An assessment of Leptospiral infection in livestock associated with contact frequency with wildlife in the Manawatu region in New Zealand

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Abstract

Ongoing research in the host laboratory in New Zealand has highlighted that wild animals are a likely source of leptospiral infection in livestock populations, leading to ineffective herd-level vaccination and maintained leptospirosis infection. This has impact on food production, abortion rates and leads to the increased risk of leptospiral spill-over into human populations. The goal of this research project was to estimate the contact frequencies between livestock and wildlife in the Manawatu region, to quantify the prevalence of different serovars of *Leptospira* in the associated livestock and to determine whether correlation exist between Leptospiral prevalence and wildlife contact frequency. Motion-sensitive cameras were installed around two farms to capture contact between livestock and wildlife and analysed to evaluate the number of contacts over time and thereby estimating contact frequency. At the same time, blood and urine samples were taken from livestock on these farms, and laboratory-based microscopic agglutination testing (MAT) and urine culturing were used to demonstrate which serovars of *Leptospira* were present.

The camera data showed direct and indirect contact possible between deer and livestock and indirect contact between possums and livestock in the beef & sheep farm. In the dairy farm mainly deer were observed and, according to the camera data, chances for direct contact between deer and livestock would be very low. Indirect contact would still be possible, but with a 25 times lower chance than on the beef & sheep farm. Possums were not observed at the dairy farm. Twice as many rats were observed on the beef & sheep farm, whereas mice were more abundant on the dairy farm. The serovars that were mainly observed in the beef & sheep farm as well as the dairy farm were Hardjobovis and Pomona. Both serovars have deer as maintenance hosts. There is a possibility that both serovars can be transferred from wild deer to the livestock, with a 25 times higher chance to occur in the beef & sheep farm. The difference for the different serovars between the two farms were small and did not exceed over 15%. Serovars Pomona and Ballum gave a higher titre in the dairy farm, serovars Hardjobovis, Copenhageni and Tarassovi in the beef & sheep farm. Overall the seroprevalence for Hardjobovis in both farms was the highest, followed by Pomona. The seroprevalence for Copenhageni was low in both farms, with no significant difference. For Tarassovi the seroprevalence for the beef & sheep farm was twice as high as for the dairy farm, whereas this was the other way around for Ballum. Further work investigating the prevalence of different leptospiral serovars in wildlife will be necessary to truly interpret the risk associated with exposure to wildlife.

1. Introduction

1.1 Leptospirosis

Leptospira are motile, obligate aerobic spirochetes with an optimal growth temperature of 28-30°C. Historically, serological taxonomy divided *Leptospira* into two species: *L. interrogans* (pathogenic) and *L. biflexa* (non-pathogenic). Based on surface antigens, the pathogenic species can be split into 24 serogroups and over 250 serovars (Cerqueira & Picardeau 2009). Genetic and genomic classification schemes also divide *Leptospira* variants into a number of different genomospecies, but serovar-based taxonomic references will be used throughout this report (Levett 2001).

Leptospira can persist in humid environment for months to years (Miller, Wilson & Beran, 1991). Survival is variable between strains, with the *L. interrogans* serovars being more adapted to environmental survival than the *L. borgpetersenii* serovars (Bulach et al. 2006). *Leptospira* are able to survive in alkaline soils, mud, swamps, streams, rivers and even in diluted milk. Survival of pathogenic *Leptospira* depends on factors as pH and temperature. In general, they are sensitive to heat, dryness, acid and basic disinfectants (Faine et al. 1999). High rainfall and flooding are favourable for leptospirosis outbreaks, which occurred in sheep flocks in New Zealand after floods (Jackson et al. 2005).

The most common serovars in livestock in New Zealand are *L. borgpetersenii* serovar Hardjobovis and *L. interrogans* serovar Pomona. Hardjobovis is regarded to be host-adapted and therefore subclinical in cattle (Marshall & Manktelow 2002). Sheep are considered to be sporadically infected with Hardjobovis, but recent research shows an increasing occurrence which may be an indication that Hardjobovis becomes adapted to sheep as well (Dorjee *et al.* 2008). *L. interrogans* serovar Pomona sporadically infects cattle and sheep with occasionally high lamb or calf mortality and abortion (Dreyfus & Wilson 2013).

Apart from Pomona and Hardjobovis there are four other endemic serovars of Leptospirosis in New Zealand. All the endemic serovars and their known (maintenance) hosts are listed in table 1.

Table 1. The endemic serovars in New Zealand and their known (maintenance) host animals (Ayanegui-Alcerreca et al., 2007; Dorjee et al., 2008; Marshall & Manktelow, 2002)

Serovar	Maintenance host animals
L. borgpetersenii Hardjobovis	Cattle, deer, sheep
L. interrogans Pomona	Pig, deer
L. interrogans Copenhageni	Norway rat
L. borgpetersenii Ballum	Black rat, mouse, hedgehog
L. borgpetersenii Balcanica	Possum
L. borgpetersenii Tarassovi	Pig

Infection can occur through exposure to urine or aborted tissue of infected animals, or indirectly through contact with contaminated surface water or soil (Hartskeerl *et al.* 2011). Infection with *L. interrrogans* serovars is usually acquired from contaminated surface water or soil, whereas infection with *L. borgpetersenii* usually requires host-to-host transmission (Bulach *et al.* 2006). *Leptospira* enter the host either through cuts or small abrasions of the skin, wet hands which causes weakening of the skin integument, mucous membranes and the genital tract (Faine *et al.* 1999).

The clinical signs of Leptospirosis vary with the grade of adaption of the serovar type to the infected species, virulence of the serovar and the current immune status of the host (Heath & Johnson 1994). Clinical signs in accidental hosts can range from mild to severe icterohaemorrhagic disease, anorexia

and can cause stillbirth or abortion (Vallée 2016; Subharat 2010). Subclinical signs involve poorer growth rate and conception rates (Vallée 2016; Subharat 2010). Maintenance hosts, where the adaption of the serovar is high to that host, usually do not develop clinical signs, but may become chronically infected and shed *Leptospira* for months or years (Smith et al. 1994; Ayanegui-Alcerreca *et al.* 2007). However, leptospirosis is a dynamic disease and strains adapt to new hosts with ecological shifts or shifts in farming practice (Hartskeerl *et al.* 2011).

Leptospirosis is a zoonotic disease and humans are considered as accidental hosts. There is very little evidence of shedding by humans. Direct human-to-human transmission is therefore negligible (Haake & Levett 2015; Levett 2001). In New Zealand, serovars Hardjobovis, Pomona and Ballum are the most common cause of human leptospirosis (Mansell & Benschop 2014). However, recent changes in the incidence of human disease caused by the serovars Ballum and Tarassovi have prompted additional research into the roles of wildlife species in the epidemiology of leptospirosis (Dr. David A. Wilkinson. 2016). Humans can be infected by contact with infected urine. (Mansell & Benschop 2014). Human leptospiral infections in endemic areas can be mild or asymptomatic. Development of more severe outcomes depend on the host susceptibility, epidemiological conditions and pathogen virulence. Clinical signs that can be observed are a severe headache, conjunctival suffusion, subconjunctival haemorrhages & icterus and renal failure. The combination of the last two is also known as Weil's disease. Organ failure can also include the lungs, liver and brain and these complications can result in death. Approximately 70,000 deaths occur worldwide from leptospirosis every year. (Haake & Levett 2015).

1.2 Current situation in New Zealand

An overview of cross-sectional studies concerning the current seroprevalence of leptospirosis in pastoral livestock at both the animal level and farm level has been given in table 1-2 of Vallée (2016). Hardjobovis prevalence in sheep ranged from 5% to 43% at the animal level (Dorjee *et al.* 2008; Dorjee *et al.* 2011) and 33% to 91% at the farm level (Dorjee *et al.* 2011; Dreyfus & Wilson 2013). Pomona in sheep ranged from 0% to 20% at the animal level (Subharat *et al.* 2007; Fang *et al.* 2014) and 0% to 74% at the farm level (Subharat *et al.* 2007; Dreyfus *et al.* 2011). Hardjobovis prevalence in beef cattle ranged from 34% to 55% at the animal level (Heuer 2007; Subharat *et al.* 2007) and 62% to 92% at the farm level (Heuer 2007; Dreyfus *et al.* 2011). Pomona prevalence in beef cattle ranged from 2% to 25% at the animal level and 0% to 72% at the farm level (Subharat *et al.* 2007; Dreyfus *et al.* 2011).

In a multi-species cross-sectional farm-study on 238 farms in New Zealand, 97% of the sheep and beef farms had at least one out of twenty animals seropositive for Hardjobovis and/or Pomona. Overall, 50% of adult sheep and 58% of adult beef were positive for either serovar (Dreyfus & Wilson 2013). Both farm and animal seroprevalence increase with the age of the animal.

In 2013, 59 human cases of leptospirosis were notified in New Zealand, of which 18 were farmers or farm workers (The Institute of Environmental Science and Research Ltd. 2013). According to the PhD thesis written by Dreyfus, this number is likely underestimated by approximately forty fold (95% CI 16-56) (Dreyfus & Wilson 2013). This is because human leptospirosis usually remains undiagnosed, or is diagnosed too late because of its non-specific clinical signs. Therefore prevention is the most efficient control measure. There is no human vaccination available in New Zealand, so prevention should be realised by protection from urine of infected animals and prevent the animals from shedding (Mansell & Benschop 2014).

Ongoing research in PhD projects by Moinet (unpublished data), Yupiana (unpublished data) and Valléé (2016) in the host laboratory has highlighted that wild animals are a likely source of leptospiral infection in livestock populations, leading to ineffective herd-level vaccination and maintained

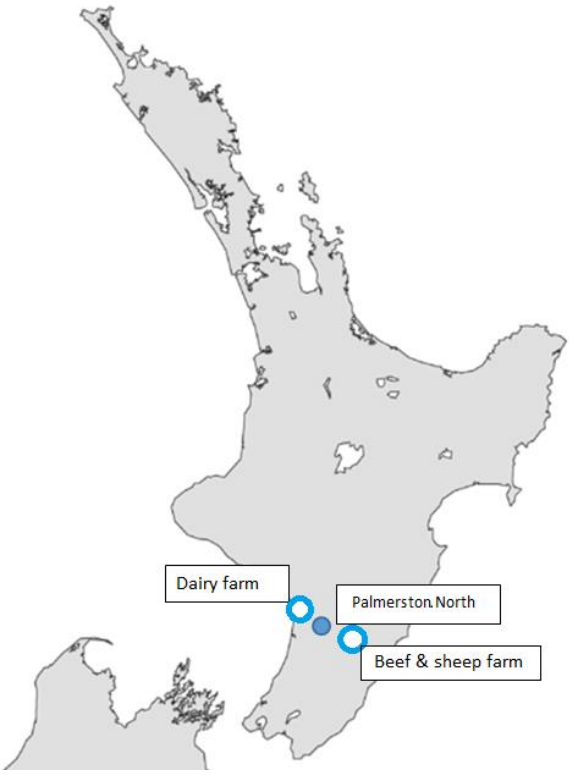
leptospirosis infection. This has impact on food production, abortion rates and leads to the increased risk of leptospiral spill over into human populations (Dr. David A. Wilkinson. 2016).

The goal of this research project is to estimate the contact frequencies between livestock and wildlife in the Manawatu region, that have contrasting surrounding habitats, to quantify the prevalence of different serovars of *Leptospira* in the associated livestock and to determine whether correlation exist between Leptospiral prevalence and wildlife contact frequency. Motion-sensitive cameras will be installed around two farms to capture contact between livestock and wildlife. Camera images will be analysed to evaluate the number of contacts over time and thereby estimating contact frequency. At the same time, blood and urine samples will be taken from livestock on these farms, and laboratory-based microscopic agglutination testing (MAT) and urine culturing will be used to demonstrate which serovars of *Leptospira* are present.

1.3 Fieldwork location

The presented research was conducted on 2 different farms. The first was a beef and sheep farm, situated on a 2100 hectares plot in the Tararua district. The farm consists of about 9000 sheep (lambs not included) and 560 beef cattle. The second farm was a lowland dairy farm, situated in the Rangitikei district. The herd consists of about 250 dairy cows and 300 youngstock (calves under a year not included). All the animals are farmed under typical New Zealand commercial farming conditions, grazed on pasture all year round. Both farms were selected through previous research, which had shown that leptospirosis was present in livestock at both sites (Vallée 2016, Yupiana unpublished data).

Fig. 1. Map of the North island of New Zealand with the location of Massey University and the approximate locations of the participating farms



2. Material and methods

This research consisted of two parts, wildlife trapping for proving the presence of wildlife and estimating densities and livestock sampling for microscopic agglutination tests (MAT's).

2.1 Wildlife trapping

In order to prove the presence of wildlife and estimate relative densities four cameras were installed at strategic locations across the studied farm sites. The cameras were Bushnell Trophycam Ultra HD (model 119774), which can be attached to a tree and are camouflaged. They were placed close to deer tracks, but not in regions where traps were placed in order to limit possible human interference.
On the beef & sheep farm the first camera was initially placed about one kilometre from the hut in a small non-fenced bush with a sheep track. After five days this was changed to a place about 200m further on the edge of bush and grassland. The second camera was initially placed in an open field facing the bush where camera 1 was placed in. After five days this was changed to a site facing a main farm track. Another eight days later this was changed to a site facing a pasture where sheep were walking at that moment. The other two cameras have been placed further up a hill based on the advice of a local hunter. One camera was placed facing the open field, the other one facing a fence on the edge of bush and open field.
On the dairy farm the first and second camera were placed on the edge of the forest in a swamp area with clearly visible deer tracks. The third camera was placed about 400 metres further facing the fenceline and open pasture and the fourth camera another 200metres further facing open pasture and the fenceline from a different angle. The positions relative to the farm paddocks are represented in appendix 1 for the dairy farm and appendix 2 for the beef & sheep farm.
The cameras were set to camera mode and were motion-triggered with a high LED trigger sensitivity. Apart from that time-captured photos were taken every five minutes during a fieldscan from 5 to 7am and from 6 to 8pm, representing dawn and dusk.
A single camera trap night was defined as the 24hr period between 12pm and 12pm. For camera traps, animal captures were measured as the number of animals that are observed in each motion-triggered and time-captured interval that falls within the period of each camera trap night. When capturing motion-triggered images, cameras acquired images at twenty second intervals when animals remain in view over extended periods of time.

In conjunction with contemporaneous on-site animal trapping procedures, traps were placed at strategic locations in the bush, along the fenceline and in open field at both sites. 72 Longworth traps were used for mice and set 10 metres apart. 50 Tomahawk traps were used for rats, hedgehogs and mustelids and placed 20 metres apart. 36 Havahart traps were used for possums and feral cats and were placed 50 metres apart. In order to calculate an index in this report, trap data derived from that project and camera data were used. An index is a measurement of animal incidence which is comparable between sites for similar animal species, but not between species. Based on camera captures, the index is calculated as the mean number of observed animals per camera site divided by the duration of the trapping period. For camera sites with identical capture periods this is equivalent to the total number of observed animals divided by the total number of camera trap nights. For trapping data, the number of animals was divided by the total number of traps for that species and the total number of trap nights.

2.2 Camera analysis

In order to analyse the camera images a custom-written graphical user interface was designed in MatlabR2016b by D. A. Wilkinson, using the image processing toolkit. This was used for the enumeration of animal observations within field images. Animals were assigned to one of six categories; rodents (rats and mice), possums, deer, sheep, cattle and other (including birds, cats, mustelids, hedgehogs etc.). For each motion triggered and timed capture image, numbers of animals

belonging to each category were assessed manually in MatlabR2014b. Data were logged and summarised using custom-written code and used to generate tables and graphs in MatlabR2014b summarising the total number of observations per camera, number of observations per day, number of observations per hour and the frequency of direct contact among observed species. Direct contact is defined as "observing species X at a single camera followed by species Y at that same camera within one hour after observing species X". This was enumerated for all combinations of X and Y.

2.3 Livestock sampling

In order to measure leptospiral infection status of the livestock on the two farms, blood samples were taken to obtain serum, which was analysed in the laboratory using MAT-analysis.
Correct sample sizes were calculated for each group based on previously observed seroprevalence measured by *Vallée et al.* (2015) for Pomona when *Leptospira* was present in sheep & beef farms, by Harland *et al.* (2013) for working dogs and by Moinet *et al.* in dairy cattle (article in preparation). The expected seroprevalence was 20% for beef & sheep, 20% for working dogs and 50% for dairy cattle. Assuming that the proportions of seropositive animals in each farm and group would be as expected, the formula in Dohoo *et al.* (2009) was used for estimating the expected seroprevalence with 10% absolute precision and a 95% confidence interval and to calculate the sample size in each group based on that. The calculated sample size for the different groups is represented in table 2.

Table 2. The calculated sample size using the formula in Dohoo et al. (2009) for estimating the expected seroprevalence with 10% absolute precision and a 95% confidence interval

Group	Expected seroprevalence (%)	Approximate group size (#)	Sample size (#)	Sample date
Working dogs	20	25	18	23/05/2017*
1 Year old ewes (hoggets)	20	2600	61	23/05/2017*
2 Year old ewes (2-tooths)	20	2300	60	05/04/2017
Mixed age ewes	20	4000	61	10/03/2017
1 Year old beef cattle (R1)	20	160	45	-
2 Year old beef cattle (R2)	20	100	39	05/04/2017
Mixed age beef cattle	20	300	52	-
Milking cows	50	250	70	29/03/2017
1 Year old dairy cattle (R1)	50	150	59	10/04/2017
2 Year old dairy cattle (R2)	50	150	59	10/04/2017

**As can be observed in this table, not all the groups have been sampled yet. The working dogs and hoggets were sampled very recently and are therefore not yet taken into account in the results in this report.*

Blood was collected by jugular venepuncture using a one inch 20G vacutainer needle and a CAT Plus Blood Collection Tube without anticoagulant (BD Vacutainer®). Blood samples were transported on ice in a cooling box to the "^{m}EpiLab" (Massey University, Palmerston North), where they were centrifuged at 1400g for ten minutes to obtain serum.

Urine samples were taken in order to check for *Leptospira* isolates, indicating leptospiral shedding. In order to determine whether each *Leptospira* serovar is circulating within that group with 80% power and 95% confidence, 20 urine samples were collected per group (Dohoo *et al.* 2009). Given the difficulty to culture Leptospirosis increasing this number to thirty per group was attempted in order to make the chances for culturing higher, without compromising on the power of the study. Urine samples were taken by tickling the vulva until urine was voided. Samples of mid-stream urine were collected in a 60ml sterile container. 100 µl of urine per container was disposed in a plastic tube containing 5ml of Ellinghausen–McCullough–Johnson–Harris (EMJH) medium for culturing *Leptospira.* The samples and tubes were transported on ice in a cooling box to the "Epilab". In the lab 100 µl out of the first tube ("A") was diluted into another tube ("B") and again 100 µl was taken out of this tube and diluted into another tube ("C"). All the tubes were then stored in an incubator on a rotation plate at 27°C to stimulate growth of potential *Leptospira* spp. For the first four weeks after sampling, samples of the tubes were checked for the presence of *Leptospira* once a week and biweekly after four weeks, using a dark-field microscope.

2.4 MAT analysis of livestock sera

Sera were diluted at 1:6 with a sterile saline solution (0,85%), using 30 µl of serum and 150 µl of standard saline for each well to create the 96-well masterplates. These were then stored at -20°C until the MATs were performed to serologically diagnose leptospirosis. This was done for five *Leptospira* serovars (Hardjobovis, Pomona, Ballum, Copenhageni, Tarassovi). In addition to these serovars, one recently isolated fieldstrain of Ballum, obtained from a mouse kidney lepto isolate in the dairy farm in November 2016, was used in order to test the similarity of MAT titre estimations between field and reference strain cultures. MAT-analysis was performed as described by Fang *et al.* 2014. The antigens used for the MAT were cultures of a maximum of a week old containing approximately 10^8 Leptospira/ml ("leptobottles"). One ml of this culture was then diluted in 100ml of EMJH medium and placed in a 27°C incubator for 2/3 days to stimulate proper growth. Eight 2-fold serial dilutions of 25µl of each masterplate serum in standard saline covering the titre range from 1:24 to 1:3072 were prepared in a 96-well plate. A plate containing 25 µl of standard antiserum and 25 µl of standard saline were used as a positive and a negative control and prepared in the same way as described above. In each well, 25 µl of EMJH mediated culture was added. The plates were then placed in re-sealable plastic bags and incubated at 37°C for approximately 2 hours, after which the degree of inactivation of the *Leptospira* through the presence of antibodies was determined, using a dark-field microscope. The endpoint titre was the dilution at which approximately 50% of the *Leptospira* were inactivated.

In order to group and analyse the MAT results, all the samples and associated MAT results were entered in a leptospirosis database, created by A. Fayaz using Microsoft Access 2010. Queries were created for different groups and exported in .txt files. All the .txt files were then uploaded into MatlabR2014b, using custom-written code by D.A. Wilkinson in order to create violin plots for the comparison of the different queries.

2.5 Statistical analysis

The seroprevalence of a group was calculated per serovar as the number of animals that had a titre ≥48, which is the recommended cut-point (Blackmore 1982), seroprevalence and 95% confidence intervals were calculated for each group using the MatlabR2016b statistics toolkit.
The proportions of seropositive animals per farm were compared, using Microsoft Excel 2010. In order to calculate the difference between the two farms and the confidence interval of the comparisons, the XLSTAT add-in for Excel was installed and a parametric Z-test comparing two proportions was used.

3. Results

3.1. Camera trapping

Exposure to native bush is uneven along the farming boundary, meaning that the total exposure of livestock to wild animals will be determined by a combination of farming rotation practises (which will also impact total animal densities), wild animal ranges and seasonal effects. Camera trapping methodology was designed to confirm the presence of different wild animal species at each site, and to provide an index measurement of animal incidence which is comparable between sites. Independent evaluation of animal farming densities and rotation timings, as well as literature reviews to determine likely animal ranges for different wild species were then integrated to assess potential contact frequency between sites, and the direct/indirect nature of the contacts between wildlife and livestock

3.1.1. Maps and farming practises

Due to the restricted home-ranges of different wild animals (which, in general, are proportional to the body size of the animal), we only anticipate direct contact between livestock and wild animals in those areas that fall within the home range distance of the bush-exposed boundaries of the farm. Thus, paddock layouts, usage and rotational schemes will impact the total proportion of time in which livestock are exposed to wild animal reservoirs of infection. Thus, land-use information was collected from each site to assess the potential for farming practises to modify the contact rates between wild animals and livestock.

The beef & sheep farm covers a total of 2065 hectares, including 320 hectares of native bush. In the autumn and winter time the cattle (200) and ewes (3000) are in one mob and enclosed at 3 cattle per hectare and 48 sheep per hectare and stay in one paddock for three days. From September to January (spring and summer) the livestock is set stocked at five to six animals per hectare. The rotation scheme is 25 days in the autumn and 50 days in wintertime. The difference is due to the seasonal variation in grass growth.

The dairy farm has a rotation scheme in which the dairy herd is moved to a new paddock after every milking, i.e. twice a day. The herd consisted of 228 cattle at the moment of sampling and the paddocks have a mean size of 2 hectares, therefore the cattle are enclosed at 114 per hectare. There are 35 paddocks, which creates a rotation schema of 17.5 days. Youngstock are housed on a different farm and therefore are not taken into account here by calculating the stock density.

A paddock map of the beef & sheep farm is represented in appendix 1 and a paddock map of the dairy farm in appendix 2.

3.1.2 Density of sites

In order to get an overview of the density at both farms the total observation rate and the spreading throughout the day were analysed. The total number of observed animals per camera for the beef & sheep farm is represented in table 3 and for the dairy farm in table 4. In the beef & sheep farm cameras 1 and 2 were placed for a total of 17 trap nights, cameras 3 and 4 were placed for 12 trap nights. At the dairy farm all cameras were in position for 12 trap nights.

Table 3. The total observation rate for the beef & sheep farm, split up per camera

	rodents	possums	deer	cattle	sheep	other
Camera 1 (location 1)	0	0	6	0	0	1
Camera 1 (location 2)	0	0	2	0	0	2
Camera 2 (location 1)	0	1	0	0	0	6
Camera 2 (location 2)	0	0	2	0	0	2
Camera 2 (location 3)	0	0	6	0	3300	4
Camera 3	0	63	202	0	0	57
Camera 4	0	14	2031	0	0	53

As can be observed in table 3, there were no rodents observed by the cameras. Cameras 1 and 2 were moved to different locations after low numbers of observations. Possums and deer were frequently observed by cameras 3 and 4, as well as "other" species. These "other" species included hare, bats, birds, feral cats and hedgehogs. Camera 2 (location 3) was positioned in a paddock where sheep were grazing at that time, hence the high observation numbers of sheep. Cattle were not seen in these pastures during the trapping dates.

Table 4. The total capture rate for the dairy farm, split up per camera

	Rodents	possums	deer	cattle	sheep	other
Camera 1	0	0	29	0	0	17
Camera 2	0	0	59	0	0	1
Camera 3	0	0	2	300	0	0
Camera 4	0	0	0	0	0	2

The total capture rate for the dairy farm in table 4 shows that there were no rodents and possums captured on camera. Deer were seen at camera sites 1 and 2. In the pasture bordering the pasture that was faced by camera 3 the dairy herd grazed for half a day, hence the high numbers of cattle.

To get an idea of the number of observations over the different dates, an overview of observations during the trapping dates for the beef & sheep farm is represented in figure 2 and for the dairy farm in figure 3. For the beef & sheep farm there has to be taken in consideration that camera 3 and 4 were not placed until the 8th of March.

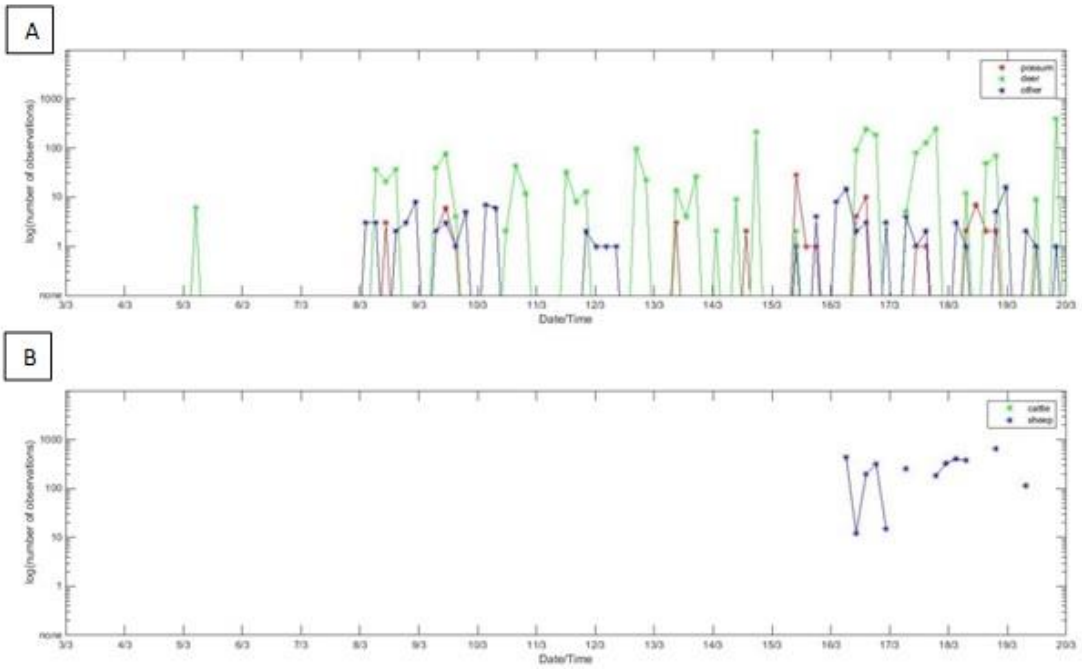


Fig 2. An overview of the total observations over time at the beef & sheep farm, represented on a log scale. Graph A gives an overview of the wildlife, graph B of the livestock captured by the cameras.

Low numbers of wildlife were observed at the beef & sheep farm in the first 5 days (figure 2A). This changed drastically after placing camera 3 and 4 on the 8th of March. From the 8th onwards the observations of wildlife occurred evenly through time. Possums, deer and others were observed around the same time, in particular from the 15th of March onwards. Deer were constantly observed at evenly spaced intervals of approximately 12 hours. It is therefore likely that they have a routine. Sheep observed in high numbers for 5 days (figure 2B), around the same dates when high numbers of deer were observed.

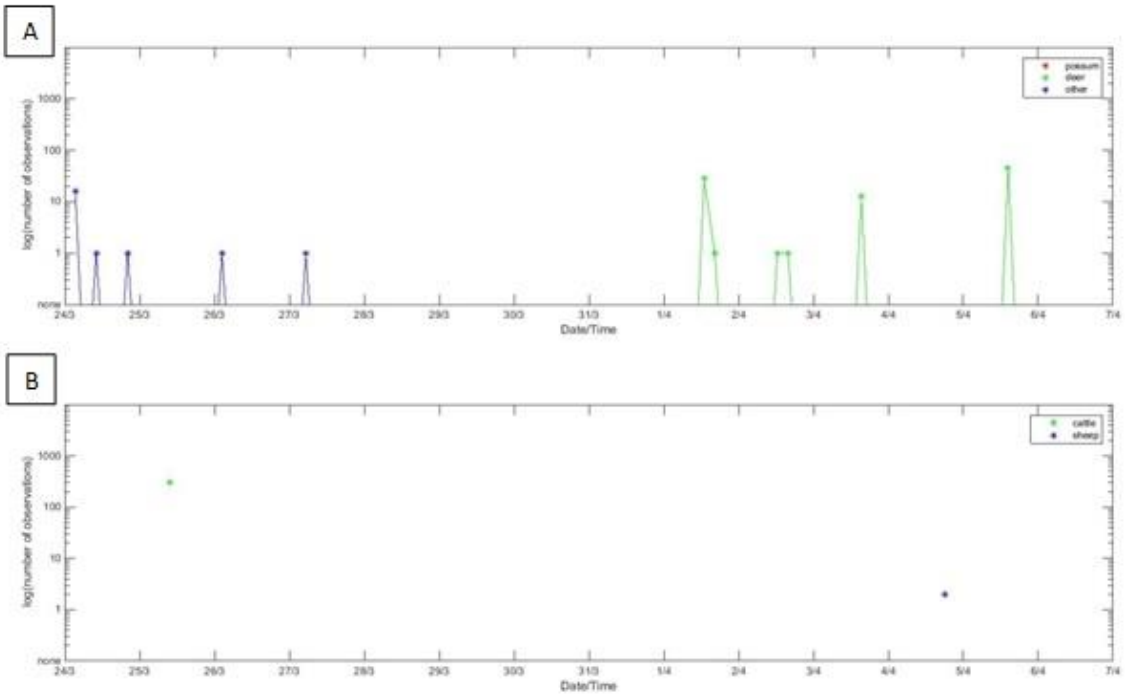


Fig 3. An overview of the total observations over time at Dairy farm, represented on a log scale. 3A gives an overview of the wildlife and 3B of the livestock captured by the cameras.

At the dairy farm, deer were present in the second week of camera trapping and others only in the first week with low numbers (figure 3A). The observations are again evenly spread out over the days. Cattle were present for half a day at one camera and therefore only show up as a single dot in figure 3B. They were present on a day when there were no deer observed.

3.1.3 Direct versus indirect contact

Transmission can occur either through direct or indirect contact. As can be concluded from figure 2 and 3 livestock and wildlife are present at the same place in both farms and for the beef & sheep farm on the same day. Background information represented in appendix 3 shows whether the observed species are either diurnal or nocturnal, which influences the likelihood of direct contact amongst them. An overview of the direct contacts between the different species is represented in table 5 for the beef & sheep farm and in table 6 for the dairy farm.

Table 5. An overview of direct contact between the different species within one hour in the beef & sheep farm

	Possums	**Deer**	**Sheep**	**Other**
Possums	11	24	0	1
Deer	24	10558	6	8
Sheep	0	6	17445	132
Other	1	8	132	127

Appendix 3 shows that deer and possums in the beef & sheep farm are mostly active in the evening and during the night, whereas sheep are mostly active during the day. Note of interest is that around 8PM and 9PM, 1PM and 2PM and 8AM there were sheep as well as deer and possums active, making direct contact likely to occur at those moments. Table 5 shows that this is indeed the case with six possibilities of direct contact happening between deer and sheep in the same pasture. No direct contacts between possums and sheep were like to occur, due to different cameras trapping the two species.

Table 6. An overview of direct contact between the different species within one hour in the dairy farm

	Deer	**Cattle**	**Sheep**	**Other**
Deer	2	0	0	0
Cattle	0	219	0	0
Sheep	0	0	4	0
Other	0	0	0	2

As can be observed in appendix 3 table 2 the deer were present at night time, whereas the dairy herd was actively grazing in the evening, so chances of active direct contact would be low. Table 6 shows that there is no possibility of direct contact between livestock and wildlife at the cameras sites.

3.1.4 Index of the observations

In order to compare the two farms an index has been created, using the camera observations as well as trap captures, presented in appendix 4. The index shows the number of observations per night for both farms, corrected for the number of cameras and the number of traps. The index is presented in table 7.

Table 7. Total number of observations per night, comparing both farms

	Beef & sheep farm	Dairy farm	Fold-difference in observations	Higher number of observations
Deer	187.0	7.5	25	Beef & sheep farm
Mouse	0.10	0.25	2	Dairy farm
Rat	0.05	0.03	2	Beef & sheep farm
Possum	6.5	0.0	NA	Beef & sheep farm
Cattle	0.0	25	NA	Dairy farm
Sheep	194.1	0.0	NA	Beef & sheep farm
Other	10.1	1.7	6	Beef & sheep farm

Based on the index calculations we estimate that deer are 25 times more abundant in the beef & sheep farm than in the dairy farm, mice 2 times more abundant in the dairy farm, rats 2 times more abundant in the beef & sheep farm and that possums are present on the beef & sheep farm, but entirely absent on the dairy farm. This last observation is the most interesting one to be drawn from this table.

3.1.5 Conclusion camera trapping

In the beef & sheep farm wildlife (predominantly deer and possums) as well as livestock (sheep) were observed by the cameras. Sheep were observed around the same dates as wildlife and closer inspection showed low numbers observed around the same time of the day. When having a look at direct contacts, derived from the different cameras, a low incidence of direct contact was happening between deer and sheep and no incidence of direct contact was happening between possums and sheep due to the appearance on different cameras.

In the dairy farm there were deer, as well as cattle observed. The cattle were observed for only half a day, when no deer observations were made. The deer were observed in that following night, when the cattle were still in the same pasture. However, the deer showed up on a different camera, making the chances of direct contact between livestock and wildlife low. Since they are both being been observed by the cameras, indirect contact would still be possible.

Deer were observed in both farms, with a 25 times higher chance on the beef & sheep farm. Rats were observed more on the beef & sheep farm, whereas mice were more abundant on the dairy farm. Possums were not observed on the dairy farm at all. This conclusion can be represented in a diagram for both farms, whereby the arrows from the same species can be compared with each other on the two farms. The diagram is represented in figure 4.

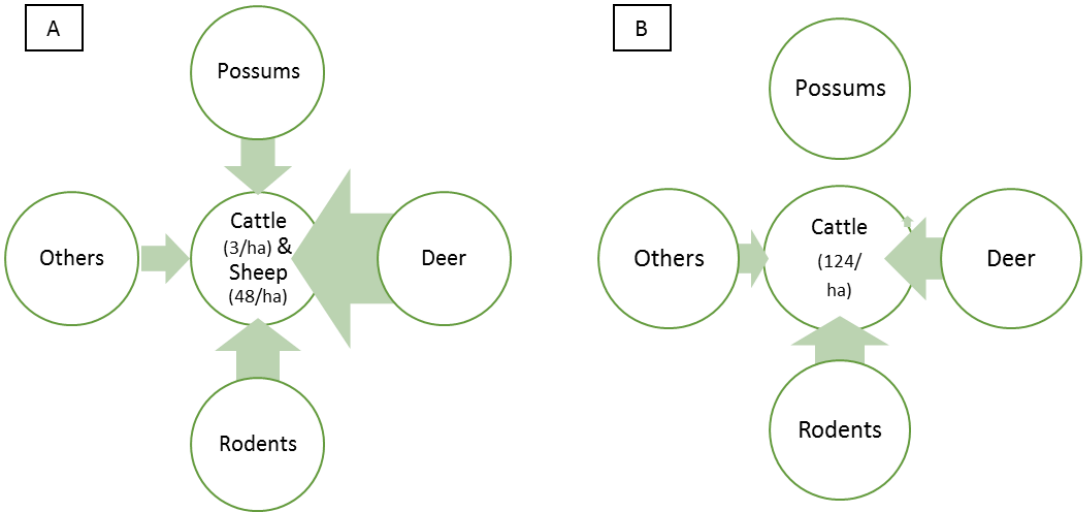


Fig 4. Diagrams representing contact frequency between livestock and wildlife on the beef and sheep farm (A) and the dairy farm (B). The arrow represent the number of contacts, whereby the same species can be compared between the 2 farms

3.2 Urine cultures

In order to evaluate whether leptospirosis was actively shed by infected animals, urine samples were taken. As can be observed in table 2 blood samples and thereby urine samples of different groups were taken at different times. This means that certain urine cultures have been checked over longer periods of time than others. Heavily contaminated cultures were excluded, since it was impossible to see whether there were any *Leptospira* present. Due to difficulties with collecting urine samples and high levels of contamination, the amount of urine samples was constrained and no urine samples were taken from the 2-tooths, hoggets and dogs.

The cultures from the mixed-aged ewes in the beef & sheep farm were checked four times. At the first time seven of the original eleven samples, three first dilutions and one second dilution were contaminated. At the last time, 6 weeks after sampling, there were only 6 second dilutions of the original 11 samples and their dilutions left and these ones also turned out to be highly contaminated at that point. The non-contaminated dilutions were all negative. Five urine samples from the rising 2 year old beef cattle were taken and the cultures were checked three times. Eight of the fifteen cultures were contaminated, the other ones were negative.

Thirty samples were taken from the milking herd at the dairy farm. The cultures from the milking herd in the dairy farm were checked three times. The first time all were negative, the last time 95% of the first dilutions were contaminated, the second and third dilutions were all negative. Thirty samples were taken from the rising one year old youngstock and thirty from the rising two year olds. The cultures were checked twice, with 95% of the first dilutions already contaminated at the first check, two weeks after sampling, and the rest negative. The second time the remaining first dilutions and 10% of the second dilutions were contaminated as well, the rest was all negative.

The conclusion that can be drawn from these results is that the livestock likely did not shed leptospirosis in their urine.

3.3 MAT-analysis serum

In order to get an overview of the titres for leptospirosis and the titre distribution on the two farms, MATs were carried out for serovars Hardjobovis, Pomona, Copenhageni, Tarassovi and Ballum in each group.

3.3.1 Titre distribution total farm: beef & sheep farm versus dairy farm

An overview of the results comparing the beef & sheep farm with the dairy farm per serovar are represented in violin plots in figure 5. The total number of samples was 167 for the beef & sheep farm and 203 for the dairy farm.

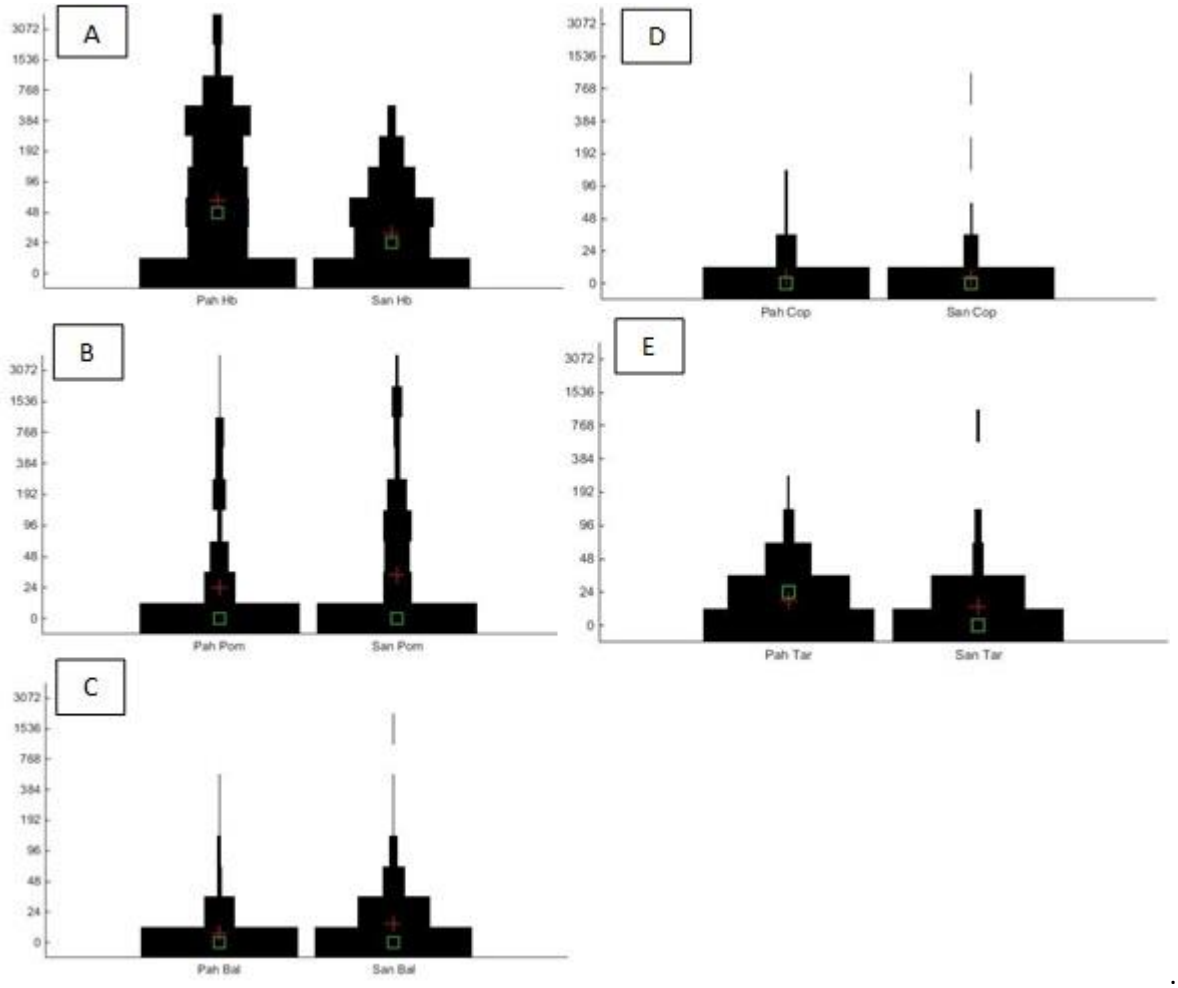


Fig 5. An overview of the titre distribution for serovars Hardjobovis (A), Pomona (B), Ballum (C), Copenhageni (D) and Tarassovi (E), comparing the beef & sheep farm (Pah, n=167) with the dairy farm (San, n=203). Violin plots depict observation frequency for each titre value using vertically stacked bars.

The overview in figure 5 shows that in all serovars most animals had no titre for the tested serovars. Serovar Hardjobovis accounted for the biggest spread in distribution, mainly in the beef & sheep farm, where animals had a titre up to 3072. Titres for serovar Pomona reached 3072 in both farms, but this was formed by a smaller percentage of animals as for Hardjobovis, therefore creating a mean titre of 0 for both farms. For serovar Ballum both farms had low titres, with an occasional high one, mainly in the dairy farm. For serovar Copenhageni the same accounts as for Ballum, with the occasional high occured in the dairy farm. For Tarassovi a higher number of animals had a titre in the beef and sheep farm than in the dairy farm, giving a mean titre of 24 for the beef & sheep farm and 0 for the dairy farm.

3.3.2 Titre distribution all groups in detail per farm

In order to get a good overview and possible differences between species, the farms were split up in the different sampling groups. All the groups for the beef & sheep farm are represented per serovar

in figure 6. Histogram overviews of the different serovars per group are given in appendix 5 for the beef & sheep farm and appendix 6 for the dairy farm.

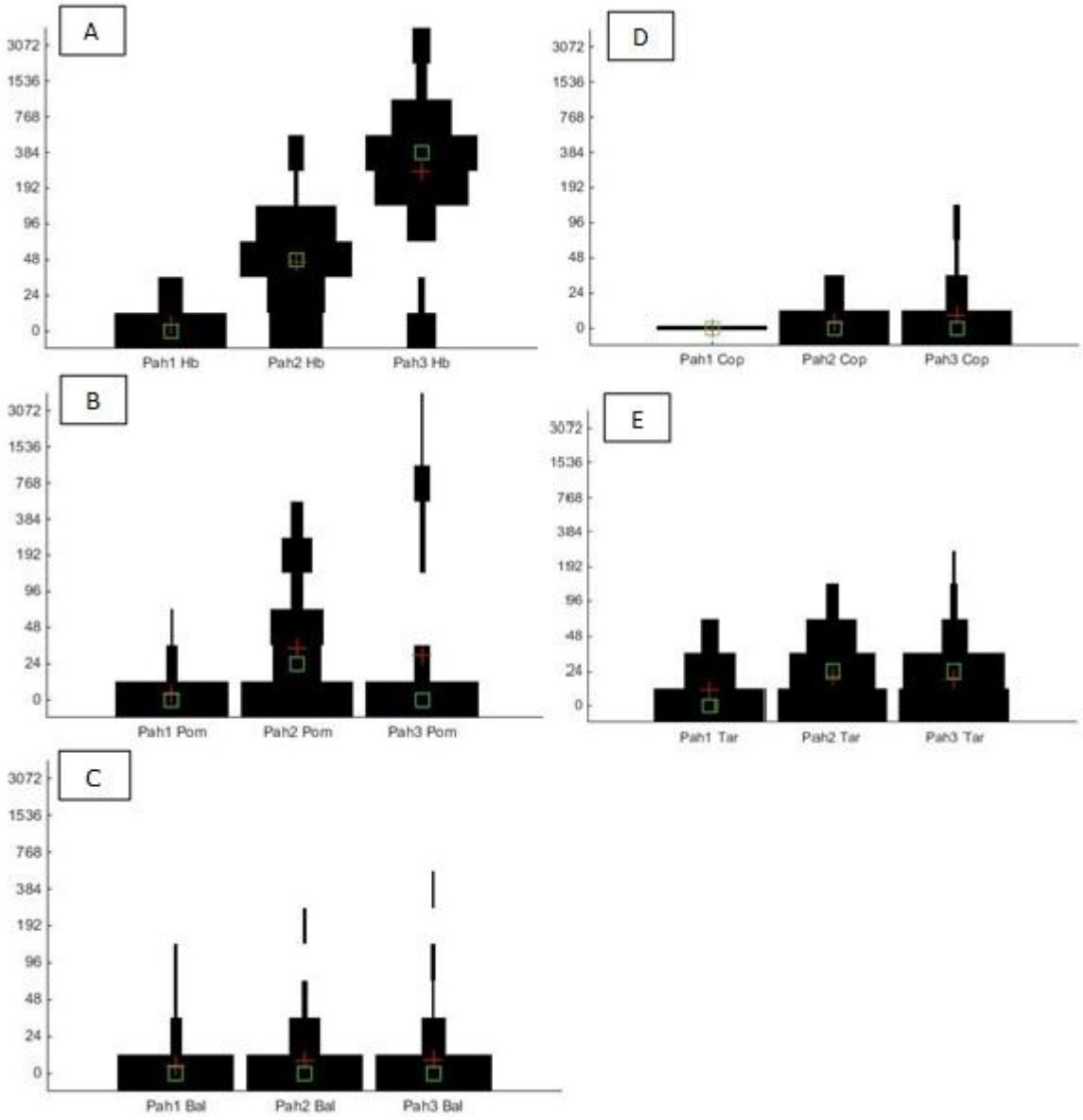


Fig 6. An overview of the titre distribution at the beef & sheep farm per group for serovars Hardjobovis (A), Pomona (B), Ballum (C), Copenhageni (D) and Tarassovi (E). Pah1 = R2 cattle (n=45), Pah2 = mixed-aged ewes (n=61), Pah3 = 2-tooth ewes (n=60). Violin plots depict observation frequency for each titre value using vertically stacked bars.

As presented in figure 6A, the titre distribution for Hardjobovis is very different for the sheep compared to the cattle. The cattle mainly had no titre, whereas the sheep predominantly had titres of 48 and over, with a mean titre of 384 in the 2-tooth ewes. The same accounts for Pomona (figure 5B), but with less sheep having high titres, therefore creating a mean titre of 24 for the mixed-aged ewes and 0 for the 2-tooth ewes.

An overview of the different groups in the dairy farm is represented in figure 7.

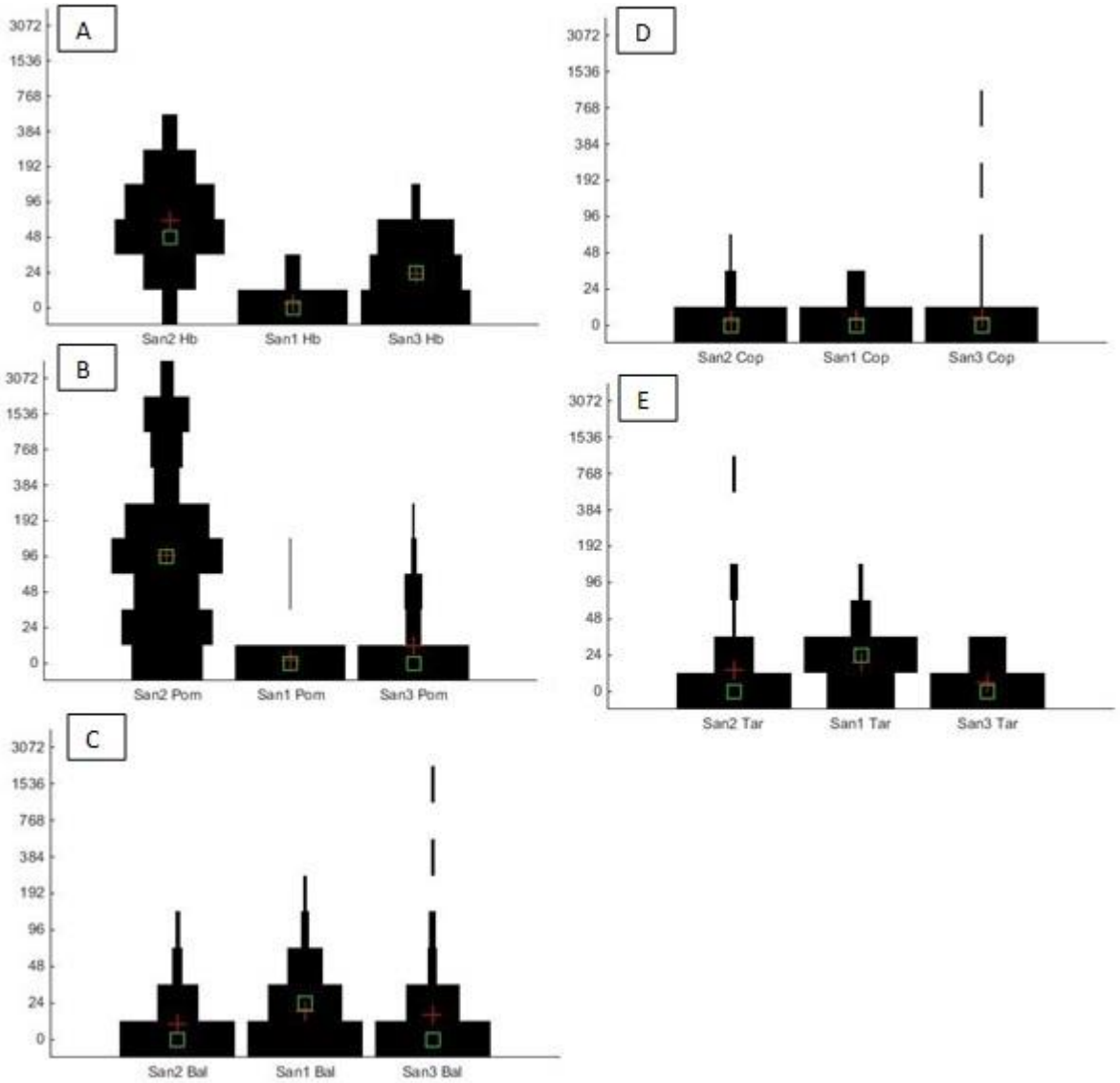


Fig 7. An overview of the titre distribution at the dairy farm per group for serovars Hardjobovis (A), Pomona (B), Ballum (C), Copenhageni (D) and Tarassovi (E). San2 = dairy herd (n=83), San1 = R2 youngstock (n=60), San3 = R1 youngstock (n=60). Violin plots depict observation frequency for each titre value using vertically stacked bars.

The most interesting result that can be obtained from figure 7 is the difference in titre distribution for serovars Hardjobovis and Pomona between the dairy herd and the youngstock. For Hardjobovis (figure 7A) the number of dairy cattle with no titre is low compared to the youngstock, creating a mean titre of 48 for the dairy herd. The rising 2 year olds have much lower titres than the rising 1 year olds, creating a mean titre of 0 for the rising 2 year olds and 24 for the rising 1 year olds. For Pomona (figure 7B) there are dairy cows that have titres up to 3072, whereas most of the youngstock have no titres at all, creating a mean titre of 96 for the dairy herd and 0 for both youngstock groups. The distribution for serovars Ballum, Copenhageni and Tarassovi is very alike among the three groups. Noticable is the few high ones for the rising one year olds for serovars Ballum and Copenhageni.

3.3.3 Ballum vs Ballum fieldstrain

Research has shown that in vitro strains of *Leptospira* attenuate and might therefore lose their virulence during serial *in vitro* culture. This is due to frequency changes of alleles in genes related to signal transduction and metabolism (Lehmann *et al.* 2016). In order to test whether this results in different titres, MATs on all the livestock plates have been carried out for the culture strain of Ballum

as well as a local Ballum field strain. The field strain is obtained from a mouse in the dairy farm in November 2016. The MAT results for the beef & sheep farm and the dairy farm are represented in figure 8.

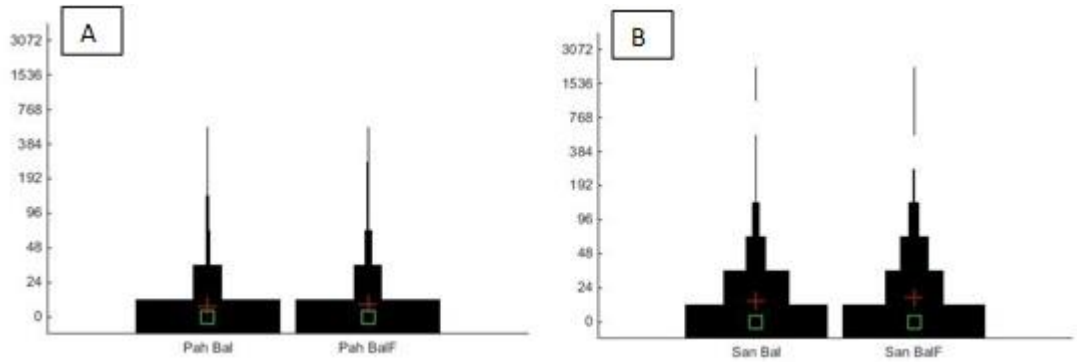


Fig 8. Serovar Ballum compared with a fieldstrain of Ballum for the beef & sheep farm (A, n=167)) and the dairy farm (B, n=203). Bal = serovar Ballum, BalF = Ballum fieldstrain. Violin plots depict observation frequency for each titre value using vertically stacked bars.

Figure 8 shows strong similarity in titres between the *in vitro* Ballum culture and the field strain. However the field strain gave a few higher titres. The significance of these differences was interrogated using a χ^2-Test, which gave a P-value of >0.999 for the beef & sheep farm and a P-value of >0.999 for the dairy farm, with serovar Ballum being the expected range and Ballum fieldstrain the observed range. This shows that there is no statistical significant difference between serovar Ballum and the Ballum fieldstrain, and thus that difference accumulated over time due to continued *in vitro* culture were not sufficient to modify the sero-reactivity of the Ballum reference strain.

3.3.4 Seroprevalence discussion

Based on the article by Blackmore *et al. (*1982) a titre of ≥48 is the recommended cut-off point for being seropositive and thereby calculating the seroprevalence. A titre of 24 can be a result of cross-reactions. If using this cut-off of ≥48 for serovar Hardjobovis the seroprevalence is 56.9% (49.3-64.2) for the beef & sheep farm and 41.4% (34.8-48.2) for the dairy farm. However, cut-off choice as a great influence on the interpretation of the results. If the cut-off ≥96 were to be used then the estimated seroprevalence drops to 44.3% (36.9-51.9) for the beef & sheep farm and by half to 20.2% (15.1-26.1) for the dairy farm. Serovar Tarassovi shows an even more dramatic drop when changing the seropositivity criterion from ≥48 to ≥96. At ≥48 the seroprevalence for the beef & sheep farm is 16.7% (11.6-22.9), but when this is changed to ≥96 the seroprevalence drops to only 3.6% (1.4-7.2). For Pomona, Copenhageni and Ballum the same reason as for Tarassovi is valid. Therefore a cut-off of ≥48 for seropositivity is being used in this report.

3.3.5 Seroprevalence per population and per farm

In table 8 an overview of the seroprevalence is given for the five different serovars in the beef & sheep farm, per group and for the total farm. As can be observed in table 8 serovar Hardjobovis is the serovar with the highest seroprevalence amongst the different groups of sheep (65.6% and 90.2%) and the total population on the farm (56.9%). Sheep have a high seroprevalence for Hardjobovis in general (77.9%), reasonably high for Pomona (30.0%) and Tarassovi (19.7%) and low for Copenhageni (4.1%) and Ballum (5.7%). The cattle have their highest prevalence for serovar Tarassovi (8.9%), but are in general low for all serovars.

Table 8. The seroprevalence (titre ≥48) in percentages for the five different serovars for the beef & sheep farm, per group and for the total farm

	# Sampled	Hardjobovis *(CI)*	Pomona *(CI)*	Copenhageni *(CI)*	Tarassovi *(CI)*	Ballum *(CI)*
Total farm	167	56.9 *(49.3,64.2)*	22.8 *(16.9,29.5)*	3.0 *(1.1,6.4)*	16.8 *(11.6,22.9)*	5.4 *(2.6,9.5)*
Total sheep	122	77.9 *(70.0,84.6)*	30.3 *(22.7,38.8)*	4.1 *(1.5,8.6)*	19.7 *(13.3,27.3)*	5.7 *(2.5,10.8)*
M-A ewes	61	65.6 *(53.3,76.6)*	41.0 *(29.3,53.4)*	0.0 *(0.0,04.7)*	24.6 *(15.1,36.2)*	4.9 *(1.2,12.4)*
2T-ewes	61	90.2 *(81.1,96.0)*	19.7 *(11.1,30.7)*	8.2 *(3.0,16.8)*	14.8 *(7.4,24.))*	6.6 *(2.1,14.6)*
R2 cattle	45	0.0 *(0.0,6.3)*	2.2 *(0.1,10.0)*	0.0 *(0.0,6.3)*	8.9 *(2.9,19.4)*	4.4 *(0.7,13.3)*

In table 9 an overview of the seroprevalence is given for the five different serovars in the dairy farm, per group and for the total farm. The results represented in table 9 show that the dairy herd have a high seroprevalence of 79.5% for Hardjobovis and 69.9 for Pomona. The R1 youngstock have the highest titres for Hardjobovis (30.0%), whereas the R2 youngstock are not positive for Hardjobovis at all. They show a reasonably high seroprevalence of 20.0% for Ballum.

Table 9. The seroprevalence (titre ≥48) in percentages for the five different serovars in the dairy farm, per group and for the total farm

	# Sampled	Hardjobovis *(CI)*	Pomona *(CI)*	Copenhageni (*CI)*	Tarassovi *(CI)*	Ballum *(CI)*
Total farm	203	41.4 *(34.8,48.2)*	34.5 *(28.2,41.2)*	2.0 *(0.6,4.6)*	7.4 *(4.3,11.5)*	12.8 *(8.7,17.9)*
Dairy herd	83	79.5 *(70.1, 87.2)*	69.9 *(59.6,79.0)*	1.2 *(0.1,5.6)*	9.6 *(4.5,17.2)*	8.4 *(3.7,15.7)*
R2 youngstock	60	0.0 *(0.0, 4.8)*	3.3 *(0.5,10.1)*	0.0 *(0.0,4.8)*	11.7 *(5.2,21.3)*	20.0 *(11.3,31.1)*
R1 youngstock	60	30.0 *(19.5,42.1)*	16.7 *(8.8,27.3)*	5.0 *(1.2,12.6)*	0.0 *(0.0,4.8)*	11.7 *(5.2,21.3)*

3.3.6 Comparison of seroprevalence

With the criterion for seropositivity set at ≥48 different groups can be compared with each other and decided whether the differences in proportion of seropositives are statistical significant. The first groups to compare are all groups of the beef & sheep farm versus all of the dairy farm. The results are represented in table 10. To calculate the P-value a Z-test comparing two proportions was used.

Table 10. Seroprevalence (titre ≥48) for the different serovars for the beef & sheep farm compared to the dairy farm

	# Pahia-tua	Sero-prevalence Beef & sheep farm	# San-toft	Sero-prevalence Dairy farm	Dif-ference	CI low	CI high	P-value
Hardjo-bovis	167	0.569	203	0.414	0.155	-0.419	0.783	0.001
Pomo-na	167	0.228	203	0.345	-0.117	-0.708	0.474	0.017
Copen-hageni	167	0.030	203	0.020	0.010	-0.522	0.542	0.782
Taras-sovi	167	0.168	203	0.074	0.094	-0.473	0.661	0.010
Ballum	167	0.054	203	0.128	-0.074	-0.631	0.483	0.019

As can be observed in table 10 the differences in seroprevalence between the two farms are small and do not exceed over 15%. Serovars Pomona and Ballum give a higher titre in the dairy farm, serovars Hardjobovis, Copenhageni and Tarassovi in the beef & sheep farm. Overall the seroprevalence for Hardjobovis in both farms is the highest, followed by Pomona. The seroprevalence for Copenhageni is in both farms low, with no significant difference. For Tarassovi the seroprevalence for the beef & sheep farm is twice as high as for the dairy farm, whereas this is the other way around for Ballum.

3.3.7 Conclusion MAT titres

The distribution of the MAT-titres was most widespread for serovars Hardjobovis and Pomona in both farms, with 2-tooth ewes reaching the highest titre of 3072 for serovar Hardjobovis in the beef & sheep farm. Fewer animals had high titres for Pomona than for Hardjobovis. When dividing the farms into the different sampled groups there was a clear difference considering the serovars Hardjobovis and Pomona between the sheep and cattle in the beef & sheep farm, with cattle having low titres and sheep having a widespread distribution and high titres in general. In the dairy farm the dairy herd had higher titres for serovars Hardjobovis and Pomona than the youngstock, with titres reaching up to 3072 for Pomona. Apart from that there were a few animals with high titres for serovars Ballum and Copenhageni among the rising one year old youngstock in the dairy farm.

With a cut-off titre of ≥48 for seropositivity serovar Hardjobovis gave the highest seroprevalence in both farms. In the beef & sheep farm the sheep had a high seroprevalence for serovars Hardjobovis and Pomona, reasonably high for serovar Tarassovi and low for serovars Ballum and Copenhageni. The R2 cattle in the beef & sheep farm had a low seroprevalence for all serovars. In the dairy farm the dairy herd had a high seroprevalence for serovar Hardjobovis and Pomona, whereas the rising two year olds were negative for Hardjobovis and had a seroprevalence of only 3% for Pomona. However, the rising two year olds did show a reasonable high titre for serovar Ballum compared to the rising one year olds and the dairy herd.

The difference for the different serovars between the two farms was small and did not exceed 15%. Serovars Pomona and Ballum gave a higher titre in the dairy farm, serovars Hardjobovis, Copenhageni and Tarassovi in the beef & sheep farm. Overall the seroprevalence for Hardjobovis in both farms was the highest, followed by Pomona. The seroprevalence for Copenhageni was low in both farms, with no significant difference. For Tarassovi the seroprevalence for the beef & sheep farm was twice as high as for the dairy farm, whereas this was the other way around for Ballum.

4. Conclusion

Camera data indicate that direct and indirect contact is possible between deer and livestock and indirect contact between possums and livestock in the beef & sheep farm. In the dairy farm mainly deer were observed and, according to the camera data, chances for direct contact between deer and livestock in this setting would be very low. Indirect contact would still be possible through the contamination of environmental surface water, but with a 25 times less chance than at the beef & sheep farm. Possums were not observed at the dairy farm. Rats were observed 2 times more on the beef & sheep farm, whereas mice were more abundant on the dairy farm.

The serovars that were mainly observed in the beef & sheep farm as well as the dairy farm were Hardjobovis and Pomona. As can be seen in table 1, both of these serovars have deer as maintenance hosts. There is a possibility that both serovars can be transferred from wild deer to the livestock, and at the time of observation contact rates between deer and livestock were estimated to be 25 times greater on the sheep and beef farm than on the dairy farm. The higher Ballum titres in the dairy farm compared to the beef & sheep farm could be explained by the higher abundance of mice in the dairy farm. Serovar Copenhageni titres were very low in both farms. This could be explained by the fact that most rats captured on both farms were black rats. This shows that the maintenance host for serovar Copenhageni, the Norway rat, is only present in very low numbers on both farms. Titres for Tarassovi were higher in the beef & sheep farm than in the dairy farm. The maintenance host for Tarassovi is the pig, which are, according to camera trapping data, not present in both farms. This difference can therefore not be explained by camera trapping data.

5. Discussion

5.1 Camera trapping data

A comment that has to be made about the camera trapping data is that not all the observations are individual animals. Twenty seconds after triggering the camera could be triggered again, therefore there can be multiple photos of the same animals. This was mostly the case for deer and sheep, that tend to graze on one place for a longer period of time. However, in figure 1 the trapping data is evenly spread through the dates. This reduces the bias that is due to multiple images of the same deer. The fact that the observations of deer occur at evenly spaced intervals (approximately every 12 hours) shows that they are constantly present in this area, and likely have a routine.

5.2 Movement of deer and possums

Amos *et al.* 2014 have GPS tracked 25 wild red deer for 3 consecutive years at Cresbrook Dam Reserve, south-eastern Queensland, Australia. They found an overall home range of 410 (±88) hectares (ha) for dams and 1506 (±536) hectares for stags. When taking these home ranges into consideration, table 5 and 6 give an understatement of the possibilities of direct contact. In the beef & sheep farm, the total farm covers about 2000ha. With the stags covering up to 1500ha, it means that approximately 75 % of the studied farm is likely to be covered by the deer's home range (see appendix 7). The rotation scheme for the livestock in the beef & sheep farm, as explained in paragraph 3.3.1, is in total 75 days in the autumn. Seventy five percent of the time the livestock is in pastures covered by the deer, i.e. 56 out of 75 days. Since the diurnal/nocturnal table in appendix 3 shows that the livestock and deer are active around the same time and table 5 shows the possibility of direct contact, there is possible direct and indirect contact for 56 days. For the dairy farm the home range of the deer cover the whole farm, so there is constantly the possibility of contact. Since the diurnal/nocturnal in appendix 3 shows that the livestock and deer are active around a different time of the day and table 6 shows no direct contact, the contacts occurring between the cattle and deer are likely to be indirect.

According to Whyte *et al.* 2013 the home range of possums varies from 1 to 12 ha, depending on the density of the possums. The handbook of New Zealand mammals describes that larger movements have been observed when going downhill into pastures, up to 24.6 ha (King 1990). The area the possums cover in the beef & sheep farm is represented in appendix 7. It shows that the possums observed by camera 3 and 4 cover one paddock and the possums observed by camera 2 (loc1) cover 3 paddocks of which 2 can be seen as one due to their size. This means that livestock are only likely to come into contact with possums when grazing in one of three different paddocks in the rotation scheme. Given that the livestock stays in one paddock for three days the total days at risk is 9 days in the rotation cycle of 75 days. The diurnal/nocturnal table in appendix 3 shows that possums and livestock are active around the same time there is possible direct and indirect contact. In the dairy farm there were no possums observed or trapped.

5.3 Urine cultures

In paragraph 3.2 the results show no indication of shedding of leptospirosis in urine of the livestock. However, since most urine cultures got contaminated, it is questionable whether this is a reliable source to detect the shedding of leptospirosis. To reduce the chances of contamination and increase the chances of finding leptospirosis, using abattoir kidneys would be recommended. Apart from that doing PCRs on urine or kidney samples to detect leptospirosis and only culturing the positive samples would be another recommendation.

5.4 MAT titres

For serovar Hardjobovis it has to be taken into account that serovar Balcanica is from the same serogroup (Hebdomadis) and can therefore give cross-reaction when using serology as a determinant (Hathaway *et al.* 1978). Serovar Balcanica has been isolated from deer (Flint 1988), as well as possums (Hathaway *et al.* 1978). This could influence the Hardjobovis titres.

Vallée (2016) reported a seroprevalence with a maximum of 97% for Hardjobovis in the hoggets at docking time (December), decreasing to 82% for 2-tooths at scanning time (July) at the beef & sheep farm. Around breeding time (April) the 2-tooths had a seroprevalence of 95% for Hardjobovis and 43% for Pomona (see appendix 8). Since the present sampling time was around April as well, it is best to compare those results with the present one. The present results show 90% seroprevalence in the 2-toots for Hardjobovis and 20% for Pomona. The Hardjobovis is comparable with the seroprevalence found by Vallée, Pomona is just half of the seroprevalence found by Vallée.

The dairy herd in the dairy farm was previously visited in March 2015 to investigate the leptospirosis state of the livestock after three of the farm staff were hospitalised with confirmed leptospirosis in January and February 2015 (Harvey, unpublished data). Forty cattle from the dairy herd and a selection of the youngstock were randomly sampled during milking. Around half of the rising 2-year old heifers had titres for Ballum, although the majority of these titres were ≤1:48. In the dairy herd 16 of the 40 animals had titres ≥1:48 for Hardjobovis, including 10 animals with a titre of 1:768. There were 16 animals that were seroprevalent for Pomona as well as Hardjobovis. On the 17th of March all of the milking and non-milking cattle received a sensitising vaccine dose for Pomona and Hardjobovis, using the bivalent vaccine Leptoshield©. A booster was given a month later. Treatment with long-acting amoxycillin was delayed until dry-off in late May. The youngstock and dairy herd are still being vaccinated annually and should therefore not have high titres anymore. According to Subharat (2010) some studies have observed that antibody titres induced by vaccination were in general lower in magnitude than titres induced by infection. For example, Allen *et al.* (1982) observed Hardjobovis MAT titres ranging from 32 to 512 in vaccinated animals, compared with a range of 128 to 8192 in naturally infected unvaccinated animals. One thing that has to be taken into consideration is that the

youngstock in 2015 did not receive antibiotic treatment and are part of the milking herd now. They might be the animals with the high titres and/or chronically infected shedders that keep the field infection going.

5.5 Recommendations for the future

By comparing two contrasting farming environments in the Manawatu region data has been produced that informs as to exposure between livestock and wild animals that may influence the transmission of leptospirosis between different animal hosts. The result show that livestock likely come into contact with deer and rodents in a dairy setting, and deer, possums and rodents in a sheep and beef setting. Sheep and beef farms are commonly more exposed to native bush, and in this instance direct contact was estimated to be more likely between livestock and wildlife due to the associated farming practices. However, farmed animal densities are typically lower on sheep and beef farms, which may reduce the risk of epidemic communicable disease. In these settings the prevalence of infection of both wildlife and non-wildlife associated leptospiral serovars has simultaneously been measured, and shown to be high for both wildlife and non-wildlife associated serovars Hardjobovis and Pomona on both farms, moderately high for wildlife-associated serovar Ballum in the dairy setting and for wildlife-associated serovar Tarassovi in the sheep and beef setting and low for wildlife-associated serovar Copenhageni in both settings.

Further work investigating the prevalence of different leptospiral serovars in wildlife will be necessary to truly interpret the risk associated with exposure to wildlife. The multi-host nature of leptospirosis epidemiology means that transmission pathways leading to infection are complex. The data this research has produced will help to disentangle the individual contributing factors of different host species to infection in livestock – which, in New Zealand, is the main contributing source to leptospirosis infection.

6. Acknowledgements

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I probably forgot some people, so I want to thank anyone in general at IVABS, you all made me feel very welcome. The morning teas with delicious home-made baking on Friday morning and tea time pints on Friday evening are definitely something I will miss. You all made my time in New Zealand sweet as!

7. References

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Appendix 1. The location of the camera traps in relation to the different paddocks in the dairy farm

Appendix 2. The location of the camera traps in relation to the different paddocks in the beef & sheep farm

Appendix 3. Camera observations for the beef & sheep farm and dairy farm, spread out per hour, all cameras together

Beef & sheep farm

	possums	deer	sheep	other
0h	9	25	0	4
1h	10	99	25	3
2h	3	101	175	3
3h	0	42	0	0
4h	6	188	0	0
5h	1	323	0	0
6h	2	392	0	4
7h	0	805	2	14
8h	0	57	912	0
9h	0	0	263	3
10h	0	0	209	2
11h	0	0	41	10
12h	0	0	58	3
13h	0	0	19	15
14h	0	0	6	4
15h	0	2	22	4
16h	0	0	165	11
17h	0	0	217	2
18h	0	0	150	5
19h	0	0	643	6
20h	0	36	381	13
21h	8	156	12	6
22h	24	18	0	8
23h	14	5	0	0

Dairy farm

	deer	cattle	other
0h	0	0	0
1h	0	0	0
2h	0	0	0
3h	76	0	0
4h	0	0	0
5h	14	0	0
6h	0	0	0
7h	0	0	1
8h	0	0	0
9h	0	0	0
10h	0	0	0
11h	0	0	0
12h	0	0	16
13h	0	0	0
14h	0	0	1
15h	0	0	1
16h	0	0	0
17h	0	0	0
18h	0	0	0
19h	0	240	1
20h	0	60	0
21h	0	0	0
22h	0	0	0
23h	0	0	0

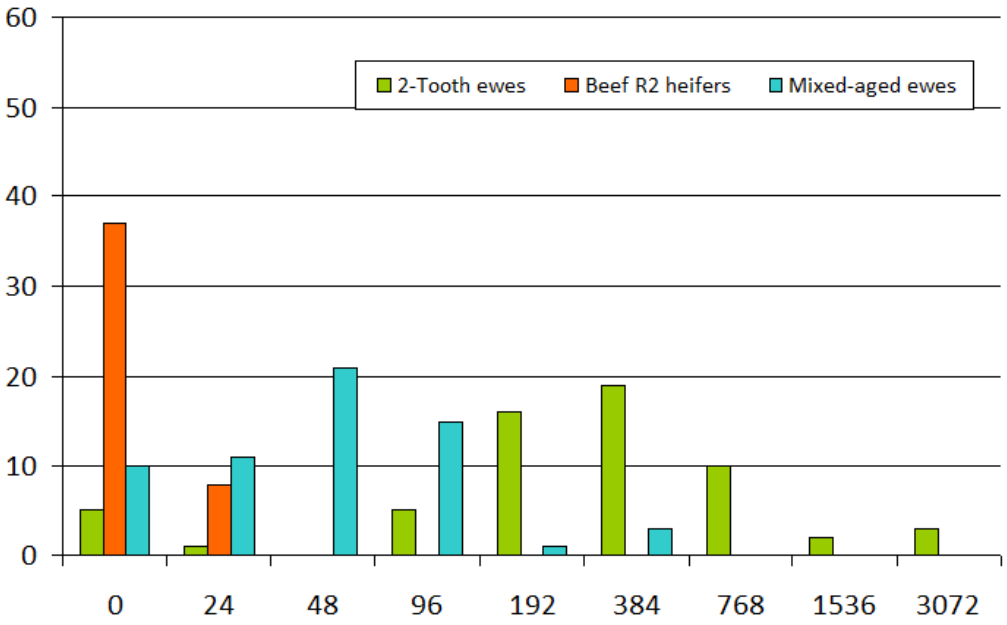
Species that were not observed were taken out of the table; rodents and cattle for Beef & sheep farm, rodents, possums and sheep for the dairy farm

Appendix 4. The total number of trap captures in 10 trap nights for both farms

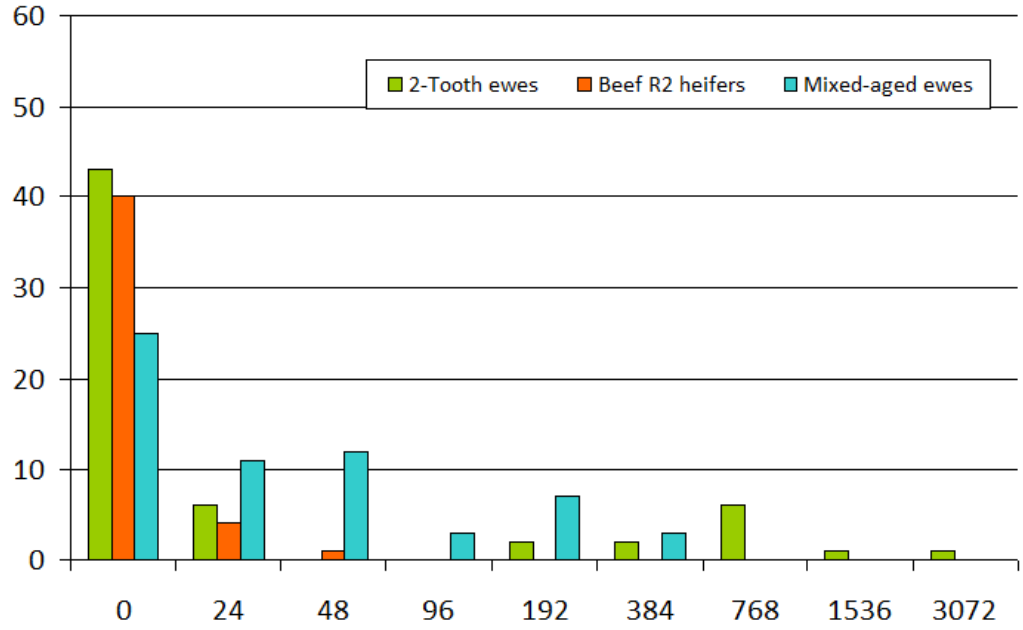
	Beef & sheep farm	**Dairy farm**
Hedgehog	5	8
Feral cat	1	1
Mouse	72	177
Rat	24	14
Possum	24	0

Appendix 5. MAT titres beef & sheep farm in histograms

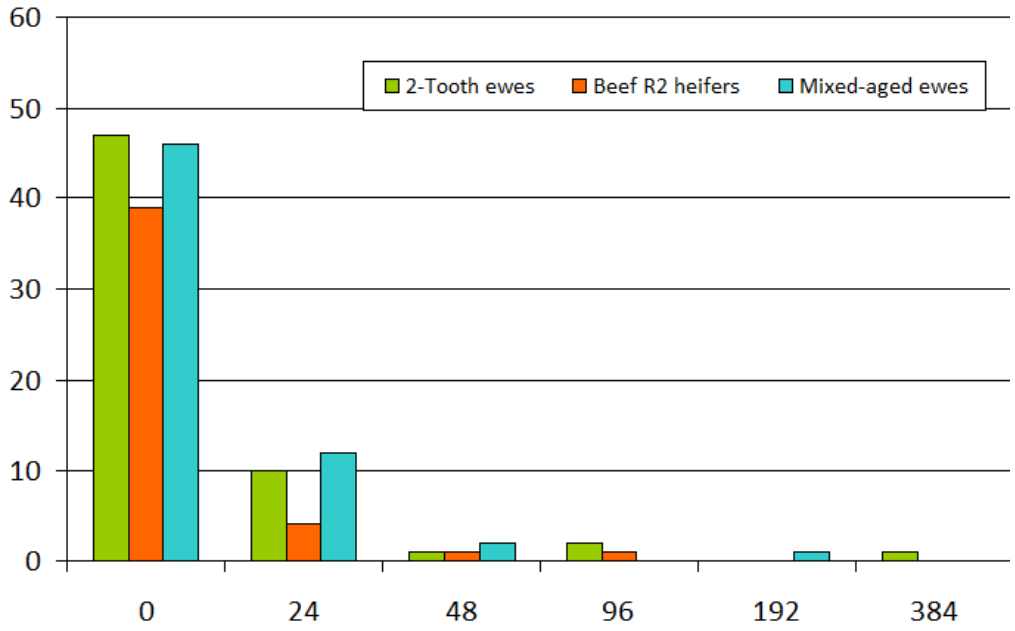
Serovar Hardjobovis



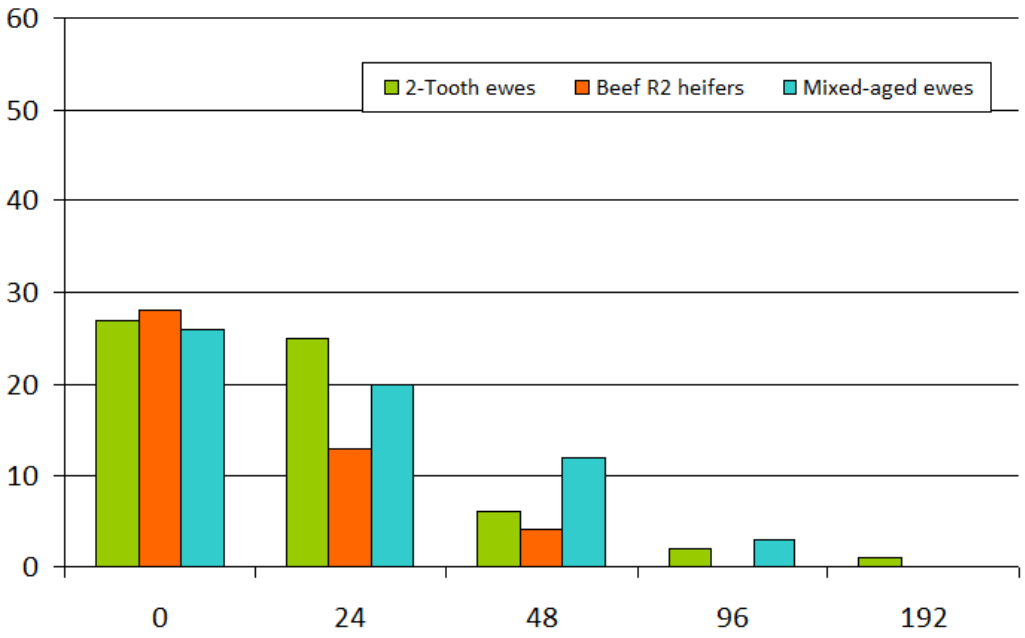
Serovar Pomona



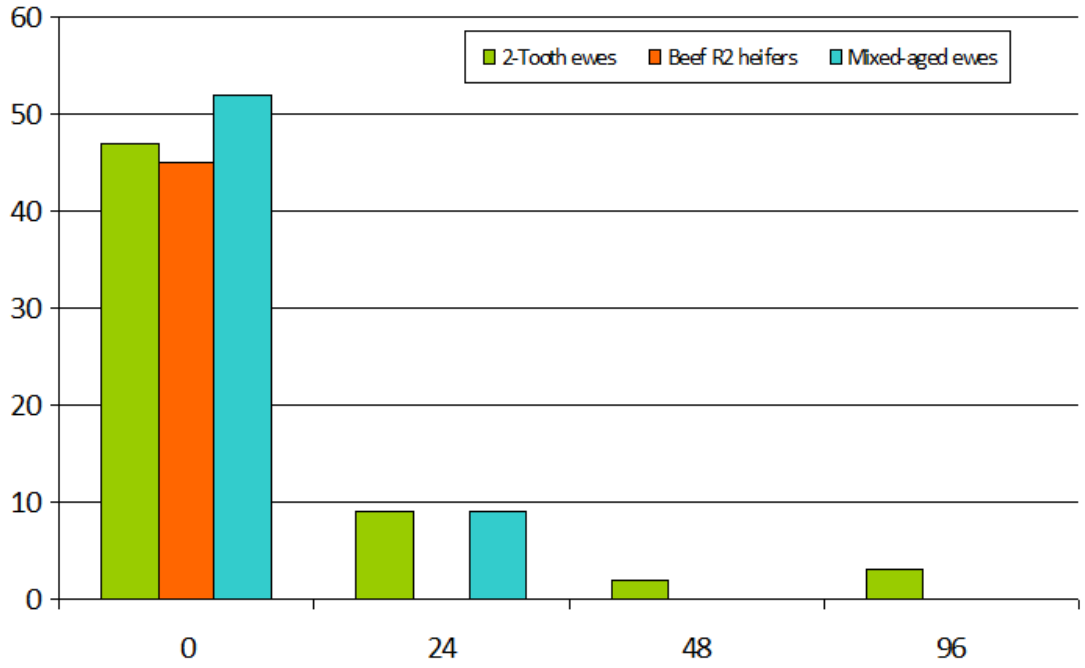
Serovar Ballum



Serovar Tarassovi

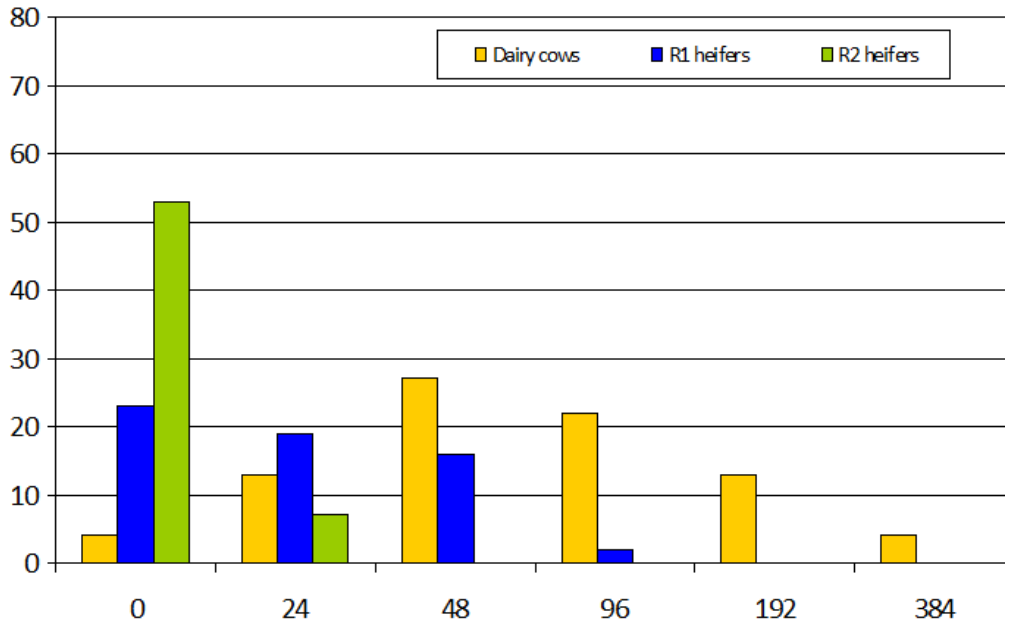


Serovar Copenhageni

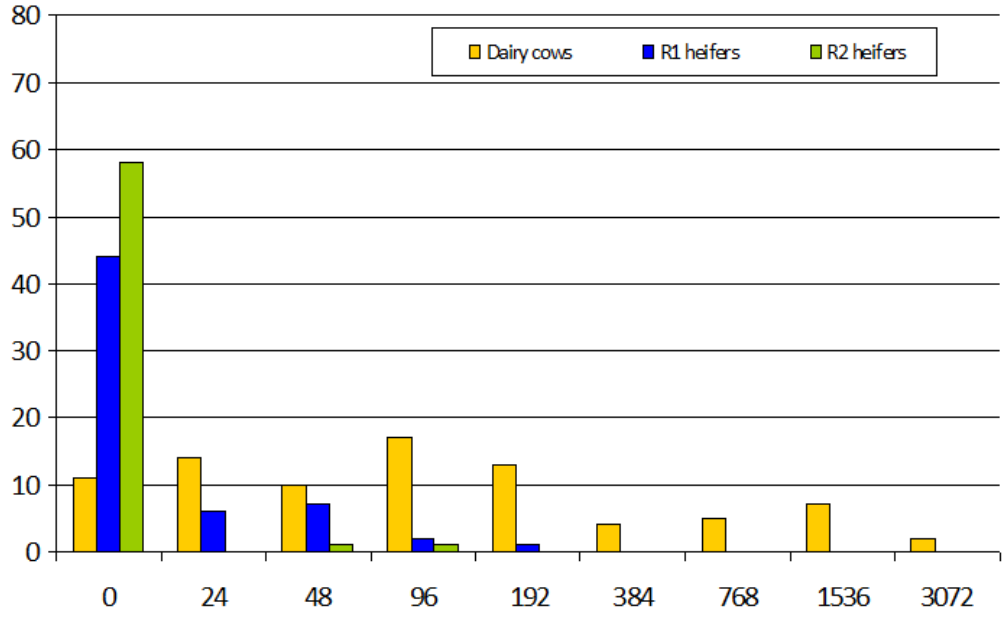


Appendix 6. MAT titres dairy farm in histograms

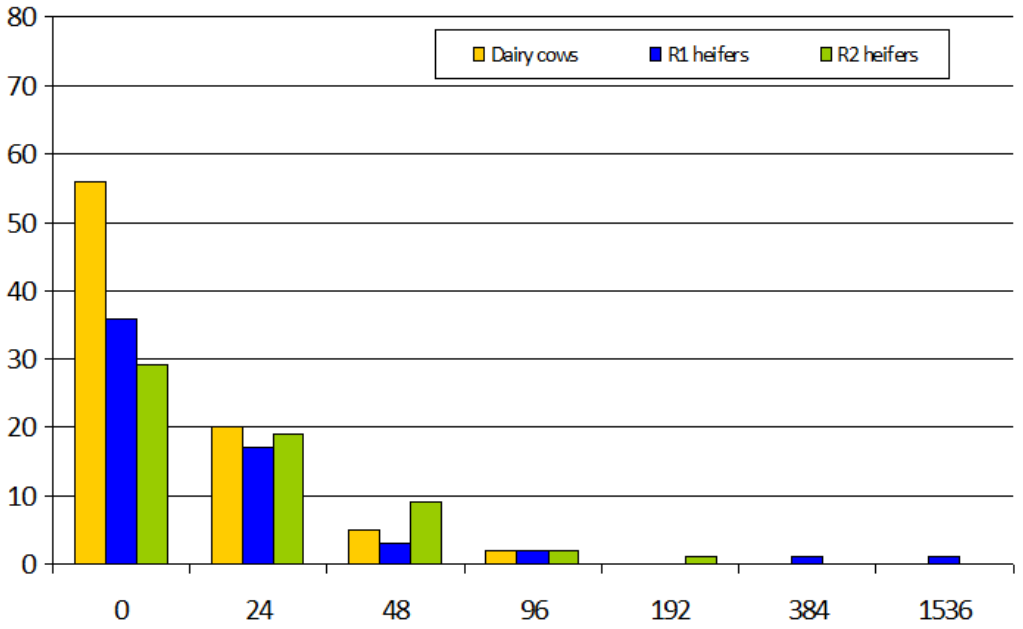
Serovar Hardjobovis



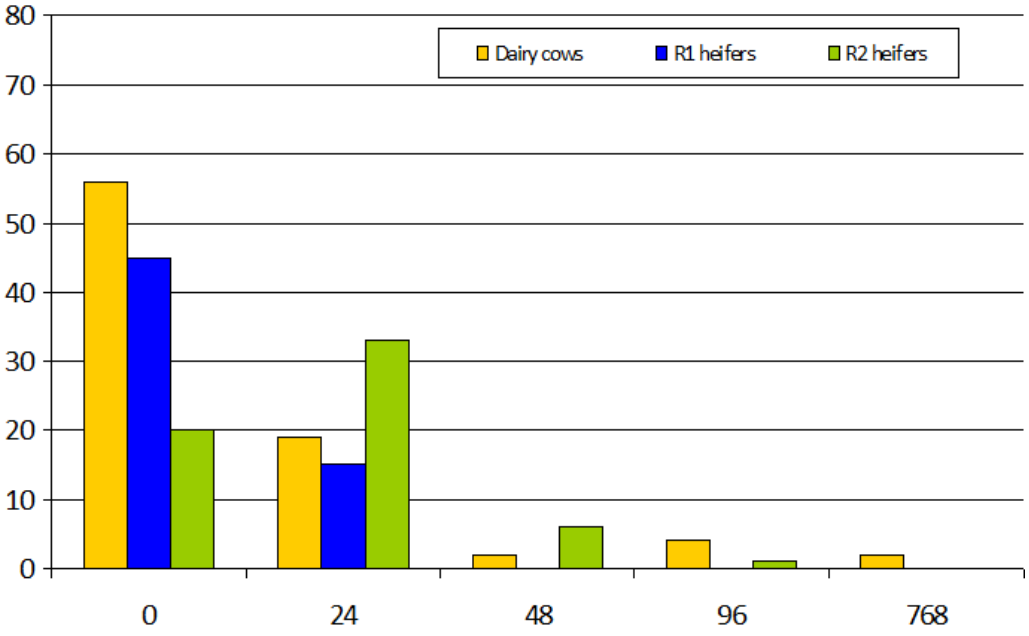
Serovar Pomona



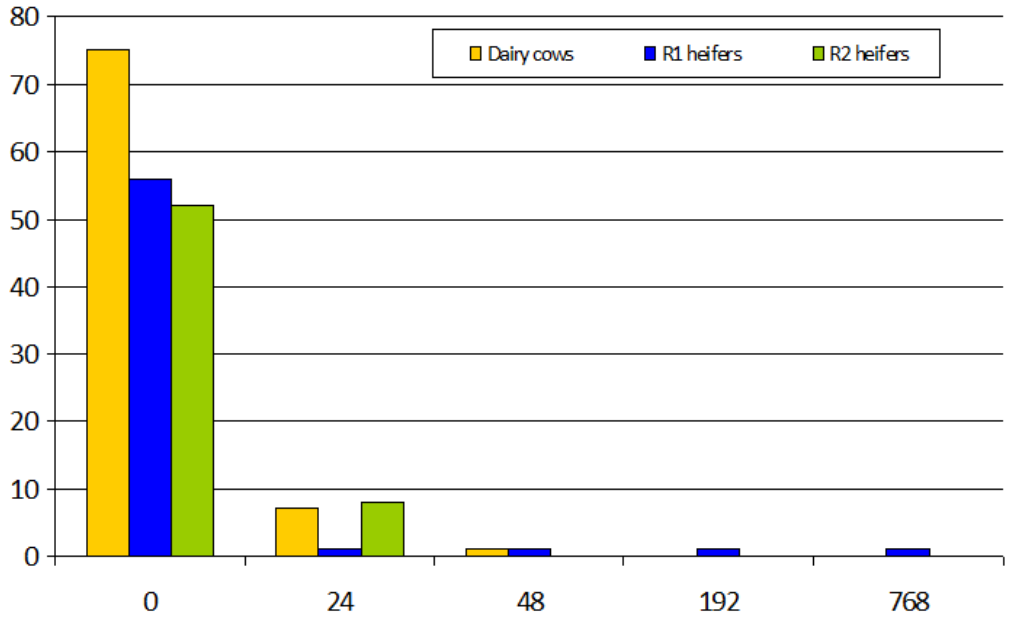
Serovar Ballum



Serovar Tarassovi



Serovar Copenhageni



Appendix 7. The coverage of the beef & sheep farm by the home range of deer and possums

*The blue lines represent the maximum home range of the hinds(small square) and the stags (big square) from the cameras where they have been observed. The purple lines represent the maximum home range of the possums.

Appendix 8. Table 5-1 Vallée et al. 2016, showing the Beef & sheep farm farm in the red frame, with the seroprevalence for serovars Hardjobovis and Pomona in sheep in the green frame

Table 5-1: Farm location, number and breed of ewes, date of weighing episodes, number of vaccinated and control sheep, Hardjo and Pomona seroprevalence (titre ≥48) and geometric mean titre (GMT, for animals with a titre ≥24) in unvaccinated controls at each sampling.

Farm	District (Island)	Number of breeding ewes	Breed	Date	Weighing episode**	Vaccination	Number vaccinated	Number of controls	Seroprevalence (%) in controls		GMT in control sheep	
									Hardjo	Pomona	Hardjo	Pomona
A	Manawatu (N)	6000	Romney/ composite	1/10/2011	LD	Sensitiser	97	197	64.6	2.0	162	73
				15/12/2011	LW	Booster	95	189	5.0	0.0	29	NA
				15/03/2012	HB		83	181	NA*	NA*	NA*	NA*
				2/08/2012	HS	Booster	84	172	17.9	5.5	856	41
B	Tararua (N)	8500	Perendale/ composite	22/11/2011	LD	Sensitiser	106	210	12.9	1.0	69	34
				17/01/2012	LW	Booster	102	193	0.0	2.1	NA	38
				30/05/2012	HB		92	175	5.1	13.1	946	665
				5/12/2012	HD	Booster	85	144	97.1	41.7	408	104
				16/04/2013	TB		73	132	95.3	43.8	301	82
				30/07/2013	TS		74	139	82.8	51.1	160	100
C	Wairoa (N)	4700	Romney/ Coopworth cross	17/10/2011	LD	Sensitiser	101	210	59.5	15.2	71	55
				1/12/2011	LW	Booster	98	207	45.6	0.0	58	NA
				26/04/2012	HB		88	190	0.0	0.5	NA	26
				6-23/11/2012	HD	Booster	76	175	98.3	18.7	293	166
				28/03/2013	TB		39	80	100.0	21.3	436	127
D	Central Hawke's	4500	Romney Texel	30/09-6/10/2011	LD	Sensitiser	78	180	2.8	0.0	41	24

*LD=lamb docking, LW=lamb weaning, HB=hoggets breeding, HD=hoggets docking, TB=2-tooths breeding, TS=2-tooths scanning