Cattle Industry Funding Scheme Final Report

Title

Define the geographical distribution of *Theileria orientalis* in the Denmark shire.

Authors

David Forshaw, Jennifer Cotter, Dieter Palmer, Daniel Roberts

Project No

888001316

Contract/Agreement No

IPO004851

Index

Cattle Industry Funding Scheme Final Report

Title

Define the geographical distribution of *Theileria orientalis* in the Denmark shire.

Authors

David Forshaw, Jennifer Cotter, Dieter Palmer, Daniel Roberts

Abstract

In order to understand the epidemiology of the new-to-WA disease, Bovine Anaemia due to *Theileria orientalis* Group (BATOG), and to provide information to allow informed advice to farmers in the future, a survey was conducted to determine the prevalence of cattle infected with *T.orientalis* in the Denmark shire over the summer of 2014-15. DNA based tests were used to detect a group sequence common to all *T.orientalis* in blood samples as well as DNA sequences specific for the types Ikeda, Chitose and Buffeli, considered to be the potential pathogenic types in Australia. Opportunistic collection of possible vector ticks was also undertaken at the time of blood collection and the same DNA based test was used to investigate the presence of the pathogen in collected ticks. The on-farm prevalence of *T.orientalis* Ikeda, the type of the parasite considered the most pathogenic and the cause of the recent epizootic of disease in the eastern states, was determined to be 30-70% with a wide distribution through the shire. Prevalence of the Chitose type was <30% and the Buffeli type was not detected. Two types of ticks were detected: *Haemaphysalis longicornis*, the bush tick, and *Ixodes australiensis*, the scrub tick. These potential vectors were widely distributed in the shire. The widespread prevalence of the organism and its presumed tick vector (*H longicornis*) indicate that organism spread is well advanced and likely to continue quickly to achieve its potential range. Increased incidence of BATOG is expected in the Denmark shire over the coming calving seasons. The low prevalence of the Chitose strain and lack of evidence of infection with the Buffeli strain suggest that there could be some unique aspects of *Theileria orientalis* infection dynamics in WA compared to the eastern states.

Introduction

Theileria orientalis is a protozoan parasite that infects red blood cells in cattle resulting in destruction of red blood cells and severe anaemia. This results in a clinical syndrome characterised by weakness, lethargy and inappetence with abortions and deaths affecting usually only a small percentage of a herd[1, 2] (BATOG). BATOG has also been called 'benign' theileriosis in contrast to the more severe disease ‘East Coast fever’ caused by T. parva and ‘tropical theileriosis’ caused by T. annulata. The parasite is spread between cattle in Australia by the bush tick Haemaphysalis longicornis although there may be other unidentified vectors [3].

BATOG has historically been recognised in Australian cattle and was attributed to “Theileria buffeli” infection1. Initially, vector specificity was used to differentiate the 'benign Theileria' into types sergenti/buffeli/orientalis [4, 5]. Since then, genotype grouping has classified the organism as Theileria orientalis with a number of different types including the ‘Buffeli’ type that was identified originally in Australia. Since 2006, there has been a dramatic increase in the number of cases of BATOG in eastern Australia especially NSW and Victoria [1,2]. Molecular analyses revealed types of Theileria orientalis other than the Buffeli type were present. These types had never before been recognised in Aus-

tralia and it was subsequently demonstrated that the 'Ikeda' type in particular, was associated with clinical disease [6, 7] . The average cost of BATOG in NSW has been estimated at $19,783 per affected farm with a range of $300 - $204,000 [8]. A study of affected dairy cows showed significantly less milk production, milk fat and milk protein at 100 and 305 days lactation but reproductive performance was unaffected [9]. Disease was found in NZ in 2012 [10] and a single case was detected in SA in 2014 (Jade Hammer pers com). Cases have been identified in the Atherton tablelands in Queensland (Graham Bailey pers com).

Before this study was undertaken, two cases of BATOG were identified in WA in the summer of 2012-13 and a further four cases in the summer of 2013-14, all within the Denmark shire.

The cases of BATOG in WA are likely to be due to a recent introduction of the agent because the disease is quite distinctive and we believe that it would have been quickly recognised by producers and vets. However, the potential impact of the disease in WA was unclear because we did not know if the disease had been present for some time and has spread unnoticed and we did not know the range of the putative vector or the effect of seasonal conditions on this range. Veterinarians need to be informed about the status of the disease in WA in order to advise farmers of the situation and if possible, formulate prevention and control strategies suitable for the state.

We decided to focus on the Denmark shire for our survey because; all cases of BATOG up until this time had been confined to the shire, we had good information of the number of herds and the number of cattle in the shire, most of the bushtick identifications made in the DAFWA labs had been from the shire and our understanding of bush tick biology suggested that the environmental conditions in the Denmark shire were best suited to the tick[11].

Materials and Methods

Survey design

At the time of planning the survey, only four cases of BATOG had been identified in WA (fig 1). A major determinant of the likely distribution of future cases was the presence of the known vector, the bush tick *H.longicornis*. Following the discovery of bush tick in Walpole in 1983, computer modelling indicated that in average years at Walpole there would be inadequate summer rainfall to allow populations of *H.longicornis* to increase to numbers sufficient to cause clinical problems. However ticks could survive in sheltered locations such as swampy areas, thick scrub and on long dense pasture[11].

As the Denmark shire contained the locations of both the known cases of BATOG and the areas where climate was deemed to be most favourable to *H.longicornis* and we had access to data detailing cattle numbers and herds, the Denmark shire was chosen as the study area.

By sampling 10 animals per herd and sampling 30 herds we calculated that we would be have 90-95% confidence of detecting organisms at a prevalence rate of 25-30% on the farm [12].

In the Denmark shire, there are 359 properties with brands, 337 of which are calculated to be big enough to have more than 10 cattle. When 30 properties are surveyed using this protocol, the results enable a broad classification into low (0-30), medium (30-70) and high (>70%) prevalence.

Blood Smear Examination

Blood smears were made from each cow sampled. Smears were stained with diff quick stain and examined at 1000X magnification.

Blood PCR Testing

Our initial cases of BATOG were confirmed by the NSW Department of Primary Industries and we imported their testing regimen into our laboratory[6]. The test is a conventional PCR test. Recently published real time PCR testing has proved to be more sensitive ….. than this test and was used on the initial cases confirmed in NSW in verifying their new test but this test was not available to us at the time of the survey[13.] Genotyping of Theileria types was by PCR conducted using the previously published method used at the NSW DPI laboratories. Due to budget constraints, we tested for three types of *T.orientalis* only: *T.orientalis* Ikeda, the putative cause of the recent epizootic of BATOG in the eastern states. *T.orientalis* Chitose, a strain that may be is implicated in some cases of BATOG and *T.orientalis* Buffeli, a strain considered to be a benign endemic Australian strain.

Tick collection

Ticks were collected using four collection methods. On survey properties, cattle were examined for ticks and on 22 farms an attempt was made to collect questing ticks on pasture by netting, CO_2 traps and flagging. Ticks were manually collected and labeled from individual cows at the time of collecting the blood sample. In addition, some ticks found on animals were sent in by veterinary practitioners working in the Denmark area. Areas suitable for flagging were identified as the last areas grazed where ticks were found on examination of the cows or areas where cows had grazed in last month with lush pasture, adjacent native vegetation or wetter areas. Transects of 100m plus were walked

while dragging the flags. All ticks were measured for length, cut in half longitudinally, one half of the tick frozen at -20^{o}C (for DNA analysis) and the other half fixed in formalin (for possible histological examination). Some whole ticks were submitted already fixed in 70% ethanol and were then stored as entire ticks in 70% alcohol. Each collected tick was given an individual identifier so that they can be linked to location, collection method and the individual animal if collected off an animal.

Tick identifications:

Every tick was identified individually using well established morphological criteria and identification keys [14, 15, 16]. Life cycle stages and sex were also determined (larvae, nymphs, males and females). Attempts were made to record the feeding stage of each tick (unfed, fed, engorged) using subjective judgement. The size of each tick was determined by measuring the length.

Tick PCR:

Tick extraction for PCR analysis.

DNA of tick halves stored frozen at – 20^{o} C were extracted with the Qiagen Dneasy blood and tissue kit using the standard protocol for animal blood and cells on the Qiacube (Qiagen,Venlo, Limburg, Netherlands). PCR amplification was conducted as described above for blood samples.

Questionnaire

A questionnaire designed to address putative risk factors for introduction and spread of disease onto the property was filled in with the manager of the cattle on each property (appendix 1).

Results

Smear Examination

Stain precipitate in a significant proportion of smears rendered interpretation of blood smear examinations invalid.

Cattle blood - PCR

Of the 309 cattle bled, 106 (34%) were positive in the group test for *Theileria orientalis*.

70 cattle (23%) were positive with the specific test for *T.orientalis* Ikeda. Positive cattle were found on 14/31 (45%) farms. (figures 1 and 2).

10 cattle (3%) were positive with the specific test for *T.orientalis* Chitose. Positive cattle were found on 2/31 (6%) of farms.

On one farm, one animal had a dual infection with *T.orientalis* Ikeda and *T.orientalis* Chitose. On this farm, another three cattle were positive for type Ikeda only and four cattle were positive for type Chitose only.

No cattle were positive with the specific test for *T.orientalis* Buffeli.

Twelve cattle (4%) from four farms (13%) were positive in the group test but negative in the typing tests. On one farm, all ten animals had this result. On one farm, only one animal had this result, all others were negative. On one farm only one animal had this result but six other animals were positive for type Ikeda.

Using these results, we calculate a prevalence rate of 30-70% for *T.orientalis* on farms in the Denmark shire. 30-70% for *T.orientalis* Ikeda, <30% for *T.orientalis* Chitose and <30% for *T.orientalis* Buffeli.

Type Ikeda was found over a wide range through the shire.Type Chitose was found on only two farms within 5km of each other in the west of the shire (fig 3).

Figure 1. PCR results - cattle

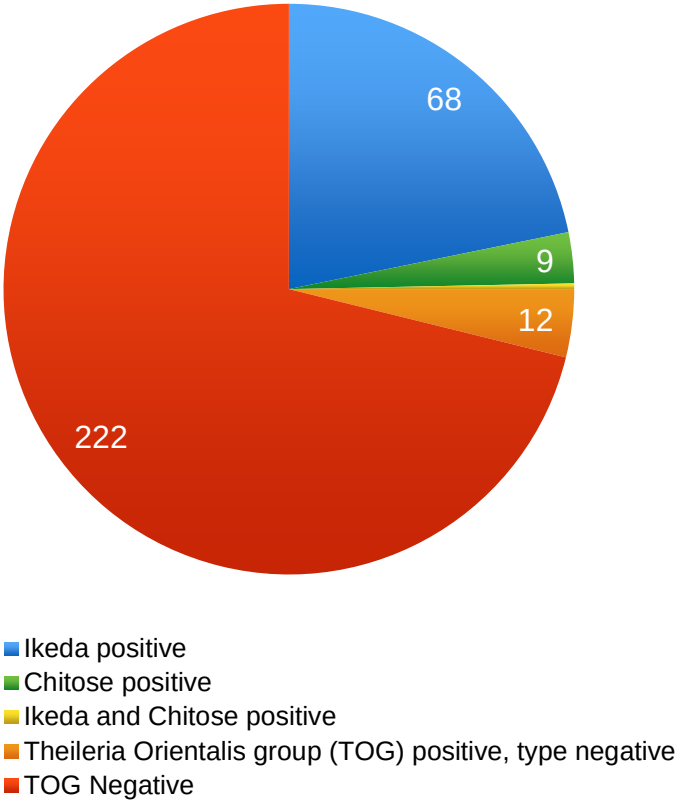
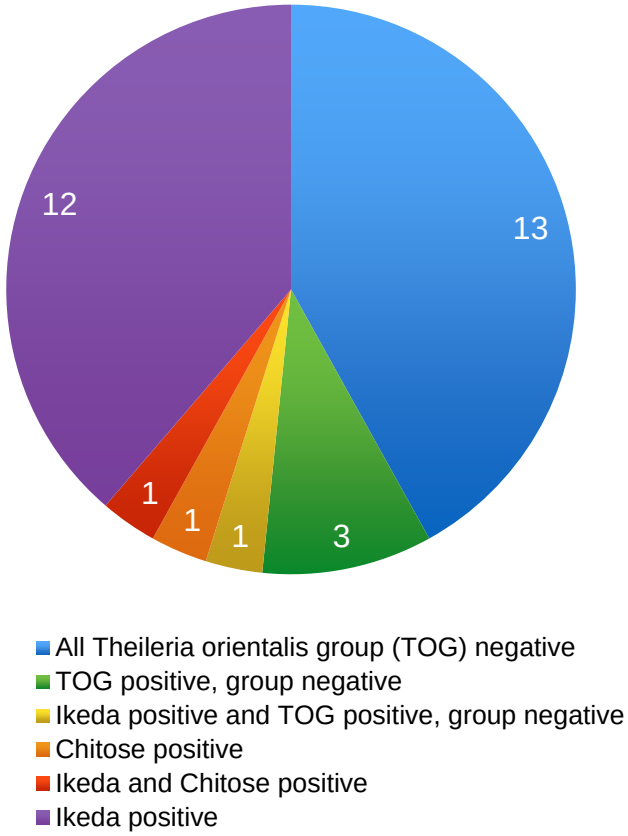


Figure 2. PCR results - status by property



Ticks:

Ticks were detected on 18 of the 31 survey properties. Ticks were found on cattle on 16 of these 18 properties. On one property ticks were only found questing (detected by flagging) and on one property the only tick found was detected by the farmer on his dog.

H longicornis ticks were found on 17/31 farms and *I australiensis* were found on 10/31 farms. Both ticks were found over a wide range through the shire (fig 3).

Over the summer of 2014-15 ticks from another 16 properties were opportunistically collected either by the local veterinarian or during farm visits for disease investigations. Most of these ticks found on non-survey properties were on cattle but on two properties ticks were detected by flagging and trapping only.

The most successful way to collect questing ticks was through flagging (on 4 of the 31 survey properties and on 3 other properties) followed by netting (2 on survey properties and one on a non-survey property). Carbon dioxide trapping of ticks was successful on one (non-survey) property only. However, it was interesting that on this property, ticks were only found by trapping and flagging and not on cattle although *T.orientalis* had been detected in cattle previously.

A total of 305 individual ticks were collected during the course of the project. Most were collected on the survey properties (203 ticks) and the remaining were opportunistically collected during the

course of disease investigations or were submitted by private veterinarians. The majority of the ticks were collected off animals.

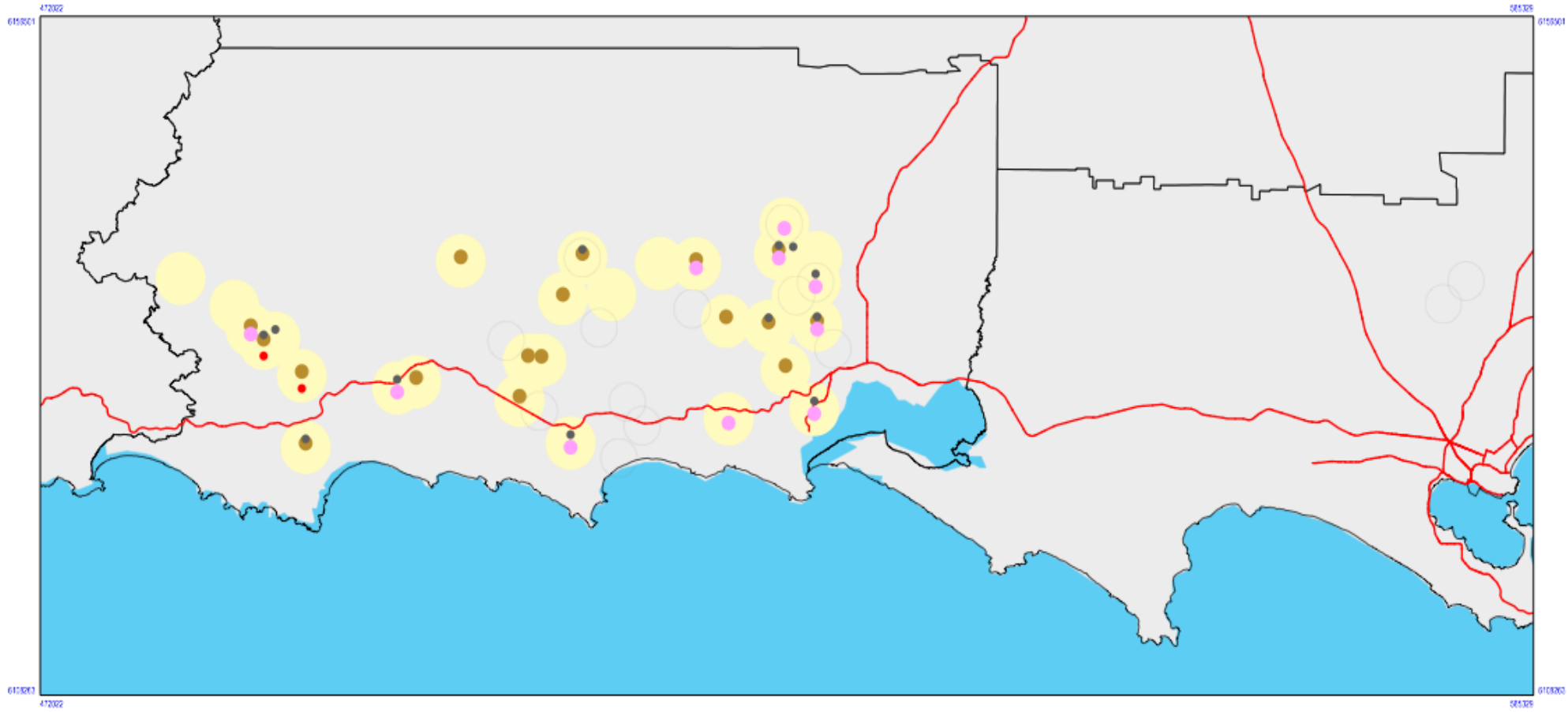
Table 1. Types of *T.orientalis* and species of ticks on survey properties

Number of properties with	*Haemaphysalis longicornis*	*Ixodes australiensis*	*H longicornis and I australiensis*	no ticks found	total
***Theileria orientalis* group (TOG) negative**	6	2	1	4	13
Ikeda positive	3	4	2	3	12
Chitose positive	0	1	0	0	1
Ikeda and Chitose positive	1	0	0	0	1
Ikeda positive and TOG positive, group negative	1	0	0	0	1
TOG positive, group negative	1	0	1	1	3
total	12	7	4	8	31

All *H. longicornis* ticks were either nymphs (13 ticks,) or females (265 ticks) with no male ticks being found. The collection of *I. australiensis* on the other hand included 4 males, 7 nymphs and 20 females. No tick larvae of any species were found during the course of the investigation. Nymphs were found throughout the time of the survey. There was no seasonal pattern to the size of the ticks (figure 4). Only about 10% of the ticks (32 ticks) were engorged although the majority were collected off animals.

To this date 100 ticks including 81 ticks collected from all cattle that tested positive for Theileria were examined by PCR for the presence of Theileria DNA. All tested negative.

Theileria orientalis, Haemaphysalis longicornis and Ixodes australiensis survey in cattle in the Denmark shire - 2014/15



Legend

- *T. orientalis* Ikeda infection
- *T. orientalis* Chitose infection
- Scrub tick
- Bush tick
- Confirmed clinical cases
- Sample properties
- LGA boundaries
- Major roads and highways

N

0 4 8 12 16 20

Kilometres

Department of Agriculture and Food

Compiled by: DAFWA - Geospatial Information Services Group

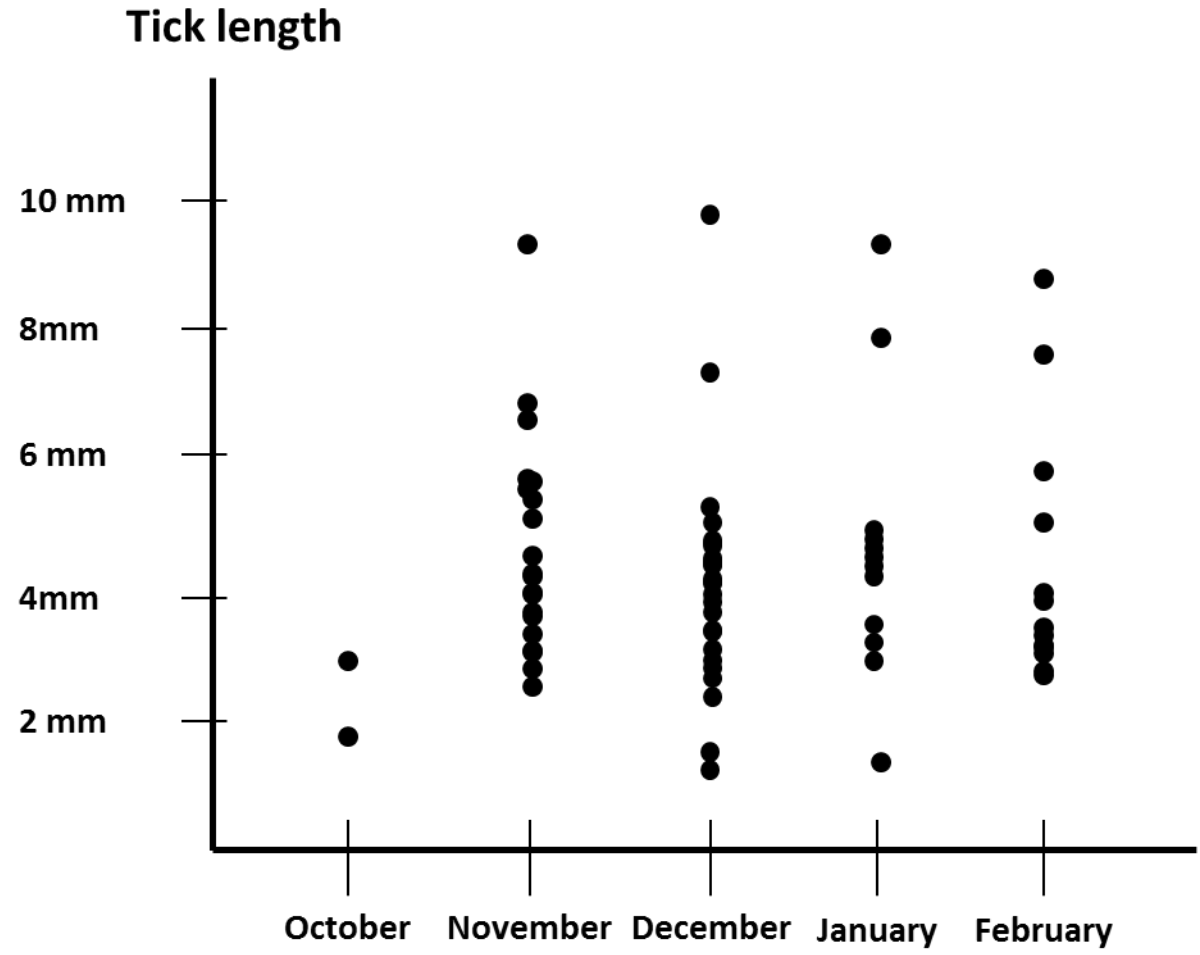
Date: 11 May 2015 JOB 2015138

Projection: Universal Transverse Mercator, Zone 50

Horizontal Datum: Geocentric Datum of Australia 1994

Vertical Datum: Australian Height Datum 1971

Figure 4. Tick size (length in mm) in relation to collection time (month of the year)



Questionnaire

The Mantel-Haenszel Chi-squared test for stratified tables was used to see if the introduction of cattle in the last two years (2013 and 2014) was a significant risk factor in infected herds. Introduction of cattle in the last two years was a risk factor in infected cattle herds - the odds ratio was 5.07 (1.43-18.03), p= 0.028.
The introduction of cattle was a significant risk factor in 2013 - odds ratio 6 (1.0025 to 35.9089) p = P = 0.0497 but introduction of cattle in 2014 was not associated with increased risk.

Other possible methods of introducing *Theileria orientalis* into a herd – pregnancy testing and use of a vet – were not significant risk factors.

Factors that were thought could potentially increase the spread of *Theileria orientalis* within the herd – marking method, hygiene, or lice – were not significant risk factors.

The acres-per-cow and the average density on infected herds of 3.1 acres per cow was similar to non-infected of 3.4 acres per cow and stocking density was not a significant risk factor.

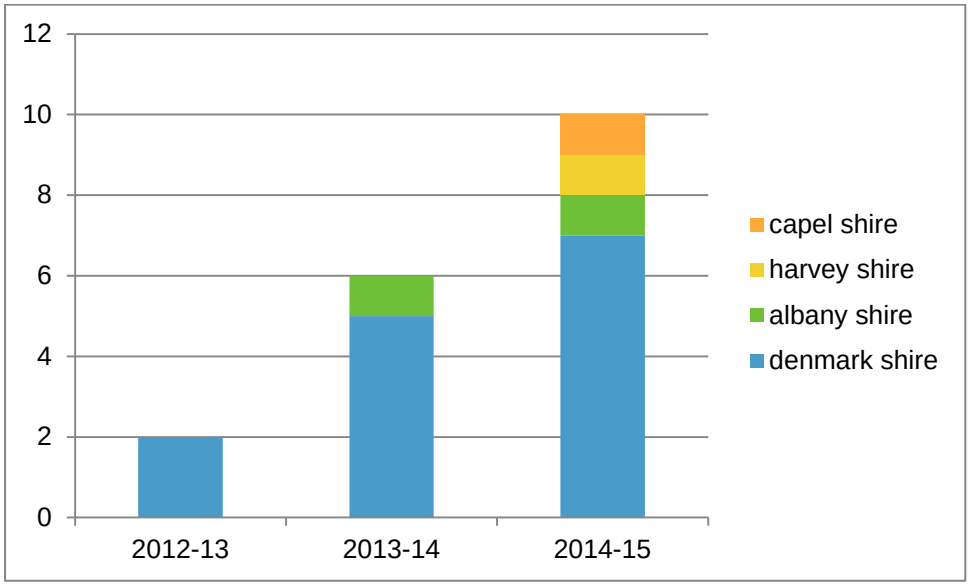
Discussion

Anaemia as a syndrome in adult cows is distinctive and unusual in the WA context. We believe that BATOG probably would have been reported to veterinarians early on in the course of an epizootic. In addition to the six cases confirmed before the commencement of the study, a further nine cases were diagnosed (including on two of the properties that were surveyed) by the end of the summer of 2014-15 suggesting that the survey had been conducted close to the beginning of an epizootic (fig 4). By the time of completion of the survey, disease awareness was much improved and we received a number of anecdotal reports of disease that were not investigated. Consequently, the number of farms on which there have been clinical cases is probably greater that the number on which laboratory testing had been conducted. Cases have now been diagnosed in the Albany, Harvey and Capel shires.

We concluded that *T.orientalis* type Ikeda infection was present on 30-70% of farms in the Denmark shire. In addition, considering that all of the clinical cases of BATOG from the Denmark shire diagnosed in the DAFWA laboratories consisted of only Ikeda type infection, we concluded that *T.orientalis* Ikeda infection was widespread in the shire even though only a low number of clinical cases had been detected at the commencement of the survey (fig 3).

Figure 5

Cases of BTOG diagnosed at the Animal Health Laboratory DAFWA



T.orientalis type Chitose was detected on two farms indicating that on less than 30% of farms in the Denmark shire, cattle are infected with Chitose type. On one farm, dual infection with Ikeda and Chitose types was noted. This result indicates that on less than 30% of farms cattle are infected with both types.

In other Australian states, different rates of infection and type prevalences have been noted. For example in a 2010-11 in a survey of herds in Queensland, NSW and Victoria, where clinical BATOG was not present, survey samples from 516 cattle in 50 herds were analysed using the same group

PCR as used in our survey. *T. orientalis* prevalence was 23.7% in NSW, 56.8% in Queensland and 34.0% in Victoria with variability among regions of each state. Chitose type was the most common and widespread type (19.1–43.7%), with Buffeli present in all states at a lower prevalence (10.8–24.8%). The prevalence of Ikeda type infection was variable; it was present in three of five regions in Queensland (North, South and South East; prevalence 3.4-15.4%), in only one of the surveyed regions in NSW (North Coast; prevalence 74.2%) and in only one animal in Victoria [6].

In contrast a 2010-11 study of 73 herds in NSW with animals clinically affected by BATOG, 88% tested positive to Ikeda type and 54% of cattle tested positive to Chitose type. In this study, 52% of cases had dual infections with Ikeda and Chitose types, and Buffeli type was found in 19% of cattle[17].

In a study conducted in Victoria between 2010-2012, testing of 301 blood samples from 19 farms on which BATOG had been diagnosed between 2010 and 2012 using a different PCR test directed against the same gene as the PCR used in this study detected *T.orientalis* DNA in 70.8% (213/301) of the samples. Genotypes Ikeda, Chitose, Buffeli and type 5 were detected in 91.1%, 32.9%, 2.4% and 1.4% of 213 blood samples, respectively. The distribution of these four genotypes varied among the 19 farms. The genotype combinations chitose/ikeda , buffeli/ikeda, chitose/ikeda/buffeli, chitose/ikeda/type 5, and buffeli/chitose/ikeda/type 5 were detected in 21.6%, 0.47%, 2, 0.47% and 2 of the 213 samples tested, respectively [7].

It is difficult to compare our own results with these surveys as different cohorts of animals are being studied. However, in a NSW survey published in 2013, in response to a perceived increase in BATOG cases (prevalence not stated) a stratified random survey similar to our own was done using Geimsa stained blood smears from beef cattle in the northern tablelands of New South Wales to estimate *Theileria* infection prevalence. *Theileria* organism were seen in smears from 33 of the 46 herds sampled, which gave a herd prevalence of 72% for this study. Approximately 18% of herds were in the medium or high prevalence group using criteria whereby herd prevalence was deemed zero, low, medium or high with 0/10, 1-3/10, 4-6/10 and 7-10/10 smears positive respectively[18]. Using these criteria and our positive pcr results, our overall prevalence rate is 61% with 19% of properties in the medium to high prevalence categories.

Our survey was conducted when there was a low level of clinical disease yet there is widespread infection. However, the rate of infection with multiple types of *T.orientalis* in the Denmark shire appears remarkably less than that seen in NSW and Victoria and we did not detect Buffeli type infection in this survey. Types other than Ikeda have also not been detected in any of the clinical cases submitted to DAFWA laboratories indicating a different infection dynamic in WA.

Our results showed that the bush tick, *H.longicornis*, the putative vector of *T orientalis* Ikeda, is present on more than half of the farms and was widely distributed through the Denmark shire. The scrub tick *I. australiensis* was also widespread but not as numerous as *H.longicornis*. Active stages of *H.longicornis* feed for only 3-14 days on their host and conditions suitable for adult tick development are probably only present from October to February in south coastal WA (pers comm Besier). So our examination of cattle for ticks during a very limited period is probably **not** a highly sensitive indicator of their presence.

Work in the 1980s showed that *H.longicornis, bancrofti* and *humerosa* can transmit *T.orientalis B*uffeli (then known as *T.buffeli*). More recent work has shown that the tick identified as *H.humerosa*

was actually *T.bremneri* [19]. The only species of *Haemaphysalis* identified in our survey was *H longicornis. H.bancrofti* is a common tick on cattle in NSW [18]. It seems unlikely that *H.bancrofti and bremneri*, which are subtropical species, are found in the south west of WA. In Japan where *T orientalis* Ikeda was first identified as a cause of severe cases of BATOG, *T. orientalis* ikeda DNA has been detected in three *Haemaphysalis* (including *H.longicornis*) species and two *Ixodes* species [20]. A range of *Theileria* species has been detected in wildlife in Australia but their vectors have not been studied extensively. These marsupials are from a range of ecosystems indicating that other vectors capable of transmitting *Theileria* are present in the Australian context [21].

One study in the 1980s failed to transmit *T.orientalis* (presumably Buffeli) using *H longicornis* [4]. Considering the long history of cattle movements from the eastern states to the south west of WA, it seems likely that the Buffeli type would have been imported previously but no evidence of infection has been found either in this survey or samples taken from clinical cases of BATOG. There are a number of possible reasons for this, one of which is that there is no competent vector capable of transmitting *T.orientalis* Buffeli in the south west of WA. However *T.orientalis* Buffeli is present in New Zealand where *H.longicornis* is the only tick found on cattle[18]. This suggests that at least some *T.orientalis B*uffeli can be transmitted by *H longicornis* or that there is an alternative vector in NZ. In eastern Australia, it is suggested that *H. bancrofti* is the main vector of *T.orientalis* Buffeli[19].

H.longicornis was found on one of the two farms where *T.orientalis* Chitose was found but no ticks were found on the other property.

We were not able to demonstrate the organism in ticks collected in the course of our survey. This could be due to the ticks not being infected, but perhaps more likely, the sensitivity of the test may not be adequate to detect infection. Detection of *T.orientalis* DNA in Australian ticks has been successful using real time PCR which has proven more sensitive at identifying clinically affected animals[13] but this test was not available to us.

Analysis of responses to our questionnaire revealed that the only risk factor associated with the presence of disease was the introduction of cattle. Introduction of cattle during 2013 was also a significant risk presumably because of the already widespread distribution of the organism and its potential to spread through animal movements.

BATOG is now an established disease in WA and will continue to cause disease over the range of the tick vector. How widespread the tick is, particularly on the west coast is not known. After the survey was completed, cases of BATOG were diagnosed in the Albany, Harvey and Capel shires and on the two Albany properties affected, *H.longicornis* ticks were identified. This information and previous reports of *H.longicornis* from farms in the Harvey area suggest that the distribution of the tick and therefore the susceptible cattle population is more widespread than originally anticipated. Ticks could temporarily survive long enough to transmit the disease in a suitable ecosystem in the cooler months. Whether they can establish a breeding population away from the south coast is not known. Future research may concentrate on better understanding the distribution of the bush tick on the west coast.

Extension of results

Presentation to DAFWA staff lunchtime 5/2/15

Preliminary results of findings emailed and posted to participants 25/3/15

Public seminar for farmers at Denmark Country Club 8/4/15

Presentation to DAFWA veterinary staff 26/5/15

Presentation at one day theileria workshop at the pan pacific veterinary conference Brisbane 29/5/15

Presentation to southern veterinary group 16/6/15

Presentation to south west veterinary group 27/6/15

ABC country hour interview 20/8/15

References

1. . Izzo MM, Poe I, Horadagoda N, De Vos AJ and House JK. Haemolytic anaemia in cattle in NSW associated with *Theileria* infectionAust Vet J 2010;88:45–51

2. Islam MK, Jabbar A, Campbell BE, Cantacessi C and Gasser RB. Bovine theileriosis – An emerging problem in south-eastern Australia? Infection, Genetics and Evolution 11 (2011) 2095–2097

3. Hammer JF, Emery D, Bogema DR and Jenkins C. Detection of Theileria orientalis genotypes in Haemaphysalis longicornis ticks from southern Australia. Parasites 5 Vectors (2015) 8:229

4. Stewart NP, Standfast NF, Baldock FC, Reid DJ and DeVos AJ. The distribution and prevalence of Theileria buffeli in cattle in Queensland. Aust Vet J 1992;69: 59-61

5. Callow LL. Bovine Theileriosis in Australia. In: Animal Health in Australia Volume 5 Protozoal and Rickettsial Diseases Australian Bureau of Animal Health, Canberra. 1984:169-173.

6. Eamens GJ, Bailey G, Gonsalves JR, Jenkins C. Distribution and temporal prevalence of Theileria orientalis major piroplasm surface protein types in eastern Australian cattle herds. Aust Vet J. 2013;8:332-340

7. Perera PK et al. Epidemiological survey following oriental theileriosis outbreaks in Victoria, Australia, on selected cattle farms. Veterinary Parasitology 197 (2013) 509–521

8. Bailey, G. http://www.csu.edu.au/__data/assets/pdf_file/0016/1351024/2012-BFD-Theileria-Wagga-Beef-Forum-August-2012.pdf accessed 19/7/15

9. Perera PK, Gasser RB1, Firestone SM, Anderson GA, Malmo J, Davis G, Beggs DS and Jabbar A. Oriental theileriosis in dairy cows causes a significant milk production loss. Parasit Vectors. 2014;19:7:73.

10. McFadden AMJ et al. An outbreak of haemolytic anaemia associated with infection of Theileria orientalis in naïve cattle. New Zealand Veterinary Journal 2011;59;79–85

11 Besier RB and Wroth R. Discovery of the tick Haemaphysalis longicornis in Western Australia. Aust Vet J 1985;62:205-206

12 Cannon RM and Roe RT. Livestock disease surveys: A field manual for veterinarians. Australian Government Publishing Service, Canberra, 1982.

13 . Bogema DR, Deutscher AT, Fell S, Collins D, Eamens GJ and Jenkins C.Development and Validation of a Quantitative PCR Assay Using Multiplexed Hydrolysis Probes for Detection and Quantification of Theileria orientalis Isolates and Differentiation of Clinically Relevant Subtypes. J. Clin Microbiol 2015;53:941-950

14. Roberts FHS. Australian Ticks, CSIRO publishing Australia 1970.

15. Barker SC and Walker AR, ZOOTAXA 3816 Ticks of Australia. The species that infest domestic animals and humans Magnolia Press Auckland New Zealand 2014.

16. Roberts FHS. A systematic study of the Australian Species of the genus Ixodes (Acarina: Ixodidae) Aust J Zool 1960;8:392-485

17. Eamens GJ, Gonsalves JR, Jenkins C, Collins D, Bailey G.Theileria orientalis MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. Vet Parasitol 2013;191:209-17.

18 Biddle A et al. A survey to determine the prevalence of Theileria spp. in beef cattle in the northern tablelands of New South Wales Aust Vet J 2013;91:427–431

19. Allen CG. Theileriosis and ticks; the role of the vector, and its control. Presentation at Australia/New Zealand workshop on Bovine Theileriosis, Pan Pacific Veterinary Conference, Brisbane 2015.

20. Yokoyama N et al Genetic diversity of Theileria orientalis in tick vectors detected in Hokkaido and Okinawa, Japan. Infection, genetics and evolution 2012;12:1669-1675

21. Lee JY1, Ryan UM, Jefferies R, McInnes LM, Forshaw D, Friend JA, Irwin PJ. Theileria gilberti n. sp. (Apicomplexa: Theileriidae) in the Gilbert's potoroo (Potorous gilbertii). J Eukaryot Microbiol 2009;56(3):290-5.

Appendix 1

Questionnaire protocol

Theileriosis – initial phone calls to randomly selected participant (Identifies farm suitability and preparedness to participate?)

Hello ….

- DAFWA veterinarians are investigating a new disease in cattle called 'Theileriosis'. This is to be done by collecting information by questionnaire, cattle blood samples and the collection of ticks from pastures. The sample points are randomly selected properties in the Denmark Shire. Information gained will help us work out the extent and spread of the disease. Theileriosis, is a disease that affects only cattle and has only been found in the Denmark Shire in the last 2 years. (post out information sheet on the history of disease and this project)
- Participating farmers will be advised of the findings on their farms as soon as information on Theilerisosis infection is known. All information gathered from your property will remain confidential. DAFWA has won funding from the Cattle Industry Compensation Fund to carry out this work, after competing with other research proposals.
- Your property has been randomly selected and I would like to ask a few questions to work out whether you are able to participate?

Eligibility Criteria

- You can participate by allowing us to collect bloods from ten mature cows that have been on the property for 3 or more years? Do you have cattle meeting these criteria?
- Participation will require responding to a short questionnaire about cattle and ticks to help provide information on the spread of disease and occurrence of ticks which will improve understanding of the disease in the Denmark area.
- Cattle yards and a crush will be needed. Do you have cattle handling facilities and a crush? Blood collection will entail a visit by a Department veterinarian. You will be asked to bring the agreed mob of cattle to the yards for blood collection and this will likely take a couple of hours on the day of the visit.
- Tick collection involves walking over the property in areas like creek lines, bushy undergrowth, pathways where wildlife traverse, pasture paddocks and cattle camp areas with flags, sweep nets and the positioning of a tick trap at two sites for 20 minutes each. An opportunity to examine dogs and horses that live on the property for ticks would be useful. The aim is to collect and identify ticks that are present. Would you be open to facilitating that on your farm?
- Would you like to participate?
- A visit to the farm will be required to carry out blood collection from cattle and tick collections from pasture.
- Under DAFWA animal ethics requirements we need assurance you had the capacity and experience to successfully euthanase an animal were it necessary. Do you have a firearm suitable for euthanasing cattle and would you be prepared to do this? Would you be willing to sign an agreement to this effect?

- Can I make a day and time appointment for the visit. (contact David Forshaw on 98928471or Jenny Cotter of 98928421).

Theilerosis Survey Questionnaire 2014 2015

Disease Events

1. In the last 2 years have you had any deaths in cows on this property? (please circle / indicate)

   a) 2013 –yes / no……,
   b) 2014 –yes / no.....……..,

2. If yes did you use a vet to investigate the cause of death in these cows – yes / no

3. If yes for using a vet – was it a DAFWA or private vet (circle)

4. When did these deaths occur?

   a) name months in 2013? …….
   b) name months in 2014? ……

5. Were any of these cow deaths
   a) within three months of calving?- yes/no,……. if yes indicate which months?.....
   b) or at calving? -yes / no, ……..if yes indicate which months?.....
   c) or three months after calving? Yes / no,……if yes indicate which months……
   d) or in mature cows in early pregnancy? –yes / no……If yes indicate which months? .....

6. In the last 2 years on this property have you had any deaths in young cattle? Yes / no …….
   a) if yes at what ages did the death occur? .....

7. In the last 2 years on this property have you seen unexplained symptoms in cattle on your property/ Yes / No

If yes were any of these symptoms seen in your cows?

a)

- pale/ white colouration of gums –yes / no…….

- pale / white colouration of tissues of the eye – yes / no

- pale / white colouration of the vulva – yes / no……

.

- abortion – yes / no……

- laboured breathing – yes / no……

- not eating – yes / no ……

- loss of body condition yes / no ……

- wobbly gait – yes / no ……..

- falling behind the mob when being moved – yes / no ……

- separation from the mob -yes / no……..

- yellow colouration of skin e.g. udders – yes / no……..

- red coloured urine yes / no……..

-eating soil yes / no……..

Herd health and Husbandry

8. In last two years on this property have you had cattle pregnancy tested? Yes /no……
    a) If yes by vet – yes / no
    b) if yes by lay tester – yes / no
    c) if yes by yourself / family member – yes / no
9. In the last two years on this property have private vets undertaken any surgical procedures on your cows yes / no……..
10. If yes – what type of procedures were undertaken?
    …….
11. Do you undertake synchronising / insemination on this property? yes / no ……..
    A) if yes is this done by ET technicians who attend the farm? yes / no.....
    a) if yes is it done by self or family member? yes / no.

…….....
12. Do other husbandry personnel attend the farm? – yes / no
    a) If yes – who? .....
13. Do you mark calves individually? yes / no…….
    a) if yes how are implements cleaned? …….
    b) if no do you carry out mob calf marking? yes / no……..
    c) if yes how are implements cleaned?.....
14. Do you vaccinate your calves or cows? – yes / no…….
    a) if yes are vaccination guns used? yes / no……..
    b) or if by syringe, is a new needle used for each injection? yes / no …….
15. Are syringes or needles ever re-used on the farm? Yes / no
    a) if yes when does this happen? .....
16. At calving assistance, are chains, ropes and calf pulling equipment cleaned between use? yes / no
    a) if yes how are they cleaned .....
17. In the last two years have cattle lice occurred on this property? yes / no ……
    a) if yes have you treated for lice? yes / no.....

Breeding patterns

18. On this property how many cows are over 2 years of age? .....
20. On this property in which month does calving begin? …….

Stocking

21. How many acres are available for grazing? ……..

Normal Movements

22. Have you introduced cattle to this property
    a) in 2013? Yes / no
    b) if yes what was the source?.....
    c) or 2014? Yes / no
    d) if yes what was the source?.....
23. Have you ever imported cattle to this property from another state – yes / no
    a) If yes - which year and what was the source? .....

Other animal movements

24. In the last 2 years have horses, sheep, goats, alpaca, deer, camel, buffalo moved onto this property? Yes / no

    .....

    a) if yes are they one off movements? Yes / no……

    b) regular back and forth movements yes/no ……..

25.In the last 2 years have horses, sheep, goats, alpaca, deer, camel, buffalo moved off this property to another location? Yes / no

……

Tick observations

26. Have you seen ticks on cattle or other animals on this property in the last 2 years? yes / no.

    .....

27. On this property has there ever been a time when you noticed massive numbers of ticks on? Yes / no

    a) if yes in which year did this happen? ……

Would you like to add anything?

.....

.....

.....

.....