of this work were obtained despite some significant unavoidable challenges. Firstly, the secondary datasets (Carlson and Morrison, 2009) used here for model fitting to estimate parameter values, were not well suited for this purpose. Such mis-matches are often the case with secondary datasets which were collected for purposes different from current use (Ancker et al., 2011). Secondly, the limited technical information on the monitoring system telemeters (for reasons of confidentiality) presented a challenge when trying to understand and optimise their performance.

7.1 Modelling findings

The case-based SEIDPRS model predicted the observed cases very well, as would have been expected to some extent as the parameterization of the baseline model was largely based on model fitting to the very same data (Chapter 2). The near-exact prediction of the observed cases on this particular farm granted acceptable credibility to the fitted parameter values. Through model sensitivity analysis, it was deduced from the farm data that: (i) For optimal production, FAMACHA© evaluations needed to be conducted at short intervals of approximately 7 days as opposed to the current recommendation of 14 days (Van Wyk and Bath, 2002); and practically all the animals had to be evaluated at each evaluation event to evade significant production losses at the end of the *H. contortus* season; (ii) Prolonged application of FAMACHA©-based TST strategy led to overall reduction in *H. contortus* cases with time, and stabilisation of case numbers towards the end of the *H. contortus* season, as a result of treatment of the high FAMACHA©-score, high egg shedding individuals. Also, gradual immune development amongst ewes in the flock could have supported this stabilisation, as TST might have encouraged the development of immunity (Van Wyk et al., 2006; McClure, 2012), with the consequence of fewer cases towards the end of the *H. contortus* season This limited the predictive power of the model, since the number of cases became few in spite of conditions that were favourable in principle for transmission; and (iii) Under FAMACHA©-based TST

strategy application, cases did not appear to play a significant role in the epidemiology of the parasites. This could be because pasture contamination levels late in the grazing season (e.g. density of accumulated L3) become progressively less affected by the number of eggs shed onto pasture from later, high-FAMACHA© cases, for instance, by lengthened periods of development between egg deposition on pasture and larval development to the infective L3 stage, on the one hand, and, on the other, by the full development in the host being delayed, owing to the phenomenon of hypobiosis. Hypobiosis of *H. contortus* larvae in the abomasum of small ruminants in semi-arid areas has been documented before as a survival mechanism particularly during the cold and dry months (Bonfoh et al., 1995; Gatongi et al., 1998).

The significant association shown between the predicted force of infection (FOI), i.e. the number of *H. contortus* infections acquired at each FAMACHA© evaluation event by susceptible ewes, and the independently obtained weather element values at each evaluation event for the period of investigation (Chapter 3), as expected (O'Connor et al., 2006), adds structural validity and measurement reliability to the SEIDPRS model as developed and used to predict *H. contortus* cases observed on this particular farm. Although the SEIDPRS model seemed to predict the total cases well, the model fitting to the observed data at each evaluation event was not exact and this is probably to be expected in a system in which increased challenge is met by increased treatment through TST, as well as movement of animals to less infective pastures, as these have the potential to mute the disease signal on which the model relies.

Potential limitations of the model include that several simplifying assumptions were incorporated, something which is not rare to modelling real-life events (Vynnycky and Edmunds, 2008), as modelling is not an exact science. For example, the effective contact rate (beta), i.e. rate at which contact between an infectious and a susceptible ewe through pasture in the same reproductive status group would result in transmission of infection, was assumed to be constant throughout the period of investigation. This is probably unrealistic, as beta is dependent on host and pathogen behaviour (Vynnycky and White, 2010). The behaviour of the ewe hosts in this study would have been influenced by the daily on-farm husbandry management routines,

while parasite behaviour was influenced by weather elements (temperature and rainfall) as established in Chapter 3. Since husbandry management practices and weather elements varied throughout the period of investigation, it is to be expected that beta would also have varied accordingly throughout. However, since force of infection (FOI) is a function of both beta and infectious proportion of ewes in each group at each FAMACHA© evaluation event, the lack of variability in beta was absorbed by the variability in the estimated infectious proportion at each evaluation event during model fitting to the observed data. This enabled adequate fitting of the model to the data, hence the satisfactory overall prediction of the observed cases by the model. But it also means that the infectious proportion in all three of the reproductive status groups was either overestimated or underestimated at each evaluation event to accommodate the constant beta value.

Since FOI is expected to vary even between farms in the same locality, it means the data-driven model is likely to have poor external validity (Carlson and Morrison, 2009). That is to say that the study findings might not hold for other farms in other places and at other times. However, the model structure, as written, appears to hold well as it responded well to sensitivity analysis with rational output, indicating robust internal validity (Rothman et al., 2008; Carlson and Morrison, 2009). Therefore, field application of the model study results is recommended as follows: (i) Harmonisation of broader FAMACHA©-based TST husbandry management practices across farms using the husbandry practices that obtained on the study farm as a reference, since they were reasonably applied to recommended standards as previously validated on the same farm by Reynecke et al. (2011c). This will ensure applicability of the developed model structure to the harmonised farms, as development of the model structure was based on both on-farm husbandry management practices and epidemiology of *H. contortus*; (ii) Then, for farms with similar husbandry management routines and in a locality that experiences similar rainfall and temperature as the study farm, parameter values obtained by fitting the model to the study farm data may be adopted across all those farms to run the model and use the model output for decision support on sustainable control of *H. contortus* infection. This is so because external validity would have been improved by having similar husbandry management practices and climatic conditions across all those farms; and (iii) For farms with similar husbandry management practices as the study

farm, but experiencing different climatic conditions, it is recommended that before using the model output for decision support for sustainable control of *H. contortus* infection, a 'burning-in' period should be observed, during which case data are collected alongside relevant climate and management factors, so that the model parameters and relationships are built up for those particular farm. Whether farmers would be willing to provide a season's worth of data to calibrate a model before gaining decision support benefits from it is open to question. Nevertheless, this latter strategy would seem to be more realistic than attempting to reduce inevitable variation in husbandry practices and TST application in the field.

7.2 Activity level monitoring findings

The prototype activity level monitoring system consisting of the current generation of telemeter components, as described in Chapter 4, showed adequate ability to transmit analytically identifiable and distinguishable serial activity level scores at points of transition from one activity event or health condition to another. In Chapter 5, a distinguishable algorithm of serial activity level scores denoting periods of resting and running were obtained on change point analysis with a clearly defined point of transition from resting to running. Change point analysis of serial (longitudinal) activity level score data of a goat before onset of lameness, during lameness and after recovery from lameness showed a downward transition trend in its activity score count with onset of lameness and an upward transition trend in activity level score count with gradual recovery from lameness after treatment. Also, the system was able accurately to distinguish major events characterising the daily husbandry management routine of a flock of ewes. However, the extent of natural events observed at pasture during the period of study were too limited to adequately test the system across the range of animal activity events expected at pasture. Immediate application of the longitudinal activity level monitoring findings is feasible as the activity monitoring system incorporates an alarm which can be set such that when activity level score appears above the established upper range for any of the observed natural events at pasture during this work, a generic (non-specific) alert can be sent to the stockmen indicating an opportune need to attend to the tagged individual animal or flock. Pending successful mapping of activity level score ranges associated with each FAMACHA© score to the tags, they could conceivably be used

longitudinally to indirectly indicate transition between FAMACHA© scores over the course of a whole *H. contortus* season. If successful, this would be a remarkable milestone towards automated animal health monitoring (Helwatkar et al., 2014) with respect to *H. contortus* control, as it signifies a potential to relieve the labour intensiveness constraint associated with FAMACHA©-based TST application (Van Wyk et al., 2006), and be expected to lead to a highly probable positive spinoff of increasing global adoption and sustenance of TT and TST strategies in a **s**IPM framework.

Although the above promising leads were established, there were also identified shortcomings of the prototype activity monitoring system during the study, both at hardware and software levels. At hardware level, foremost was the significant performance difference between the tags, possibly caused by rapid, special adaptation to the needs of the investigation or poor quality control along the assembly line. Approximately 41% of the tags experienced malfunctions, mainly as a result of untimely breakdowns of the tag casing and loss of the enclosed components (Chapter 4 and 6). Performance difference between the tags made them a poor measurement tool to use for data capture in cross-sectional studies, where attributable risks across animals had to be measured simultaneously at a point in time, because of random measurement error effects (Chapter 6).

Although the prototype system generated near real-time streaming data, i.e. almost continuous at intervals of a minute, at software level it was realised that there was no existing program to conduct streaming data analysis (Gaber et al., 2005) to detect insidious conditions at pasture and give immediate actionable early warning alert to the stockmen. In this study the data had to be first stored in a web-based server, downloaded manually at set intervals and analysed historically for significant change points. Therefore, delay in diagnosis and mitigation against any insidious changes with potential loss of production is implicit in the current approach to analysing the transmitted activity level data. Hence this aspect merits special attention in future investigation of the potential of automated detection of changes in animal health conditions with the use of a low-cost, relatively unsophisticated system, as used in the present investigations, as this is a sine qua non for use under resource-poor conditions of developing farming.

7.3 Value added by the study to TST and TT approaches

In this study a near-holistic quantitative analysis of the FAMACHA©-based TST strategy as currently being recommended for application in South Africa by Van Wyk and Bath (2002) was achieved. Although such analysis has been attempted before by others (Kaplan et al., 2004; Reynecke et al., 2011c), this was the first study, through use of an SIR-type modelling tool, to simultaneously evaluate decision support on husbandry management practices and climatic conditions, as critical input variables in the application of FAMACHA©-based TST. From the analysis, critical decision outputs with potential to lead to a more efficient way of applying the FAMACHA©-based TST strategy were identified as being: (i) The interval between FAMACHA© evaluation events; (ii) Competency in conducting FAMACHA© evaluations; and (iii) Proportion of the flock to evaluate at each evaluation event to avoid significant production losses over the course of a whole *H. contortus* season. It was also deduced from the critical analysis of the FAMACHA©-based TST strategy, as currently being recommended for application in South Africa, that almost all the animals needed to be evaluated at each evaluation interval on this particular farm to avoid significant losses at the end of the *H. contortus* season. At least for this farm, the above deductions support the conclusion by Van Wyk and Reynecke (2011) that the current way in which the FAMACHA©–based TST is being applied has limited flexibility to reduce the associated labour intensiveness. Labour intensiveness reduction under TST application has been identified as the critical step to achieve towards encouraging extensive TST adoption and sustenance across countries and farms within countries (Van Wyk et al., 2002; Van Wyk et al., 2006). Reliance of the model fitting on detailed case data means that the conclusions reached might differ for other farms under different husbandry management practices or experiencing different rainfall and temperature compared to the study farm. As a consequence, there was low external validity of the study (Rothman et al., 2008; Carlson and Morrison, 2009) to yield broadly generalisable recommendations for FAMACHA©-based TST application.

Remote animal health monitoring through activity level in this study has shown promising leads towards alleviation of the labour intensiveness constraint associated with TST strategy of controlling parasite infection, in particular helminths. The

verified ability of the prototype activity monitoring system to offer dependable, remote and near real-time longitudinal individual animal monitoring as well as whole-flock monitoring, through representative tagging of animals within a flock, offers a great deal of potential reduction in labour costs when compared to continuous monitoring of animals at pasture to guard against predators or other loss. As projected by Van Wyk et al. (2006) and Van Wyk and Reynecke (2011), and now supported by the present investigations, risk-based flock or individual animal evaluations, with activity level score acting as a 'universal' risk indicator for the 'need-to-be-evaluated' or as a generic remote diagnostic tool, can add value through improved efficiency and efficacy of targeted management practices (TST and TT) with a drastically reduced labour requirement. This has potential for applicability well beyond worm infections, to include other debilitating diseases and general husbandry management routines.

Longitudinal activity monitoring indicated potential for the prototype system to detect onset of and recovery from lameness in goats in this study (Chapter 5), and was suggested or shown by others elsewhere through field trials as the most hopeful indicator to remote data capture for the detection of a host of animal diseases and behaviours that include: (i) Parasite-induced inappetence by remotely monitoring jaw movement (Forbes et al., 2007); (ii) Oestrus in dairy cows (Xu et al., 1998; Firk et al., 2002; López-Gatiusa et al., 2005; Løvendahl and Chagunda, 2010; Valenza et al., 2012; Helwatkar et al., 2014); and (iii) Lameness, mastitis, fever, displaced abomasum, ketosis, milk fever, retained placenta, clinical acidosis and heifer pneumonia (Mottram et al., 2008; Hogeveen et al., 2010; Helwatkar et al., 2014). Notwithstanding the fact that only one animal was monitored for lameness in this study, effective detection by the prototype system in this study as well as by others (Helwatkar et al., 2014) is promising and presents a lead for possible use in passive surveillance for globally important economic diseases, such as foot-and-mouth disease (Knight-Jones and Rushton, 2013). This is so, particularly in sub-Saharan Africa, where extensive rearing systems are still in practice, with their inherent problem of limiting contact time between owners and their livestock.

7.4 Future needs, potentials and prospects from this study

A validated case-based SEIDPRS model of this nature can be used on-farm in the future in the form of a 'black box', with proportion of cases predicted acting as a guideline upon which actionable response feedback is based. Minimal and readily available information like: (i) Total rainfall; (ii) Average temperature; (iii) Size of flock in each reproductive class; (iv) Number of cases diagnosed in the previous immediate evaluation event in each group; and (v) Time in days since the previous evaluation event, will have to be entered at the user interface for the model to predict future cases (risk). The actionable outputs, which will be dependent on where the predicted proportion of future cases falls on a predetermined scale of case proportion thresholds, may include: (i) Continuing with TST treatment at the same or different interval; (ii) Increasing the range of FAMACHA© scores to treat; and/or (iii) Switching to a different treatment drug (Van Wyk and Bath, 2002).

Telemeter robustness, particularly the tags, and tag standardisation, are essential to ensure performance compatibility between tags. This would enable the activity level monitoring system not only to be useful for longitudinal data capture where each tag acts as its own control, but also to be useful for comparative study data capture involving a number of tagged animals. The promising leads shown through longitudinal animal health monitoring in this study makes it possible to map activity level score ranges correlated to each FAMACHA© score to the tags, then use those scores to monitor individual animals serially over a *H. contortus* season. However, it would be mandatory to have activity level score thresholds correlated to each FAMACHA© score for each tag in order to gain useful serial animal health monitoring for infection with *H. contortus*. However, unless computer hardware and software systems can be developed (as discussed below) this could be tedious to apply at field level, particularly for large flocks where large numbers of tags have to be deployed. Hence, for practical field application of activity level score as a risk indicator for any condition of interest, there is a need to first have all tags sufficiently standardized to enable setting of activity level score thresholds applicable to all tags for any condition of interest, such that any variability in the measured activity level can be attributed to the subjects under remote monitoring but not to the tags themselves.

There is also a need for future developments to focus on developing compatible data stream mining software, to perpetually analyse the data for specific pattern recognition and give actionable feedback without delay and need for data storage capacity. The near real-time streaming of data from the system makes it conducive for novel analysis techniques like pattern recognition and machine learning (Bishop, 2006). Future integration of artificial intelligence (machine learning) capability into the streaming data analysis software may be essential to enable timely diagnosis of risk patterns associated with particular behaviours or diseases. This can lead to establishment of near-perfect diagnostic algorithms for specific animal activities like grazing and some health conditions like lameness, such that the system can be enabled not only to be capable of non-specific alerts, but also for offering remote diagnostic capabilities for various health conditions most likely to be experienced by animals at pasture.

7.5 Potential for adoption of techniques pursued in this study

In today's world where agricultural production is confronted with increased reduction in profit margins in the face of strict enforcement of adherence to environmental, social, welfare and safety regulations, farmers have no option but to reduce production costs and yet optimise on their physical output (Sorensen et al., 2011). Therefore, there are limited choices the world over but to embrace information and communication technology (ICT) and electronic decision support systems which have already shown great promise for achieving set agricultural production goals, with compliance to obtaining rules and regulations (Godwin et al., 2003). These decision support systems are largely interchangeable between livestock and cereal production, albeit with modification. With positive attitudes towards decision support systems already having been shown both in cereal and animal production enterprises (Lawson et al., 2011; Garforth et al., 2013), there is hope that the current work in progress on activity level monitoring and modelling is likely to be adopted by livestock farmers, when the limitations discussed are overcome.

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APPENDIX 1

Appendix 1A: Baseline model technical details

The transmission dynamics of *H. contortus* is described using a population-based deterministic model of differential equations in dry [Figure 1A. (i)], pregnant [Figure 1A. (ii)] and lactating [Figure 1A. (iii)] ewe groups denoted by *dry* = dry, *preg* = pregnant and *lac* = lactation respectively. The explanations of the parameters and variables in this reproductive status group-structured model are provided in Tables 1A. (i) and 1A. (ii). Number of pre-infectious, infectious at the start, $t = 0$ in animal group j where j denotes dry, pregnant and lactating groups were estimated by fitting model predictions of the weekly incidence cases to the observed incidence cases data per 100 ewes per week during the estimation of unknown parameter values. All other values were estimated from below initial conditions' formulas:

Sus_ j0 = pop_ j - Preinfous_ j0 - Infous_ j0 - Reported_ j0 - Immune_ j0

*Immune_ j0 = Infous_ j0*frac_infous_non_clin_ j*

*Reported_ j0 = Infous_ j0*frac_rep_ j*

*Cum_reported_ j0 = Infous_ j0*frac_rep_ j*

Rec_ j0 = 0

The number of new cases in animal group j at time t is given by:

*infectious_rate*Preinfous_ j*frac_inftd_clin_ j*

The number of newly reported cases in animal group j at time t is given by:

*diag_rate_ j*Infous_ j*frac_rep_ j*

The number of cases in animal group j diagnosed by FAMACHA© and reported as cases at the end of the period of investigation (at time T) is given by:

Cum_reported_ j - Cum_ reported_ j0

The force of infection expressions in the 3 groups of animals is as follows for dry, pregnant and lactating ewes respectively:

force_of_infn_dry = β_dry_dry*Infous_dry + β_dry_preg*Infous_preg + β_dry_lac*Infous_lac

force_of_infn_preg = β_preg_dry*Infous_dry + β_preg_preg*Infous_preg + β_preg_lac*Infous_lac

force_of_infn_lac = β _lac_dry*Infous_dry + β_lac_preg*Infous_preg + β_lac_lac*Infous_lac

Figure 1A (i): Dry group infection transmission differential equations

d/dt (Sus_dry) = sus_rate1_dry*Rec_dry + sus_rate2_dry*Immune_dry + dry_rate*Sus_lac - preg_rate*Sus_dry - removal_rate*Sus_dry - force_of_infn_dry*Sus_dry*infected_prop

d/dt (Preinfous_dry) = force_of_infn_dry*Sus_dry*infected_prop + dry_rate*Inftd_lac - preg_rate*Inftd_dry – infective_rate*Inftd_dry - removal_rate*Inftd_dry

d/dt (Infous_dry) = infective_rate*Inftd_dry + dry_rate*Infous_lac – preg_rate*Infous_dry - immunity_rate_dry*Infous_dry*frac_inftd_non_clin_dry - diag_rate_dry*Infous_dry*frac_rep_dry - removal_rate*Infous_dry

d/dt (Immune_dry) = immunity_rate_dry*Infous_dry*frac_inftd_non_clin_dry + dry_rate*Immune_lac - preg_rate*Immune_dry – sus_rate2_dry*Immune_dry - removal_rate*Immune_dry

d/dt (Reported_dry) = diag_rate_dry*Infous_dry*frac_rep_dry + dry_rate*Reported_lac - preg_rate*Reported_dry - removal_rate*Reported_dry - recovery_rate_dry*Reported_dry*prop_dosed_dry*dose_eff

d/dt (Rec_dry) = recovery_rate_dry*Reported_dry*prop_dosed_dry*dose_eff + dry_rate*Rec_lac - preg_rate*Rec_dry - sus_rate1_dry*Rec_dry - removal_rate*Rec_dry

d/dt (Cum_reported_dry) = diag_rate_dry*Infous_dry*frac_rep_dry

Figure 1A (ii): Pregnant group infection transmission differential equations

d/dt (Sus_preg) = sus_rate1_preg*Rec_preg + sus_rate2_preg*Immune_preg + preg_rate*Sus_dry + pop_all*replacement_rate - lac_rate*Sus_preg - force_of_infn_preg*Sus_preg*infected_prop - removal_rate*Sus_preg

d/dt (Preinfous_preg) = force_of_infn_preg*Sus_preg*infected_prop + preg_rate*Inftd_dry - lac_rate*Inftd_preg - infective_rate*Inftd_preg - removal_rate*Inftd_preg

d/dt (Infous_preg) = infective_rate*Inftd_preg + preg_rate*Infous_dry - lac_rate*Infous_preg - immunity_rate_preg*Infous_preg*frac_inftd_non_clin_preg - diag_rate_preg*Infous_preg*frac_rep_preg - removal_rate*Infous_preg

d/dt (Immune_preg) = immune_rate_preg*Infous_preg*frac_inftd_non_clin_preg + preg_rate*Immune_dry - lac_rate*Immune_preg - sus_rate2_preg*Immune_preg - removal_rate*Immune_preg

d/dt (Reported_preg) = diag_rate_preg*Infous_preg*frac_rep_preg + preg_rate*Reported_dry - lac_rate*Reported_preg - removal_rate*Reported_preg - recovery_rate_preg*Reported_preg*prop_dosed_preg*dose_eff

d/dt (Rec_preg) = recovery_rate_preg*Reported_preg*prop_dosed_preg*dose_eff + preg_rate*Rec_dry - lac_rate*Rec_preg - sus_rate1_preg*Rec_preg – removal_rate*Rec_preg

d/dt (Cum_reported_preg) = diag_rate_preg*Infous_preg*frac_rep_preg

Figure 1A (iii): Lactating group infection transmission differential equations

d/dt (Sus_lac) = sus_rate1_lac*Rec_lac + sus_rate2_lac*Immune_lac + lac_rate*Sus_preg - dry_rate*Sus_lac - force_of_infn_lac*Sus_lac*infected_prop – removal_rate*Sus_lac

d/dt (Preinfous_lac) = force_of_infn_lac*Sus_lac*infected_prop + lac_rate*Inftd_preg - dry_rate*Inftd_lac - infective_rate*Inftd_lac - removal_rate*Inftd_lac

d/dt (Infous_lac) = infective_rate*Inftd_lac + lac_rate*Infous_preg - dry_rate*Infous_lac - immunity_rate_lac*Infous_lac*frac_inftd_non_clin_lac – diag_rate_lac*Infous_lac*frac_rep_lac - removal_rate*Infous_lac

d/dt (Immune_lac) = immunity_rate_lac*Infous_lac*frac_inftd_non_clin_lac + lac_rate*Immune_preg - dry_rate*Immune_lac - sus_rate2_lac*Immune_lac – removal_rate*Immune_lac

d/dt (Reported_lac) = diag_rate_lac*Infous_lac*frac_rep_lac + lac_rate*Reported_preg - dry_rate*Reported_lac - removal_rate*Reported_lac - recovery_rate_lac*Reported_lac*prop_dosed_lac*dose_eff

d/dt (Rec_lac) = recovery_rate_lac*Reported_lac*prop_dosed_lac*dose_eff + lac_rate*Rec_preg - dry_rate*Rec_lac - sus_rate1_lac*Rec_lac - removal_rate*Rec_lac

d/dt (Cum_reported_lac) = diag_rate_lac*Infous_lac*frac_rep_lac

Table 1A (i): Summary of the parameters used in the mode.

Symbol	Definition	Assumed value	Reference
preinfective_period_ j	average pre-infective period (contamination to infective stage) for pasture currently being grazed by ewes in animal group *j*, where *j* denotes dry, pregnant and lactating ewes. Because *H. contortus* transmission between ewes is mediated through infective pasture, it can range from 0 for continuously infective pasture to a maximum of 28 days [pre-patent period (19 – 21 days) + duration of development from egg to infective L3 (5 – 7 days)] where ewes at various stages of infection are brought onto a clean pasture.	estimated through model predictions fitting to observed data	(Veglia, 1915; Mendez and Cabo, 1980; Urquhart et al., 1995)
Latent_period	average pre-infectious period for ewes which is estimated to be [pre-patent period (19 – 21 days) + duration of development from egg to infective L3 (5 – 7 days)].	26.54 days	(Veglia, 1915; Mendez and Cabo, 1980; Urquhart et al., 1995)
period_to_diag_ j	average time it took an infectious clinical case in animal group *j* to be diagnosed by FAMACHA© where *j* denotes dry, pregnant and lactating groups.	7 days	Particular farm data in relation to time interval between FAMACHA© evaluations
period_to_drug_eff_ j	average time it takes for the drug used for dosing to reach its maximum activity level against the prevailing *Haemonchus* spp. parasite burden in animal group *j* where *j* denotes dry, pregnant and lactating groups	estimated through model predictions fitting to observed data	(Barragry, 1994)
lactation_period	average duration ewes are allowed to suckle their lamb (s) before weaning which was approximated to be 90 - 100 days to enable single lambing per year.	90 days	Particular farm data
gestation_period	time from conception to lambing (parturition) which is between 148 and 150 days.	150 days	(Jainudeen and Hafez, 2000; Ensminger, 2002)

dry_period	time from weaning to conception (ram introduction) which is approximated to be 16 - 30 days to enable single lambing per year	30 days	Particular farm data
residual_period_ j	average number of days post-treatment that ewes in in animal group *j* are protected from re-infection with *H. contortus* by the direct and indirect effects of the treatment drugs used where *j* denotes dry, pregnant and lactating groups	estimated through model predictions fitting to observed data	(Barragry, 1994; Van Wyk, 2001; Plumb, 2008)
period_to_immunity_ j	time interval in days from infectious status to immune status in animal group *j* where *j* denotes dry, pregnant and lactating groups	estimated through model predictions fitting to observed data	–
immune_period_ j	time interval in days from acquisition of immunity to loss of that protective immunity in ewes and becoming susceptible to re-infection (30 – 90 days) in animal group *j* where *j* denotes dry, pregnant and lactating groups	estimated through model predictions fitting to observed data	(Stoll, 1958; Levine, 1980; Smith, 2001; McClure, 2012)
Infective_rate_ j	rate at which pasture grazed by ewes in animal group *j* becomes infective where *j* denotes dry, pregnant and lactating groups	(1/preinfective_period_ j) day^{-1}	(Vynnycky and White, 2010)
infectious_rate	rate at which infected ewes become infectious	(1/latent_period) day^{-1}	(Vynnycky and White, 2010)
diag-rate_ j	rate at which clinical cases are diagnosed per unit time by FAMACAH© in animal group *j* where *j* denotes dry, pregnant and lactating groups	(1/period_to_report_ $_j$) day^{-1}	Particular farm Interval between FAMACHA©
recovery_rate	rate at which ewes recover from haemonchosis per unit time as a result of drenching in animal group *j* where *j* denotes dry, pregnant and lactating groups	(1/period_to_drug_eff_ $_j$) day^{-1}	Particular farm data

sus_rate1_ j	rate at which recovered ewes becomes susceptible to re-infection after dosing in animal group *j* where *j* denotes dry, pregnant and lactating groups	(1/residual_period_ j) day^{-1}	(Barragry, 1994; Plumb, 2008)
immunity_rate_ j	rate at which infectious and resilient ewes acquire immunity against *H. contortus* in animal group *j* where *j* denotes dry, pregnant and lactating groups	(1/ period_to_immunity_ j) day^{-1}	(Vynnycky and White, 2010)
dry_rate	rate at which lactating ewes are dried	(1/lactation_period) day^{-1}	Particular farm data
preg_rate	rate at which dry ewes become pregnant	(1/dry_period) day^{-1}	Particular farm data
lac_rate	rate at which pregnant ewes lactates or lamb	(1/gestation_period) day^{-1}	Particular farm data
removal_rate	rate at which ewes are removed from the flock through culling or death per day	estimated through model predictions fitting to observed data	(Fourie and Cloete, 1993) Particular farm data
replacement_rate	rate at which ewes are replaced in the whole flock and the farmer replaced with pregnant healthy (susceptible) ewes at the rate of removal to maintain a constant flock size	(removal_rate) day^{-1}	(Fourie and Cloete, 1993)
sus_rate2_ j	rate at which immune ewes lose their immunity and becomes susceptible to re-infection in animal group *j* where *j* denotes dry, pregnant and lactating groups	(1/immune period_ j) day^{-1}	(Smith, 2001; McClure, 2012)
prop_dosed_ j	proportion of clinical cases reported by FAMACHA© in animal group *j* that is properly dosed where *j* denotes dry, pregnant and lactating groups	1	Particular farm data
dose_eff	proportion of cases that were cured from haemonchosis as a result of drenching	estimated through model predictions fitting to observed data	Particular farm data

infected_prop	proportion of ewes carrying heavy burden of worms in the flock per day in animal group *j* where *j* denotes dry, pregnant and lactating groups.	estimated through model predictions fitting to observed data	(Barger, 1985; Sreter et al., 1994; Hoste et al., 2001)
frac_infous_clin_ j	proportion of infected and infectious ewes which experience haemonchosis due to limited resistance in animal group *j* where *j* denotes dry, pregnant and lactating groups.	estimated through model predictions fitting to observed data	(Malan et al., 2001)
frac_infous_non_clin_ j	proportion of infected and infectious ewes which do not experience haemonchosis due to resilience in animal group *j* where *j* denotes dry, pregnant and lactating groups	estimated through model predictions fitting to observed data	(Malan et al., 2001)
famacha_sen	proportion of diagnosed cases by FAMACHA© (≥3 FAMACHA© score) that is truly anaemic (≤19% PCV)	estimated through model predictions fitting to observed data	(Kaplan et al., 2004; Burke et al., 2007; Reynecke et al., 2011c)
prop_invest_ j	proportion of ewes evaluated by FAMACHA© in animal group *j* where *j* denotes dry, pregnant and lactating groups	see text	Expert Opinion
frac_rep_ j	proportion of infected and infectious dry ewes which experienced clinical signs of haemonchosis and were reported as cases by FAMACHA© in animal group *j* where *j* denotes dry, pregnant and lactating groups	prop_invest*famacha_sen* frac_inftd_clin_ j	(Malan et al., 2001; Burke et al., 2007; Reynecke et al., 2011c)
Bj _j'	effective contact that is sufficient to lead to *H. contortus* transmission through pasture between an infectious ewe in group *j* and a susceptible ewe in group *j'* where *j* and *j'* denotes dry, pregnant and lactating groups	estimated through model predictions fitting to observed data	(Vynnycky and White, 2010)
force_of_infn_ j_1	rate at which susceptible ewes become infected per unit time with routine FAMACHA©-based TST application and without emergency whole-flock drenching in animal group *j* where *j* denotes dry, pregnant and lactating groups.	see text and Appendix A	(Anderson and May, 1985a; Vynnycky and White, 2010)

force_of_infn_ j_2	rate at which susceptible ewes become infected per unit time with routine FAMACHA©-based TST application, and with emergency whole flock drenching in animal group j where j denotes dry, pregnant and lactating groups.	0.001*force_of_infn_ j_1	Expert opinion
pop_ j	number of ewes in animal group *j* where *j* denotes dry, pregnant and lactating animal groups and it was assumed to be equal to number of FAMACHA© evaluations in each group scaled per 100 ewes per week.	dry = 1400 pregnant = 1400 lactating = 1400	Particular Farm data
pop_all	total number of ewes on the farm which was assumed to be equal to sum total of all FAMACHA© evaluations during the period of interest.	4200	Particular Farm Data

Table 1A (ii): Summary of the variables in the model.

Symbol	Definition
$Sus\_j(t)$	number of susceptible ewes at time *t* in animal group *j* at time *t* where *j* denotes dry, pregnant and lactating groups
$Preinfous\_j(t)$	number of infected ewes at time *t* in animal group *j* at time *t* where *j* denotes dry, pregnant and lactating groups
$Infous\_j(t)$	number of infectious ewes at time *t* in animal group *j* at time *t* where *j* denotes dry, pregnant and lactating groups
$Immune\_j(t)$	number of ewes which have become immune at time *t* in animal group *j* where *j* denotes dry, pregnant and lactating groups
$Reported\_j(t)$	number of ewes which have been reported as cases by FAMACHA© at time *t* in animal group *j* where *j* denotes dry, pregnant and lactating groups
$Rec\_j(t)$	number of recovered ewes in animal group *j* at time *t* as a result of the direct effect of the drench where *j* denotes dry, pregnant and lactating groups
$Cum\_reported\_cases\_j(t)$	cumulative number of reported cases in animal group *j* which have occurred in the farm by time *t* where *j* denotes dry, pregnant and lactating groups

Appendix 1B: Model fitting details

The expression below was used in the calculation of the deviance after model data fitting using maximum likelihood:

$$= 2 \left\{ \text{log likelihood of the saturated model – log likelihood of observing the data set if “my” model was correct} \right\}$$

$$= 2 \left\{ \sum_{t=0}^{7} \sum_{j} [\, R_{jt}\, \ell n(r_{jt}) + (N_{jt} - R_{j})\, \ell n(1 - r_{jt})\,] - [\, R_{jt}\, \ell n(\hat{r}_{jt}) + (N_{jt} - R_{jt})\, \ell n(1 - \hat{r}_{jt})\,] \right\}$$

where the above variables are defined in Table 2B.

Table 1B: Fitting process variable definitions.

Variable	Definition
N_{jt}	The number of ewes in reproductive status group *j* which were evaluated in week *t*.
R_{jt}	The number of ewes in reproductive status group *j* which were reported (observed) as cases of haemonchosis during FAMACHA© evaluation in week *t*.
r_{jt}	The proportions of ewes in reproductive status group *j* which were reported (observed) as cases of haemonchosis during FAMACHA© evaluation in week *t*.
$\hat{r}_{jt}$	The proportions of ewes in reproductive status group *j* which were predicted to be cases of haemonchosis by the model in week *t*.

APPENDIX 2

Appendix 2A: Stata®/IC 10 Link-function formulation .do file

/* Variation in modelled force of infection with *Haemonchus contortus* as a function of climate*/

```
. clear all
. capture log close
. set memory 32m
. set more off
. log using linkfunction.log, replace

/*exploring the panel data*/
. use linkfunction7days.dta, clear
. xtset reproclass famawave
. xtline foi
. xtline rain temp entropy, ytitle ("Four-week period values")

. use linkfunction14days.dta, clear
. xtset reproclass famawave
. xtline rain temp entropy, ytitle ("Four-week period values")

. use linkfunction21days.dta, clear
. xtset reproclass famawave
. xtline rain temp entropy, ytitle ("Four-week period values")

/*dependency of FOI on temperature, rainfall and entropy with entity (panels) random effects*/
. use linkfunction7days.dta, clear
. xtset reproclass
```

```
. xtreg foi entropy temp rainfall, re vce (conventional)
. predict r                    /*prediction of residuals for model diagnostics*/
. pnorm r
. predict foihat               /*prediction of average FOI across the three groups*/
. xtline foihat
. outreg2 using test.doc, nolabel bdec (2) rdec (2) coefastr ctitle (FOI against 7-day values) replace

. use linkfunction14days.dta, clear
. xtset reproclass
. xtreg foi entropy temp rainfall, re vce (conventional)
. outreg2 using test.doc, nolabel bdec (2) rdec (2) coefastr ctitle (FOI against 14-day values) append

. use linkfunction21days.dta, clear
. xtset reproclass
. xtreg foi entropy temp rainfall, re vce (conventional)
. outreg2 using test.doc, nolabel bdec (2) rdec (2) coefastr ctitle (FOI against 21-day values) append
/* testing to see if the choice of random effects over fixed effects model is statistically justifiable for the significant model*/
. use linkfunction7days.dta", clear
. xtset reproclass
. quietly xtreg foi entropy temp rainfall, fe vce(conventional)
. estimates store fixed
. quietly xtreg foi entropy temp rainfall, re vce(conventional)
. estimates store random
. hausman fixed random

/*Testing to see if time fixed effects are needed for the significant model*/
. use linkfunction7days.dta, clear
. xtset reproclass
```

```
. xi: xtreg foi entropy temp rainfall i.famawave, re vce (conventional)
. testparm _Ifamawave*
/*end of analysis*/
. log close
```

Appendix 2B: Summary tables for the FAMACHA© evaluation events, weather elements and model predicted force-of-infection (FOI) data

Table 2B (i): Dry ewes' summary FAMACHA©, weather and point force-of-infection (FOI) estimates data for the 1997/98 *H. contortus* season.

FAMACHA© evaluation dates	Evaluation wave number	Number of animals done at each FAMACHA© evaluation	Observed incidence cases (≥3 FAMACHA© score)	Cumulative time (days)	Scaled observed incidence cases (≥3 FAMACHA© score) out of 100 ewes per week	Four-week period*									Fitted point force-of-infection estimates (Matrix 1)
						7-day values			14-day values			21-day values			
						Ave. Temp. (°C)	Total rainfall (mm)	Entropy	Ave. Temp. (°C)	Total rainfall (mm)	Entropy	Ave. Temp. (°C)	Total rainfall (mm)	Entropy	
10-Nov-97	1	28	21	0	75	23.52	42.00	1.60	24.54	40.00	1.56	25.29	40.00	1.56	$1.93x10^{-3}$
17-Nov-97	2	41	12	7	29	25.32	30.00	1.18	23.52	42.00	1.60	24.54	40.00	1.56	2.37
24-Nov-97	3	41	18	14	44	26.42	49.00	1.47	25.32	30.00	1.18	23.52	42.00	1.60	4.63
01-Dec-97	4	61	31	21	51	26.14	64.00	1.67	26.42	49.00	1.47	25.32	30.00	1.18	5.00
08-Dec-97	5	53	30	28	57	25.40	117.00	2.00	26.14	64.00	1.67	26.42	49.00	1.47	4.70
15-Dec-97	6	29	11	35	38	24.74	107.00	1.82	25.40	117.00	2.00	26.14	64.00	1.67	4.22
29-Dec-97	7	11	0	42	0	25.56	92.00	1.60	25.46	107.00	1.78	24.74	107.00	1.82	3.76
05-Jan-98	8	8	4	49	50	26.89	59.00	1.46	25.56	92.00	1.60	25.46	107.00	1.78	3.37
09-March-98	9	13	3	56	23	28.00	47.50	1.49	27.85	44.50	1.33	28.85	59.00	1.29	3.07
16-March-98	10	21	9	63	43	27.20	53.50	1.67	28.00	44.50	1.49	27.85	44.50	1.33	2.84
23-March-98	11	15	5	70	33	26.46	43.50	1.68	27.20	53.50	1.67	28.00	47.50	1.49	2.66
30-March-98	12	46	17	77	37	27.98	26.00	1.26	26.46	43.50	1.68	27.20	53.50	1.67	2.53
06-April-98	13	39	12	84	31	27.78	23.00	1.02	27.98	26.00	1.26	26.46	43.50	1.68	2.43
13-April-98	14	28	11	91	39	27.90	17.00	1.02	27.78	23.00	1.02	27.98	26.00	1.26	2.35

* Recorded average temperature, total rainfall and rainfall entropy during the four-week period up to 7,14 and 21 days before the day of the FAMACHA© evaluation.

Table 2B (ii): Pregnant ewes' summary FAMACHA©, weather elements and point force-of-infection (FOI) estimates data.

FAMACHA© evaluation dates	Evaluation wave number	Number of animals done at each FAMACHA© evaluation	Observed incidence Cases (≥3 FAMACHA© score)	Cumulative time (days)	Scaled observed incidence cases (≥3 FAMACHA© score) out of 100 ewes per week	Four-week period*									Fitted point force-of-infection estimates (Matrix 1)
						7-day values			14-day values			21-day values			
						Ave. Temp. (°C)	Total rainfall (mm)	Entropy	Ave. Temp. (°C)	Total rainfall (mm)	Entropy	Ave. Temp. (°C)	Total rainfall (mm)	Entropy	
10-Nov-97	1	62	51	0	82	23.52	42.00	1.60	24.54	40.00	1.56	25.29	40.00	1.56	$8.63x10^{-3}$
17-Nov-97	2	71	23	7	32	25.32	30.00	1.18	23.52	42.00	1.60	24.54	40.00	1.56	14.18
24-Nov-97	3	79	14	14	18	26.42	49.00	1.47	25.32	30.00	1.18	23.52	42.00	1.60	14.91
01-Dec-97	4	96	28	21	29	26.14	64.00	1.67	26.42	49.00	1.47	25.32	30.00	1.18	15.15
08-Dec-97	5	49	18	28	37	25.40	117.00	2.00	26.14	64.00	1.67	26.42	49.00	1.47	15.13
15-Dec-97	6	75	15	35	20	24.74	107.00	1.82	25.40	117.00	2.00	26.14	64.00	1.67	14.95
29-Dec-97	7	49	2	42	4	25.56	92.00	1.60	25.46	107.00	1.78	24.74	107.00	1.82	14.68
05-Jan-98	8	49	4	49	8	26.89	59.00	1.46	25.56	92.00	1.60	25.46	107.00	1.78	14.37
09-March-98	9	30	3	56	10	28.00	47.50	1.49	27.85	44.50	1.33	28.85	59.00	1.29	14.05
16-March-98	10	116	58	63	50	27.20	53.50	1.67	28.00	44.50	1.49	27.85	44.50	1.33	13.74
23-March-98	11	88	40	70	45	26.46	43.50	1.68	27.20	53.50	1.67	28.00	47.50	1.49	13.45
30-March-98	12	131	65	77	50	27.98	26.00	1.26	26.46	43.50	1.68	27.20	53.50	1.67	13.19
06-April-98	13	128	65	84	51	27.78	23.00	1.02	27.98	26.00	1.26	26.46	43.50	1.68	12.96
13-April-98	14	112	55	91	49	27.90	17.00	1.02	27.78	23.00	1.02	27.98	26.00	1.26	12.76

* Recorded average temperature, total rainfall and rainfall entropy during the four-week period up to 7, 14 and 21 days before the day of the FAMACHA© evaluation.

Table 2B (iii): Lactating ewes' summary FAMACHA©, weather and point force-of-infection (FOI) estimates data for the 1997/98 *H. contortus* season.

FAMACHA© evaluation dates	Evaluation wave number	Number of animals done at each FAMACHA© evaluation	Observed incidence Cases (≥3 FAMACHA© score)	Cumulative time (days)	Scaled observed incidence cases (≥3 FAMACHA© score) out of 100 ewes per week	Four-week period*									Fitted point force-of-infection estimates (Matrix 1)
						7-day values			14-day values			21-day values			
						Ave. Temp. (^{o}C)	Total rainfall (mm)	Entropy	Ave. Temp. (^{o}C)	Total rainfall (mm)	Entropy	Ave. Temp. (^{o}C)	Total rainfall (mm)	Entropy	
10-Nov-97	1	40	32	0	80	23.52	42.00	1.60	24.54	40.00	1.56	25.29	40.00	1.56	$6.13x10^{-5}$
17-Nov-97	2	30	15	7	50	25.32	30.00	1.18	23.52	42.00	1.60	24.54	40.00	1.56	3.00
24-Nov-97	3	27	11	14	41	26.42	49.00	1.47	25.32	30.00	1.18	23.52	42.00	1.60	2.40
01-Dec-97	4	11	7	21	64	26.14	64.00	1.67	26.42	49.00	1.47	25.32	30.00	1.18	2.26
08-Dec-97	5	7	5	28	71	25.40	117.00	2.00	26.14	64.00	1.67	26.42	49.00	1.47	2.21
15-Dec-97	6	23	6	35	26	24.74	107.00	1.82	25.40	117.00	2.00	26.14	64.00	1.67	2.18
29-Dec-97	7	29	5	42	17	25.56	92.00	1.60	25.46	107.00	1.78	24.74	107.00	1.82	2.17
05-Jan-98	8	46	11	49	24	26.89	59.00	1.46	25.56	92.00	1.60	25.46	107.00	1.78	2.16
09-March-98	9	21	4	56	19	28.00	47.50	1.49	27.85	44.50	1.33	28.85	59.00	1.29	2.15
16-March-98	10	24	13	63	54	27.20	53.50	1.67	28.00	44.50	1.49	27.85	44.50	1.33	2.15
23-March-98	11	14	7	70	50	26.46	43.50	1.68	27.20	53.50	1.67	28.00	47.50	1.49	2.15
30-March-98	12	14	7	77	50	27.98	26.00	1.26	26.46	43.50	1.68	27.20	53.50	1.67	2.15
06-April-98	13	8	6	84	75	27.78	23.00	1.02	27.98	26.00	1.26	26.46	43.50	1.68	2.15
13-April-98	14	25	21	91	84	27.90	17.00	1.02	27.78	23.00	1.02	27.98	26.00	1.26	2.16

* Recorded average temperature, total rainfall and rainfall entropy during the four-week period up to 7, 14 and 21 days before the day of the FAMACHA© evaluation.

Appendix 2C: Regression results from .log file

Table 2C (i): Dependency of interval force-of-infection (FOI) on the on-farm recorded temperature, rainfall and entropy with only entity fixed effects included for the 7-day values.

```
. use linkfunction7days.dta, clear
 (Force of infection and weather elements)

. xtset reproclass famawave
       panel variable:  reproclass (strongly balanced)
        time variable:  famawave, 1 to 14
                delta:  1 unit
. xtreg foi entropy temp rainfall, re vce(conventional)

Random-effects GLS regression                   Number of obs      =        42
Group variable: reproclass                      Number of groups   =         3

R-sq:  within  = 0.0000                         Obs per group: min =        14
       between = 0.0000                                        avg =      14.0
       overall = 0.0633                                        max =        14

Random effects u_i ~ Gaussian                   Wald chi2(3)       =     20.30
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.0001

------------------------------------------------------------------------------
         foi |      Coef.   Std. Err.       z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     entropy |  -2.243728   2.116582    -1.06   0.289    -6.392152    1.904695
        temp |   .9781476   .2782584     3.52   0.000     .4327713    1.523524
    rainfall |   .0605518   .0194017     3.12   0.002     .0225251    .0985784
       _cons |  -19.66626   9.216335    -2.13   0.033    -37.72994   -1.602574
-------------+----------------------------------------------------------------
     sigma_u |  6.0582487
     sigma_e |   1.979547
         rho |  .90353246   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

Table 2C (ii): Dependency of interval force-of-infection (FOI) on the on-farm recorded temperature, rainfall and entropy, with only entity fixed effects included for the 14-day values.

```
. use linkfunction14days.dta, clear
 (Force of infection and weather elements)

xtset reproclass
       panel variable:  reproclass (balanced)

. xtreg foi entropy temp rainfall, re vce(conventional)

Random-effects GLS regression                   Number of obs      =        42
Group variable: reproclass                      Number of groups   =         3

R-sq:  within  = 0.0000                         Obs per group: min =        14
       between = 0.0000                                        avg =      14.0
       overall = 0.0209                                        max =        14

Random effects u_i ~ Gaussian                   Wald chi2(3)       =      4.87
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.1813

------------------------------------------------------------------------------
         foi |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     entropy |   -2.34276   2.548874    -0.92   0.358    -7.338462    2.652942
        temp |   .3258325   .2999203     1.09   0.277    -.2620006    .9136655
    rainfall |   .0388227   .0210647     1.84   0.065    -.0024635    .0801088
       _cons |  -1.028889   10.12143    -0.10   0.919    -20.86654    18.80876
-------------+----------------------------------------------------------------
     sigma_u |  6.0495234
     sigma_e |  2.3232778
         rho |  .87146797   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

Table 2C (iii): Dependency of interval force-of-infection (FOI) on the on-farm recorded temperature, rainfall and entropy, with only entity fixed effects included for the 21-day values.

```
. use linkfunction21days.dta, clear
 (Force of infection and weather elements)

xtset reproclass
       panel variable:  reproclass (balanced)

. xtreg foi entropy temp rainfall, re vce(conventional)

Random-effects GLS regression                   Number of obs      =        42
Group variable: reproclass                      Number of groups   =         3

R-sq:  within  = 0.0000                         Obs per group: min =        14
       between = 0.0000                                        avg =      14.0
       overall = 0.0123                                        max =        14

Random effects u_i ~ Gaussian                   Wald chi2(3)       =      2.70
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.4401

------------------------------------------------------------------------------
         foi |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     entropy |  -4.042588   3.041515    -1.33   0.184    -10.00385    1.918672
        temp |  -.1604303   .2868602    -0.56   0.576     -.722666    .4018054
    rainfall |   .0354001   .0219189     1.62   0.106    -.0075602    .0783603
       _cons |   14.59132   10.70753     1.36   0.173    -6.395064    35.57769
-------------+----------------------------------------------------------------
     sigma_u |  6.0477341
     sigma_e |  2.3876076
         rho |   .8651551   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

Table 2C (iv): Testing to see if choice of random-effects over fixed effects model was statistically justifiable for analysis of the dependency of force-of-infection (FOI) on the on-farm recorded temperature, rainfall and entropy, using the deduced significant model based on 7-day values.

```
. use linkfunction7days.dta", clear
 (Force of infection and weather elements)

. xtset reproclass
       panel variable:  reproclass (balanced)

. quietly xtreg foi entropy temp rainfall, fe vce(conventional)
. estimates store fixed

. quietly xtreg foi entropy temp rainfall, re vce(conventional)
. estimates store random

. hausman fixed random

                 ---- Coefficients ----
             |      (b)          (B)            (b-B)     sqrt(diag(V_b-V_B))
             |     fixed        random       Difference          S.E.
-------------+------------------------------------------------------------
     entropy |   -2.243728    -2.243728       -1.78e-15               .
        temp |    .9781476     .9781476       -1.11e-16               .
    rainfall |    .0605518     .0605518        2.08e-17               .
------------------------------------------------------------------------------
                          b = consistent under Ho and Ha; obtained from xtreg
           B = inconsistent under Ha, efficient under Ho; obtained from xtreg

    Test:  Ho:  difference in coefficients not systematic

                  chi2(3) = (b-B)'[(V_b-V_B)^(-1)](b-B)
                          =        0.00
                Prob>chi2 =      1.0000
                (V_b-V_B is not positive definite)
```

Table 2C (v): Testing to see if time-fixed effects inclusion was needed for the analysis of the dependency of force-of-infection (FOI) on the on-farm recorded temperature, rainfall and entropy, using the deduced significant model based on 7-day values.

```
. use linkfunction7days.dta, clear
 (Force of infection and weather elements)

. xtset reproclass
       panel variable:  reproclass (balanced)

. xi:xtreg foi entropy temp rainfall i.famawave, re vce(conventional)
i.famawave        _Ifamawave_1-14     (naturally coded; _Ifamawave_1 omitted)
note: entropy dropped because of collinearity
note: _Ifamawave_5 dropped because of collinearity
note: _Ifamawave_14 dropped because of collinearity

Random-effects GLS regression                   Number of obs      =        42
Group variable: reproclass                      Number of groups   =         3

R-sq:  within  = 0.0000                         Obs per group: min =        14
       between = 0.0000                                        avg =      14.0
       overall = 0.1062                                        max =        14

Random effects u_i ~ Gaussian                   Wald chi2(13)      =     39.80
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.0001

------------------------------------------------------------------------------
         foi |      Coef.   Std. Err.       z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        temp |   1.637983   .3589007     4.56   0.000     .9345505    2.341416
    rainfall |   .0568496   .0151535     3.75   0.000     .0271493    .0865499
_Ifamawave_2 |   4.246952    1.31072     3.24   0.001     1.677987    6.815916
_Ifamawave_3 |   2.161695   1.252862     1.73   0.084      -.29387     4.61726
_Ifamawave_4 |   1.924253   1.242279     1.55   0.121     -.510568    4.359075
_Ifamawave_6 |   1.419565   1.406527     1.01   0.313    -1.337177    4.176306
_Ifamawave_7 |   .6824954   1.319192     0.52   0.605    -1.903073    3.268064
_Ifamawave_8 |   .1433473   1.305328     0.11   0.913    -2.415049    2.701744
_Ifamawave_9 |  -1.231044   1.471094    -0.84   0.403    -4.114334    1.652247
_Ifamawav~10 |  -.4417547   1.339723    -0.33   0.742    -3.067563    2.184053
_Ifamawav~11 |   1.182182   1.261752     0.94   0.349    -1.290806     3.65517
_Ifamawav~12 |  -.4426848   1.480628    -0.30   0.765    -3.344663    2.459293
_Ifamawav~13 |  -.0545395   1.456794    -0.04   0.970    -2.909803    2.800724
       _cons |   -40.9095   10.15259    -4.03   0.000    -60.80821    -21.0108
-------------+----------------------------------------------------------------
     sigma_u |   6.061582
     sigma_e |   1.831126
         rho |  .91637472   (fraction of variance due to u_i)
------------------------------------------------------------------------------

. testparm _Ifamawave*

 ( 1)  _Ifamawave_2 = 0
 ( 2)  _Ifamawave_3 = 0
 ( 3)  _Ifamawave_4 = 0
 ( 4)  _Ifamawave_6 = 0
 ( 5)  _Ifamawave_7 = 0
 ( 6)  _Ifamawave_8 = 0
 ( 7)  _Ifamawave_9 = 0
 ( 8)  _Ifamawave_10 = 0
 ( 9)  _Ifamawave_11 = 0
 (10)  _Ifamawave_12 = 0
 (11)  _Ifamawave_13 = 0

           chi2( 11) =   17.39
         Prob > chi2 =    0.0970
```

APPENDIX 3

Appendix 3A: Analysis commands .do file

/* Evaluation of a prototype radio frequency identification (RFID) system for remote monitoring of individual animal activity levels*/

. clear all

. capture log close

. set memory 32m

. set more off

. log using tags.log, replace

. use tags.dta, clear

/*Data preparation*/

. recode tagarrang (2 = 0) (1 = 1)

. label define label 0 "spread-out" 1 "clustered"

. label values tagarrang label

. bysort grouptotal: egen clusteredstationary = mean (dtr) if tagarrang == 1 & motion == 0

. rename clusteredstationary meandtr1

. label variable meandtr1 "mean 10 minutes' data transmission for clustered, stationary levels of tag numbers"

. bysort grouptotal: egen clusteredswinging = mean (dtr) if tagarrang == 1 & motion == 1

. rename clusteredswinging meandtr2

. label variable meandtr2 "mean 10 minutes' data transmission for clustered, swinging levels of tag numbers"

. bysort grouptotal: egen spreadoutstationary = mean (dtr) if tagarrang == 0 & motion == 0

. rename spreadoutstationary meandtr3

. label variable meandtr3 "mean 10 minutes' data transmission for spread-out, stationary levels of tag numbers"

```
. bysort grouptotal: egen spreadoutswinging = mean (dtr) if tagarrang == 0 & motion == 1
. rename spreadoutswinging meandtr4
. label variable meandtr4 "mean 10 minutes' data transmission for spread-out, swinging levels of tag numbers"
. save tagsready.dta, replace
. use tagsready.dta, clear

/*Data Analysis*/
/*Graphical exploration of raw data for trends*/
. hist dtr if tagarrang == 1 &  motion == 1, by ( grouptotal) normal percent
. hist dtr if tagarrang == 1 &  motion == 0, by ( grouptotal) normal percent
. hist dtr if tagarrang == 0 &  motion == 1, by ( grouptotal) normal percent
. hist dtr if tagarrang == 0 &  motion == 0, by ( grouptotal) normal percent

. twoway (lfitci meandtr1 grouptotal, stdp) (scatter meandtr1 grouptotal)
. twoway (lfitci meandtr2 grouptotal, stdp) (scatter meandtr2 grouptotal)
. twoway (lfitci meandtr3 grouptotal, stdp) (scatter meandtr3 grouptotal)
. twoway (lfitci meandtr4 grouptotal, stdp) (scatter meandtr4 grouptotal)

/*Test for trend*/
. nptrend meandtr1, by (grouptotal)
. nptrend meandtr2, by (grouptotal)
. nptrend meandtr3, by (grouptotal)
. nptrend meandtr4, by (grouptotal)

/*Regression analysis for the effect of numbers of tags on data transmission per 10 minutes when adjusted for disposition, movement and distance*/

/*For clustered, stationary categorical levels of tag numbers*/
. xi: xtmixed dtr || tagid: if tagarrang == 1 & motion == 0, reml var /*null model*/
. xi: xtmixed dtr i.grouptotal || tagid: if tagarrang == 1 & motion == 0
. predict resid, residuals
. predict resid_std, rstandard              /*residuals/sd(residuals)*/
```

```
. kdensity resid_std, normal            /* model diagnostics*/
. pnorm resid_std
. qnorm resid_std
. hist resid_std, normal percent
. outreg2 using test.doc, replace
. drop resid resid_std
. lincom _cons          /*calculation of mean DTR per 10 minutes for each group*/
. lincom _Igrouptota_20 + _cons
. lincom _Igrouptota_30 + _cons
. lincom _Igrouptota_40 + _cons
. lincom _Igrouptota_50 + _cons
. lincom _Igrouptota_60 + _cons
. lincom _Igrouptota_70 + _cons
. lincom _Igrouptota_80 + _cons
. lincom _Igrouptota_90 + _cons
. lincom _Igrouptota_100 + _cons

/*For clustered, swinging categorical levels of tag numbers*/
. xi: xtmixed dtr || tagid: if tagarrang == 1 &  motion == 1, reml var/*null model*/
. xi: xtmixed dtr i.grouptotal || tagid: if tagarrang == 1 &  motion == 1, reml var
. predict resid, residuals
. predict resid_std, rstandard          /*residuals/sd(residuals)*/
. kdensity resid_std, normal            /*model diagnostics*/
. pnorm resid_std
. qnorm resid_std
. hist resid_std, normal percent
. outreg2 using test.doc, append
. drop resid resid_std
. lincom _cons          /*calculation of mean DTR per 10 minutes for each group*/
. lincom _Igrouptota_20 + _cons
. lincom _Igrouptota_30 + _cons
. lincom _Igrouptota_40 + _cons
. lincom _Igrouptota_50 + _cons
. lincom _Igrouptota_60 + _cons
```

```
. lincom _Igrouptota_70 + _cons
. lincom _Igrouptota_80 + _cons
. lincom _Igrouptota_90 + _cons
. lincom _Igrouptota_100 + _cons

/*For spread-out, stationary categorical levels of tag numbers*/
. xi: xtmixed dtr || tagid: if tagarrang == 0 &  motion == 0, reml var/*null model*/
. xi: xtmixed dtr i.grouptotal || tagid: if tagarrang == 0 &  motion == 0, reml var
. predict resid, residuals
. predict resid_std, rstandard          /*residuals/sd(residuals)*/
. kdensity resid_std, normal            /*model diagnostics*/
. pnorm resid_std
. qnorm resid_std
. hist resid_std, normal percent
. outreg2 using test.doc, append
. drop resid resid_std
. lincom _cons          /*calculation of mean DTR per 10 minutes for each group*/
. lincom _Igrouptota_20 + _cons
. lincom _Igrouptota_30 + _cons
. lincom _Igrouptota_40 + _cons
. lincom _Igrouptota_50 + _cons
. lincom _Igrouptota_60 + _cons
. lincom _Igrouptota_70 + _cons
. lincom _Igrouptota_80 + _cons
. lincom _Igrouptota_90 + _cons
. lincom _Igrouptota_100 + _cons

/*For spread-out, swinging categorical levels of tag numbers*/
. xi: xtmixed dtr || tagid: if tagarrang == 0 &  motion == 1, reml var/*null model*/
. xi: xtmixed dtr i.grouptotal || tagid: if tagarrang == 0 &  motion == 1, reml var
. predict resid, residuals
. predict resid_std, rstandard          /*residuals/sd(residuals)*/
. kdensity resid_std, normal            /*model diagnostics*/
. pnorm resid_std
```

```
. qnorm resid_std
. hist resid_std, normal percent
. outreg2 using test.doc, append
. drop resid resid_std
. lincom _cons          /*calculation of mean DTR per 10 minutes for each group*/
. lincom _Igrouptota_20 + _cons
. lincom _Igrouptota_30 + _cons
. lincom _Igrouptota_40 + _cons
. lincom _Igrouptota_50 + _cons
. lincom _Igrouptota_60 + _cons
. lincom _Igrouptota_70 + _cons
. lincom _Igrouptota_80 + _cons
. lincom _Igrouptota_90 + _cons
. lincom _Igrouptota_100 + _cons
/*end of analysis*/
. log close
```

APPENDIX 4

Appendix 4A: Stata®/IC 10 analysis commands .do file

/* Activity level as an indicator of clinical infection of individual goats with *Haemonchus contortus*/

```
. clear all
. capture log close
. set memory 32m
. set more off
. log using famalink.log, replace

/*Data Preparation*/
. use famalink.dta, clear
. gen haemo = famascore
. recode haemo (min/2 = 0) (3/max = 1)
. label define negativepositive 0 "negative" 1 "positive"
. label values  haemo label
. label variable haemo "Haemonchosis status based on FAMACHA score values"
. gen logactimean = ln(actimean)
. label variable logactimean "natural log mean activity level score"

. save famalink_prepared.dta, replace
. use famalink_prepared.dta, clear

/*Exploratory analysis*/
. sum
. hist actimean, by (famascore) normal percent
. hist logactimean, by (famascore) normal percent
. hist actimean, normal percent
. hist famascore, normal percent
. twoway (lfitci actimean famascore, stdp) (scatter actimean famascore)
```

. mean actimean if txgroup == 1

. mean actimean if txgroup == 2

. mean actimean if haemo == 0

. mean actimean if haemo == 1

/*Deciding between fixed or random effects model*/

. xi: xtreg actimean i.famascore, fe vce (conventional)

. estimates store fixed

. xi: xtreg actimean i.famascore, re vce (conventional)

. estimates store random

. hausman fixed random

/*Final model fitting excluding a priori confounders*/

. xi: xtreg actimean i.famascore, re vce (conventional)

. outreg2 using test.doc, nolabel bdec (2) rdec (2) coefastr ctitle (MAL excluding a priori confounders) replace

/*Final model fitting including a priori confounders included*/

. xi: xtreg actimean i.famascore i.otherdx age i.reprostat, re vce (conventional)

. outreg2 using test.doc, nolabel bdec (2) rdec (2) coefastr ctitle (MAL including a priori confounders) append

/*Roc analysis*/

. roctab haemo actimean, graph sum

/*End of Analysis*/

. log close

Appendix 4B: Regression results from .log file

Table 4B (a): Crude regression analysis for the dependency of mean activity level on natural *H. contortus* infection burden as determined by FAMACHA© evaluation.

```
. xi: xtreg actimean i.famascore, re vce(conventional)
i.famascore       _Ifamascore_1-5     (naturally coded; _Ifamascore_1 omitted)

Random-effects GLS regression                   Number of obs      =       131
Group variable: animalid                        Number of groups   =        67

R-sq:  within  = 0.0443                         Obs per group: min =         1
       between = 0.0003                                        avg =       2.0
       overall = 0.0045                                        max =         2

Random effects u_i ~ Gaussian                   Wald chi2(4)       =      1.50
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.8273

------------------------------------------------------------------------------
    actimean |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
_Ifamascor~2 |  -1.108349   2.738951    -0.40   0.686    -6.476594    4.259896
_Ifamascor~3 |  -2.696454   3.022459    -0.89   0.372    -8.620366    3.227458
_Ifamascor~4 |  -2.168132   4.454759    -0.49   0.626     -10.8993    6.563034
_Ifamascor~5 |  -6.908635   7.072232    -0.98   0.329    -20.76995    6.952685
       _cons |   17.69838   2.346883     7.54   0.000     13.09858    22.29819
-------------+----------------------------------------------------------------
     sigma_u |  7.5588314
     sigma_e |  9.8578505
         rho |  .37025965   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

Table 4B (b): A priori confounder adjusted regression analysis for the dependency of mean activity level on natural *H. contortus* infection burden as determined by FAMACHA© evaluation.

```
. xi: xtreg actimean i.famascore i.otherdx age i.reprostat, re vce(conventional)
i.famascore       _Ifamascore_1-5     (naturally coded; _Ifamascore_1 omitted)
i.otherdx         _Iotherdx_1-2       (naturally coded; _Iotherdx_1 omitted)
i.reprostat       _Ireprostat_2-3     (naturally coded; _Ireprostat_2 omitted)

Random-effects GLS regression                   Number of obs      =       131
Group variable: animalid                        Number of groups   =        67

R-sq:  within  = 0.3033                         Obs per group: min =         1
       between = 0.0194                                        avg =       2.0
       overall = 0.1046                                        max =         2

Random effects u_i ~ Gaussian                   Wald chi2(7)       =     27.13
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.0003

------------------------------------------------------------------------------
    actimean |      Coef.   Std. Err.       z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
_Ifamascor~2 |  -1.622999   2.508053    -0.65   0.518    -6.538692    3.292694
_Ifamascor~3 |   -2.39951   2.814039    -0.85   0.394    -7.914924    3.115905
_Ifamascor~4 |  -2.042826    4.02596    -0.51   0.612    -9.933563    5.847912
_Ifamascor~5 |  -2.927747   6.506711    -0.45   0.653    -15.68067    9.825172
 _Iotherdx_2 |   4.017553   5.385873     0.75   0.456    -6.538563    14.57367
         age |    .803078   1.277739     0.63   0.530    -1.701244      3.3074
_Ireprosta~3 |   7.678474   1.586746     4.84   0.000     4.568508    10.78844
       _cons |    6.56069   8.648805     0.76   0.448    -10.39066    23.51204
-------------+----------------------------------------------------------------
     sigma_u |  8.5269361
     sigma_e |  8.5692518
         rho |  .49752486   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

APPENDIX 5

Animal ethics committee approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**An automated decision support system for on-farm management of internal parasites of small ruminants**
PROJECT NUMBER	**V075-11**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. N Babayani**

STUDENT NUMBER (where applicable)	**11321700**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**Caprine**	**Ovine**
NUMBER OF ANIMALS	**400**	**400**
Approval period to use animals for research/testing purposes		**January–December 2013**
SUPERVISOR	**Dr. E Morgan**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date **27 May 2013**
CHAIRMAN: UP Animal Ethics Committee	Signature