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Impact of feral cat control on toxoplasmosis levels in sheep as part of the Cape to City Programme

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Impact of feral cat control on toxoplasmosis levels in sheep as part of the Cape to City Programme

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Summary

Project and client

- The Cape to City (C2C) programme aims to control invasive predators (feral cats, stoats, and ferrets) across 26,000 hectares of farmland, peri-urban areas, and native bush in Hawke's Bay.
- Manaaki Whenua – Landcare Research was contracted by Hawke's Bay Regional Council (HBRC) to assess the impact of feral cat control on toxoplasmosis levels in sheep within the C2C programme area.

Objectives

- The objective of this study was to investigate the influence of a large-scale predator control programme on toxoplasmosis in domestic sheep in Hawke's Bay.

Methods

- *Toxoplasma gondii* seroprevalence of 1-year-old ewes was compared prior to and after commencement of large-scale control of predators, including feral cats.
- Predator control took place within the C2C treatment area in two stages: initial knockdown and long-term maintenance.
- Six sites were chosen for toxoplasmosis sampling in domestic sheep, including two predator control treatment sites (T1 & T2) and three non-treatment sites (NT1, NT2, & NT3). An additional site, originally intended to be a treatment site, only received limited predator control due to a change in the treatment area boundary, and so was put into its own category of 'limited treatment' (L1).
- Predator trapping data were grouped into two trapping periods, each occurring between sheep sampling dates. Period 1 and Period 2 consisted of predominantly knockdown-stage and maintenance-stage trapping, respectively.
- Blood samples were collected from approximately 60 1-year-old ewes from each site in late 2015, 2017, and 2019, with 2015 being a pre-treatment year.
- Serological tests were conducted to quantify exposure to *T. gondii* based on the proportion of sheep with a positive antibody response (seroprevalence).
- Changes in seroprevalence over time were assessed using a mixed-effects logistic regression model.

Results

- Overall, 285 feral cats were caught in the C2C treatment area during this study, along with 723 rats and 53 mice (both are intermediate hosts of *T. gondii*).
- More feral cats were caught in Period 1 than Period 2, with the opposite occurring for rats and mice.
- Overall, 24.4% (255/1,044) of all sheep sera tested were seropositive for *T. gondii*, with values among all sites and years ranging between 1.8% and 51.7%.
- The only significant change in *T. gondii* seroprevalence was an increase between 2015 and 2017, however this occurred at both the treatment and non-treatment sites.

Conclusions

- It appears that the predator control undertaken in this study did not reduce the incidence of toxoplasmosis in sheep within the treatment area. In general, high variability in seroprevalence was observed across sampling sites and years.
- A number of factors could have influenced the results presented here, including the sampling and testing methods used, as well as certain ecological characteristics of *T. gondii*.
- Following data analysis, it was made known by HBRC that some of the sheep in this study were vaccinated against toxoplasmosis, which would have reduced the likelihood of a treatment response being detected.
- While an apparent reduction in feral cat abundance occurred in the treatment sites, this reduction did not last. However, since *T. gondii* oocysts can remain infectious in the environment for long periods without the presence of cats, sheep can become infected well after any cats are removed from the area. Therefore, to increase the likelihood of observing any effects in sheep, it may be necessary to maintain the knockdown effect for longer, by extending the period of targetted cat trapping
- Infections can also be maintained within rodent populations for multiple generations due to transmission from mother to offspring. Infections in cats occur at a much higher rate from predation on infected intermediate hosts, especially mice, than from the environment. Rodent control, along with cat control, may be necessary to reduce or eliminate *T. gondii* infection in sheep.

Recommendations

General

- Continue to assess the influence of feral cat control on toxoplasmosis infection in sheep.
- Maintain feral cat numbers at low levels for a longer period of time following initial reductions.
- Incorporate control and monitoring of rodents, particularly mice, into future toxoplasmosis management strategies.

Pest control

- Increase efforts to remove feral cats from the landscape by:
  - improving the current cat control over the entire C2C treatment area, and/or
  - targeting cat control in and around the sheep sampling sites.
- Improve the capture rate of feral cats by utilising traps and techniques designed specifically for feral cats (e.g. leg-hold trapping).
- Rodents, especially mice, should also be targeted, instead of relying on by-catch using current methods.

Pest monitoring

- In addition to maintaining high-quality feral cat trapping records, continue to independently monitor changes in the relative abundance of feral cats in response to trapping efforts (e.g. via camera traps).
- Obtain a better understanding of domestic, stray, and farm cat numbers and how these compare with those of feral cats, particularly near areas grazed by sheep.
- Monitor changes in the relative abundance of rats and mice in response to trapping efforts (e.g. via tracking tunnels).

Toxoplasmosis sampling of sheep

- Continue to measure *T. gondii* seroprevalence levels in sheep by:
    - maintaining sampling for multiple years following any any reductions in cat numbers
    - sampling 1-year-old ewes
    - sampling from unvaccinated flocks only
    - using an antibody titre cut-off of 1:64 when determining seropositivity.
- Increase the number of treatment and non-treatment sites (to be decided using a power analysis).

Toxoplasmosis infection surveys in rodents

- Collect data on *T. gondii* infection status in rodent intermediate hosts through trapping and molecular analysis of tissue samples.
- Identify and compare *T. gondii* strain types between:
    - Rodents (using fresh tissue samples)
    - Sheep (using fresh tissue samples from dead animals)
    - Cats (using fresh faeces from the environment or collected from necropsies; faeces is necessary to determine strains that are actively being shed)

1 Introduction

Toxoplasma gondii is a protozoan parasite that causes toxoplasmosis in people and other animals worldwide, including in New Zealand. Felids, including feral and domestic cats (*Felis catus*), act as definitive hosts, becoming infected by preying on infected intermediate hosts and then shedding infective oocysts through their faeces into the environment (Dubey 2010).

Susceptible intermediate hosts, including most warm-blooded animals (Tenter et al. 2000), become infected by ingesting oocysts from contaminated environments, such as water, soil, or infected intermediate hosts. Some intermediate hosts, such as mice (*Mus musculus*) and rats (*Rattus* spp.), can maintain *T. gondii* infections in their populations via vertical transmission from mother to offspring (Webster 1994; Dubey 2010).

Toxoplasmosis has been recognised as a significant cause of abortion in sheep (*Ovis aries*), goats (*Capra hircus*), and pigs (*Sus scrofa*) globally (Dubey et al. 1995; Dempster et al. 2011), with the cost of toxoplasmosis to the New Zealand sheep industry in Hawke's Bay estimated at approximately $18 million in 2014 (Walker 2014).

2 Objective

The objective of this study was to investigate the influence of a large-scale predator control programme on toxoplasmosis in domestic sheep in Hawke's Bay.

3 Methods

3.1 Predator control treatment

3.1.1 Study area

The Cape to City (C2C) programme, part of the Predator Free Hawke's Bay initiative, aims to control invasive predators across 26,000 hectares of farmland, peri-urban areas and native bush in Hawke's Bay. Target species are feral cats, stoats (*Mustela erminea*), and ferrets (*M. furo*). However, other pest species are often trapped as by-catch, such as mice, rats (*R. rattus* and *R. norvegicus*), and hedgehogs (*Erinaceus europaeus*). Large-scale predator control, designed and conducted by HBRC, occurs within the C2C treatment area, which consists of three predator control operational areas (A, B, and C; Figure 1).

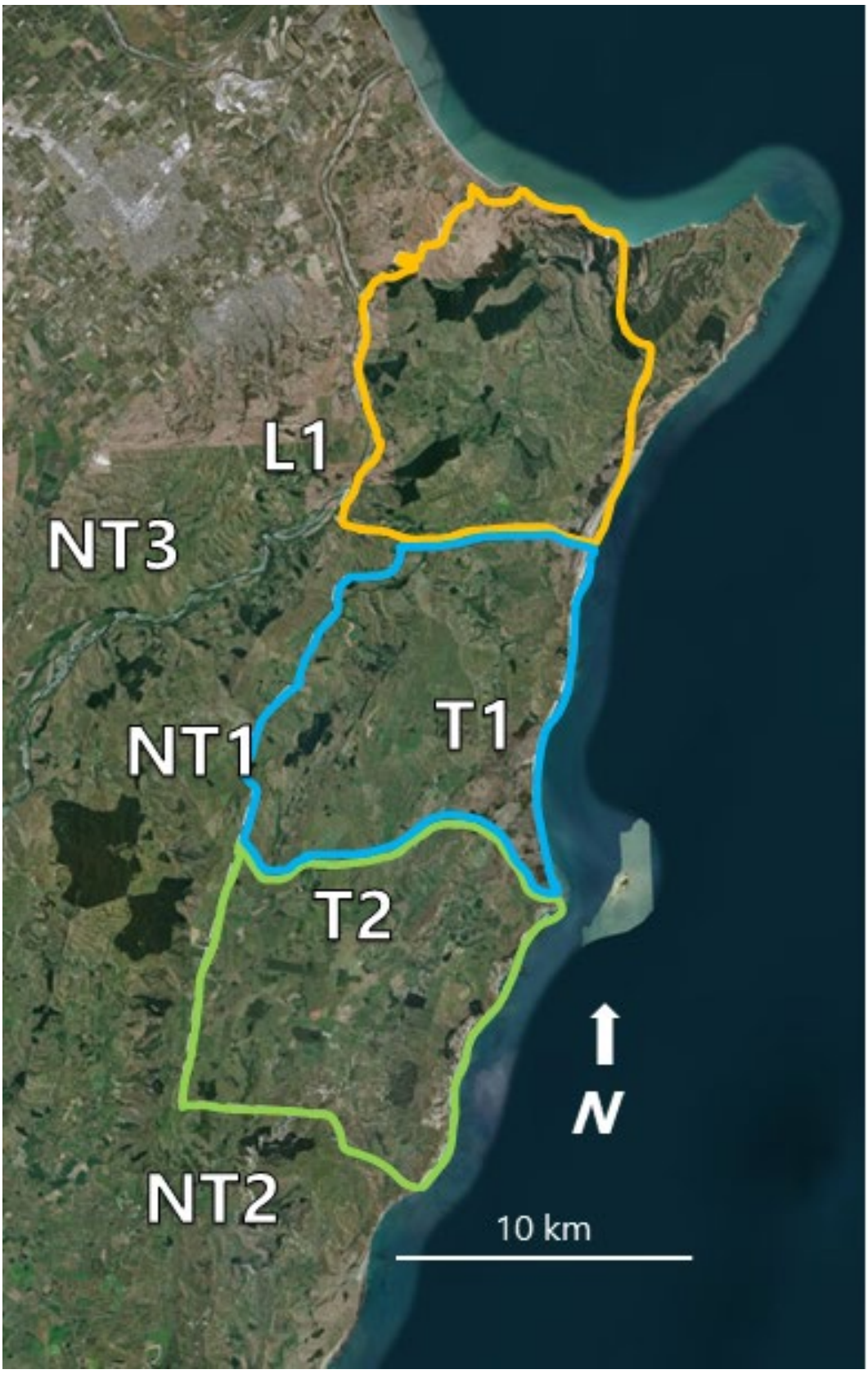

Figure 1. Map of the Cape to City treatment area showing three predator control operational areas: A (orange), B (blue) and C (green). T1 & T2 = treatment sites; NT1, NT2, & NT3 = non-treatment sites; L1 = limited treatment site (this site received a limited amount of treatment due to a change in A's boundary and so was considered separately). Site labels on maps indicate the approximate locations of sheep sampling.

3.1.2 Predator control stages

Large-scale predator control in the C2C treatment area occurred in two stages: initial knockdown, followed by long-term maintenance. The initial knockdown stage, beginning in 2016, targeted the removal of feral cats and mustelids and incorporated a mix of live traps (leg-hold and cage) and kill traps (Possum Master; Possum Master Industries Limited, Tauranga, NZ). This control effort was applied to approximately 1,000 ha trapped simultaneously for 10 nights. Traps were then redeployed into the next adjacent area in a 'rolling front'. This process was repeated across the entire 26,000-hectare C2C treatment area, from April 2016 to December 2017. Overall, cage traps, leg-hold traps, and Possum Master kill traps were deployed in approximately 1,230, 130, and 180 locations, respectively. These trap types are more effective at trapping cats than at trapping rodents. Knockdown control was mostly in Area A in 2016, and in Areas B and C in 2017. Also, some localised trapping, targeting feral cats, occurred in 2018 and 2019.

Following the knockdown stage, the second stage of control, intended as long-term maintenance of predators at low levels, consisted of a network of approximately 1,450 kill traps, predominantly podiTRAPs (Metalform, Dannevirke, NZ), deployed across the entire C2C treatment area. The traps were deployed at one per 10 hectares in Areas A and C, and one per 20 hectares in Area B of the treatment area (Figure 1). This trapping network is now checked four times a year, with traps reset and rebaited at each check. While these trap types are more effective at trapping rodents than cats, some additional feral cats were caught during this stage of the study.

3.2 *Toxoplasma gondii* in sheep

3.2.1 Sampling sites

Six sites (Figure 1) were chosen by HBRC for assessment of *T. gondii* exposure in domestic sheep (Table 1). Two sites were in areas that received large-scale predator control (treatment sites T1 and T2). The initial predator knockdown stage for sites T1 and T2 and surrounding areas occurred mostly throughout 2017, with a general north-to-south rolling front of trapping across predator control areas B and C. The traps for the long-term maintenance stage were deployed in April and June 2017 in the vicinity of sites T1 and T2, respectively. Also, some targeted trapping of cats (with leg-hold, cage, and Possum Master traps) in and around sites T1 and T2 took place in 2018 and 2019. Three sites were located outside the C2C treatment area and did not receive any predator control from the C2C programme (non-treatment sites: NT1 to NT3).

An additional site was originally planned as a third treatment site, but due to an unforeseen change in treatment area A's boundary in the early stages of the study, this site only received a limited amount of predator control and is thus considered as a limited treatment site (L1). Site L1 received some initial predator control during the knockdown stage in 2016, and 10 days of additional live trapping in 2018. Since the trapping results provided in this study are only based on those occurring within the treatment area, the numbers of animals caught at site L1 are not included in these totals. Seroprevalence results from site L1 are not included in the statistical analysis, but are included in section

4.2 for continuity reasons, as they were included in a previous progress report (Landcare Research 2017).

Table 1. Sampling times of *Toxoplasma gondii* levels in domestic sheep flocks in the C2C area. Treatment types refer to the implementation of large-scale predator control. The 2015 sampling occurred prior to the C2C treatment area receiving predator control.

		Sheep sampling dates		
Site ID	**Treatment type**	**2015**	**2017**	**2019**
T1	Treatment	18/09/2015	19/09/2017	18/09/2019
T2	Treatment	9/10/2015	26/09/2017	4/10/2019
L1	Limited treatment	27/08/2015	29/08/2017	27/08/2019
NT1	Non-treatment	1/10/2015	5/10/2017	30/09/2019
NT2	Non-treatment	16/10/2015	24/10/2017	9/10/2019
NT3	Non-treatment	19/11/2015	23/11/2017	11/11/2019

3.2.2 Trapping periods

While the predator control occurred in two stages (initial knockdown and long-term maintenance), the start and end dates for these stages were not the same as the sheep sampling dates. To account for this, predator control results (i.e. numbers and species trapped) were grouped into two trapping periods. Period 1 represents the trapping dates occurring between the 2015 and 2017 sampling events (April 2016 to September 2017), and Period 2 represents the dates occurring between the 2017 and 2019 sampling events (September 2017 to September 2019). As a result, Period 1 is predominantly knockdown control while Period 2 is predominantly maintenance control. The exact dates used to define trapping periods were based on the sheep sampling dates from site T1, because it was the first treatment site sampled each year.

3.2.3 Sampling and laboratory testing

Serological testing was undertaken to detect the presence of *T. gondii* antibodies in domestic sheep. Sampling, organised by HBRC, occurred between late August and late November of 2015, 2017, and 2019 (Table 1). The 2015 sampling occurred prior to the C2C treatment area receiving predator control. Blood samples were taken from c. 60 one-year-old ewes at each site (57, 59, 29, and 59 ewes were sampled from sites T1, T2, NT2, and NT3 in 2019, respectively). Blood samples were submitted for laboratory analysis (Gribbles Veterinary, Palmerston North) and the latex agglutination test (LAT) was used to quantify the antibody response to *T. gondii*. A cut-off titre of 1:64 was used for this study because it is considered the most appropriate titre for interpreting toxoplasmosis prevalence in sheep (Zaki 1995; Pita Gondim et al. 1999; Dempster et al. 2011; Amina et al. 2015; B. Vaatstra, Gribbles Veterinary, pers. comm.). Results are presented as seroprevalence; that is, the proportion of sheep classified as positive for the presence of *T. gondii* antibodies in their sera.

Note: for more information on antibody titres and cut-off levels, and for results presented using a cut-off titre of 1:16, as done with the previous surveys in this study (Landcare Research 2017), see the Appendix.

3.2.4 Statistical analysis

Differences in seroprevalence were assessed in relation to two independent variables: year (treated as a factor, 0 = 2015, 1 = 2017, 2 = 2019) and treatment (0 = outside C2C, 1 = inside C2C). Data from site L1 were not included in the analysis. Treatment and year effects were evaluated using mixed-effects logistic regression models using the package lme4 (Bates et al. 2015) in R version 3.6.2 (R Core Team 2019). The model was fitted using site as a grouping factor (to account for the fact that it is always the same site that is being sampled, so the presence of disease is a repeated measure). The model also tested for an interaction between these two variables. The model (seroprevalence ~ Year + Treatment + Year * Treatment) was fitted to the dependent variable: presence/absence of *T. gondii*. Bootstrapped 95% confidence intervals around mean predicted prevalence were obtained using the package ciTools (Haman & Avery 2020). A significance level of 0.05 was used for all analyses.

4 Results

4.1 Predator control treatment

Overall, 285 feral cats were caught in the C2C treatment area during the study, along with 723 rats and 53 mice (Table 2). Fewer feral cats were caught in Period 2 than Period 1 (Figure 2). The opposite was the case for rat numbers, with considerably more rats caught in Period 2 than Period 1 (Figure 3). The same trends were apparent from the trapping results from areas B and C only (Table 2). Overall, few mice were caught compared with rats (Figure 4.)

Table 2. Numbers of feral cats, rats and mice removed from the Cape to City (C2C) treatment area. Trapping dates are separated into Trapping Period 1 (April 2016 – September 2017) and Trapping Period 2 (September 2017 – September 2019).

	Predator control	**No. animals removed**		
Species	**operational area**	*Period 1*	*Period 2*	**Total**
Feral cats	A	80	13	93
	B, C	124	68	192
	All	**204**	**81**	**285**
Rats	A	71	126	197
	B, C	18	508	526
	All	**89**	**634**	**723**
Mice	A	3	14	17
	B, C	5	31	36
	All	**8**	**45**	**53**

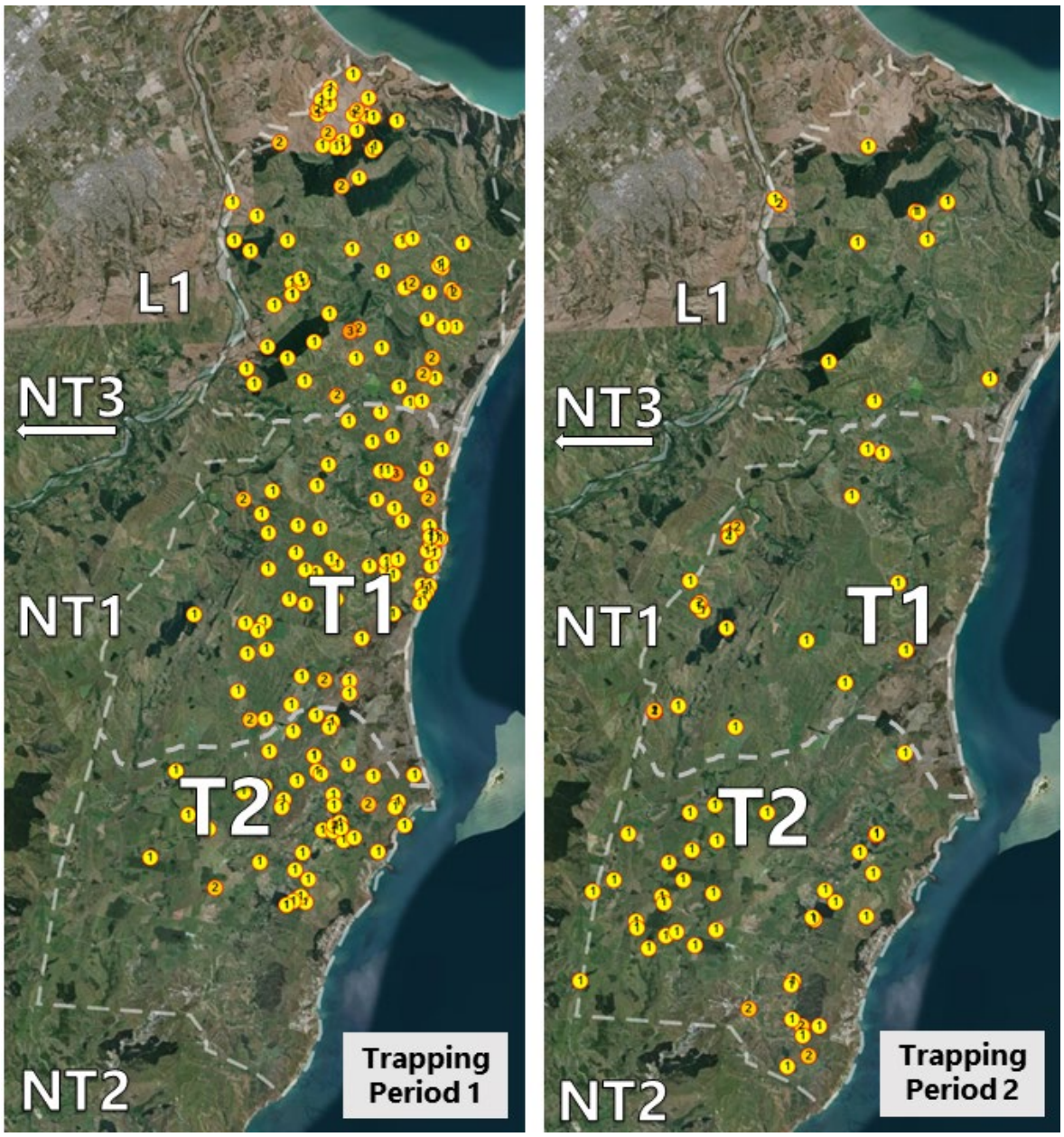

Figure 2. Locations and numbers of *feral cats* removed from the Cape to City treatment area. T1 & T2 = treatment sites; NT1 to NT3 = non-treatment sites; L1 = limited treatment site (this site received a limited amount of treatment due to a change in the treatment area boundary and so was considered separately). Site labels on maps are approximate locations of the corresponding sheep sampling sites. Trapping dates are separated into Trapping Period 1 (April 2016 – September 2017) and Trapping Period 2 (September 2017 – September 2019).

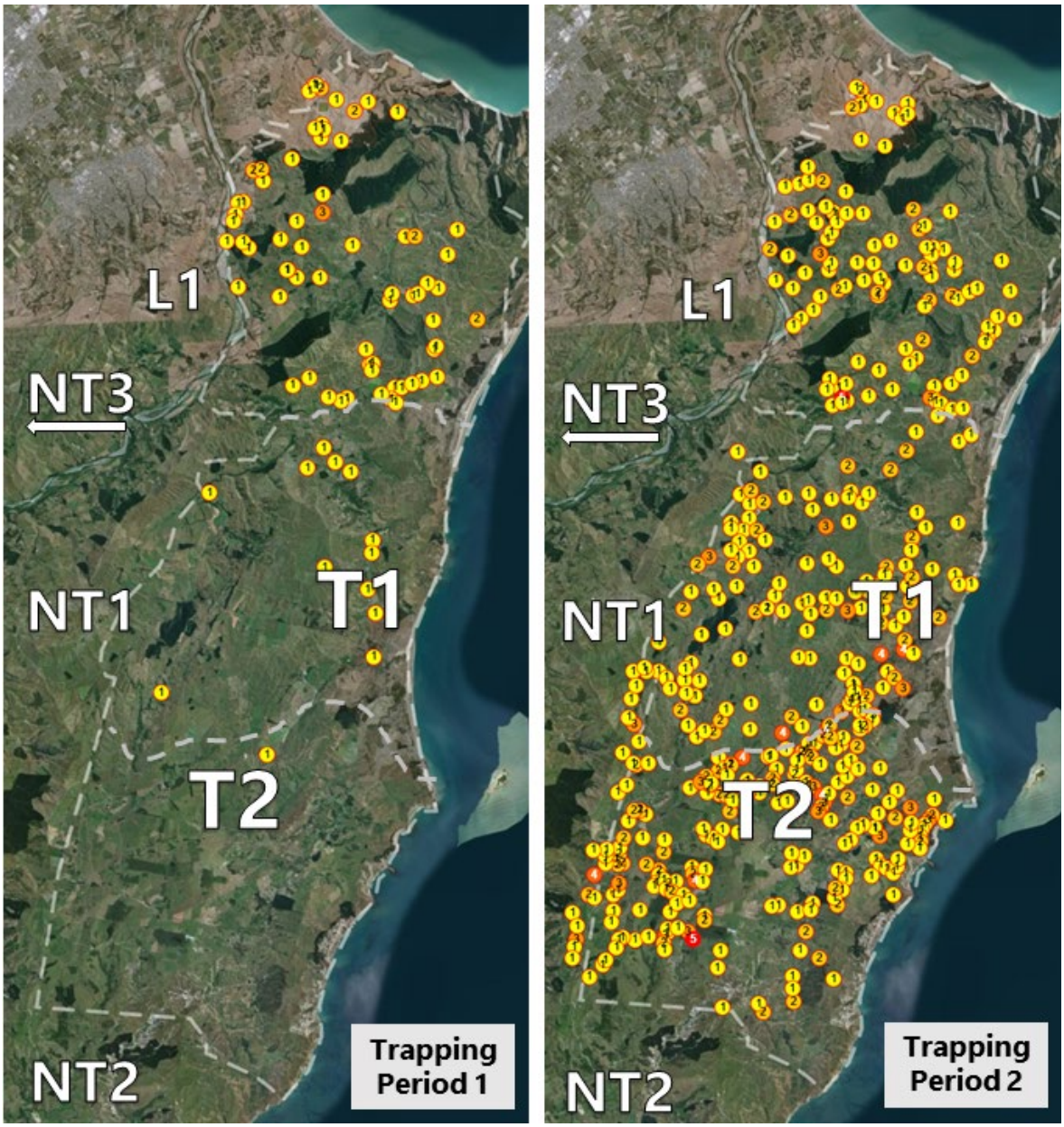

Figure 3. Locations and numbers of *rats* removed from the Cape to City treatment area. T1 & T2 = treatment sites; NT1 to NT3 = non-treatment sites; L1 = limited treatment site (this site received a limited amount of treatment due to a change in the treatment area boundary and so was considered separately). Site labels on maps are approximate locations of the corresponding sheep sampling sites. Trapping dates are separated into Trapping Period 1 (April 2016 – September 2017) and Trapping Period 2 (September 2017 to September 2019).

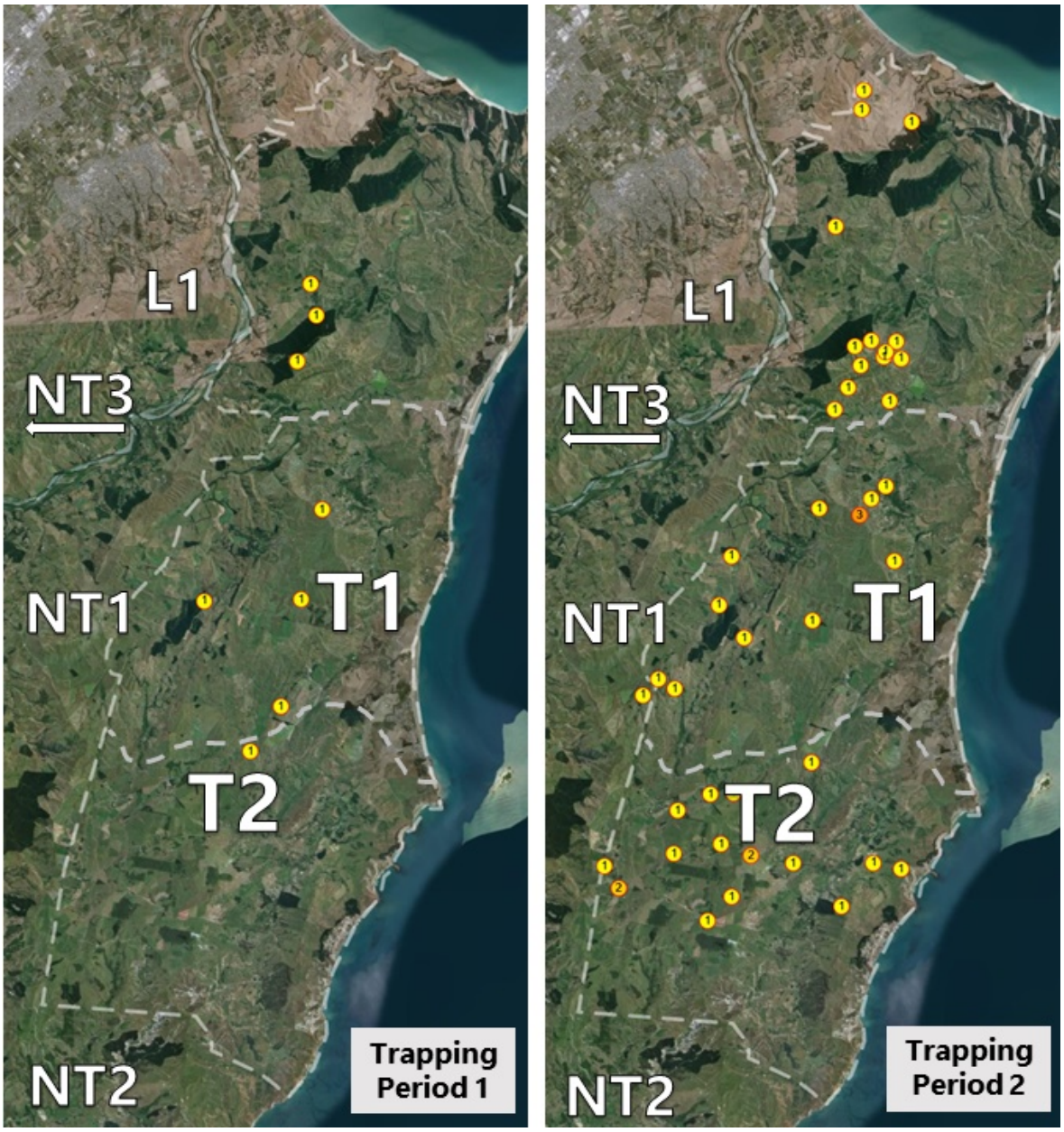

Figure 4**. Locations and numbers of *mice* removed from the Cape to City treatment area. T1 & T2 = treatment sites; NT1 to NT3 = non-treatment sites; L1 = limited treatment site (this site received a limited amount of treatment due to a change in the treatment area boundary and so was considered separately). Site labels on maps are approximate locations of the corresponding sheep sampling sites. Trapping dates are separated into Trapping Period 1 (April 2016 – September 2017) and Trapping Period 2 (September 2017 – September 2019).**

4.2 *Toxoplasma gondii* in sheep

Overall *T. gondii* seroprevalence was 24.4% (255/1,044), but values at the different sites in different years were highly variable, ranging from 1.8% to 51.7% (Table 3). The results from the statistical model suggest that the only significant change in *T. gondii* seroprevalence in this study was an increase between 2015 and 2017, but this increase was seen on both the treatment and non-treatment sites (Table 4, Figure 5). In other words, there was no observable effect of cat and rodent removal on sheep seroprevalence in this study.

Table 3. Observed seroprevalence in domestic sheep at six sites in and adjacent to the Cape to City predator control treatment area. T1 & T2 = treatment sites; L1 = limited treatment site; NT1 to NT3 = non-treatment sites. Year 2015 was pre-treatment. A cut-off titre of 1:64 was used to determine seropositivity.

Sampling year	Site	No. seropositive	Total sampled	% seropositive
2015	T1	7	60	11.7
	T2	24	60	40.0
	L1	13	60	21.7
	NT1	3	60	5.0
	NT2	4	60	6.7
	NT3	20	60	33.3
2017	T1	18	60	30.0
	T2	24	60	40.0
	L1	31	60	51.7
	NT1	2	60	3.3
	NT2	18	60	30.0
	NT3	26	60	43.3
2019	T1	1	57	1.8
	T2	27	59	45.8
	L1	11	60	18.3
	NT1	4	60	6.7
	NT2	12	29	41.4
	NT3	10	59	16.9
Total		**255**	**1,044**	**24.4**

Table 4. Statistical results from the model. An asterisk represents statistical significance with $p \leq 0.05$.

	Estimate	Std. error	z value	Pr(>|z|)	
(Intercept)	–1.978	0.532	–3.720	<0.001	*
Year1	0.719	0.280	2.564	0.010	*
Year2	0.241	0.314	0.769	0.442	
Treatment1	0.790	0.823	0.960	0.337	
Year1:Treatment1	–0.276	0.410	–0.674	0.500	
Year2:Treatment1	–0.353	0.444	–0.793	0.428	

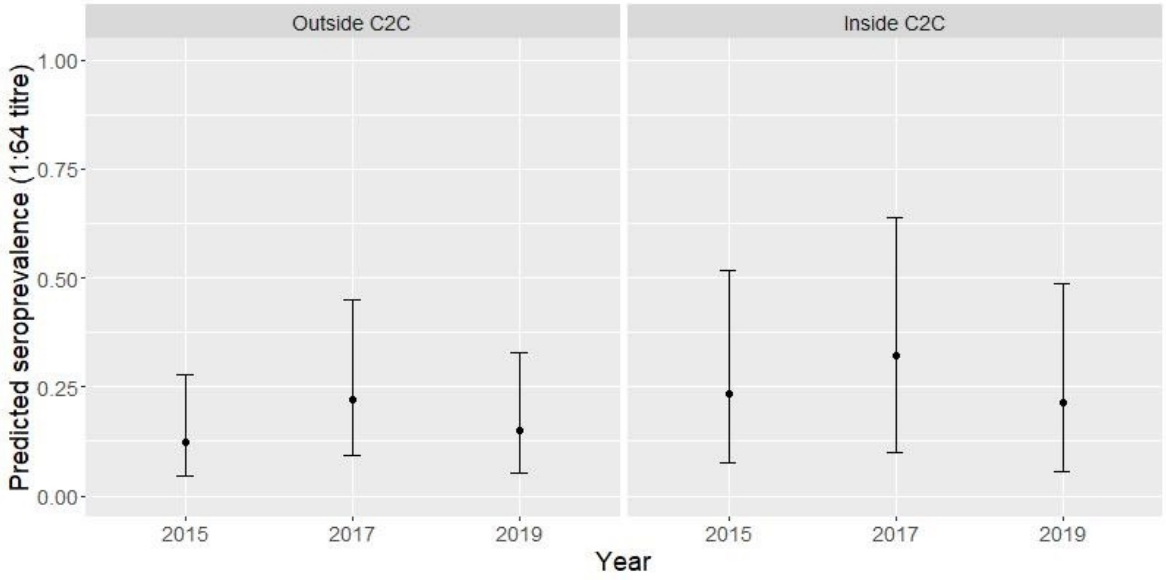

Figure 5. Predicted seroprevalence results for *Toxoplasma gondii* in sheep at treatment sites (inside C2C) and non-treatment sites (outside C2C). Year 2015 was pre-treatment. A cut-off titre of 1:64 was used to determine seropositivity. Error bars represent 95% confidence intervals, obtained by bootstrapping.

5 Conclusions

Predator control efforts undertaken in this study did not measurably reduce the prevalence of toxoplasmosis in sheep within the treatment area, but high variability in seroprevalence observed across sampling sites and years meant that the study lacked power to detect possible small treatment effects. A number of factors could have influenced the results presented here, including the sampling and testing methods used, as well as certain ecological characteristics of *T. gondii*.

Firstly, following data analysis, it was made known by HBRC that some of the sheep in the study area were vaccinated against toxoplasmosis. It appears that, at least for 2019, sheep from sites T1 and T2 were vaccinated, sheep from sites L1, NT2, and NT3 were not, with the status of sheep at site NT1 unknown. Sheep vaccinations in the area typically occur prior to mating, approximately January – March, which would have been 7–9 months before serum sampling occurred. If the susceptibility to *T. gondii* infection was altered as a result of vaccination, it would certainly complicate any interpretations of reported infection levels.

Furthermore, sheep that have previously been vaccinated against *T. gondii* can produce false-positive results at titres of 1:16, 1:32, and even 1:64 (B. Vaatstra, Gribbles Veterinary, personal communication). These titres typically decrease rapidly after a few months, since vaccine strains, unlike wild strains, do not persist and form tissue cysts. Nevertheless, this adds additional uncertainty to our interpretation of the study's findings. In the future, vaccination information should be recorded for all sheep sampled and incorporated into any study designs. Specifically, sheep sampling sites at which sheep are not vaccinated may be more appropriate for studies comparing seroprevalence levels.

More feral cats were trapped in the first half of the study than in the second half, although this coincided with a change in the type of traps used, with those more successful at catching cats deployed predominantly in the first half. Based on camera data, Glen (2020) reported that in late 2017 and 2018 feral cats were less abundant in the C2C treatment area compared to the adjacent non-treatment area, but in 2019, the camera data suggest that relative abundance was similar in both areas. In other words, an initial knockdown effect on cat abundance was observed but did not last. Since *T. gondii* oocysts can remain infectious in the environment for long periods without the presence of cats, including a year or more in water or soil under good conditions (Dumètre & Dardé 2003; Dubey 2010), there is potential for sheep to become infected for some time after cats are removed from the area.

Toxoplasma gondii can also be maintained within populations of intermediate hosts, such as rodents, in the absence of cats. These infected hosts can act as disease reservoirs that can transmit the parasite back to naïve cats, such as offspring of existing cats or individuals that have recently moved into the area. Indeed, infections in cats occur at a much higher rate from predation on infected intermediate hosts than from ingestion of oocysts from the environment (Dubey 2010). Unlike most other intermediate hosts, rodents are capable of maintaining *T. gondii* infections for multiple generations due to vertical transmission from mother to offspring. This occurs at much higher rates in mice, up to nine generations, than in rats (Beverley 1959; Dubey & Frenkel 1998). Jiang et al. (2012) report that 80% of infections in mice occur through vertical transmission, and about 95% of infections in cats occur from infected mice. As mentioned by Tompkins (2014), model simulations suggest that the amount of feral cat control required to eradicate toxoplasmosis can be greatly reduced by low-level reductions in mouse populations each year (Turner et al. 2013). Management of rodents, along with cats, may therefore be advantageous in efforts to reduce or eliminate *T. gondii* infection in sheep.

Most of the rats and mice in this study were trapped in the final two years. As with the cat trapping results, this can be attributed in part to the types of traps used, with those more successful at catching rats deployed in the latter half of the study. However, these results are also suggestive of an increase in rodents throughout the landscape as the study progressed, possibly due to decreased predation from cats. For example, Oppel et al. (2014) reported an increase in rat and mouse activity in pastures following feral cat control. A better understanding of rodent abundance and infection status may be a crucial component in the overall understanding of *T. gondii* transmission dynamics in the area. Additionally, given that different strains of *T. gondii* exist, some of which are far more pathogenic than others, characterisation of the diversity of these strains within each host species (sheep, cats, rodents and perhaps others) would increase our understanding of *T. gondii* transmission dynamics in this landscape.

Additional risk factors also exist in terms of *T. gondii* infection. The presence of domestic cats in and around the sampling sites could also be playing a role in *T. gondii* transmission. While infection is generally higher in feral cats than in domestic cats, usually due to more rodents in their diets (Dubey 2010), all cats are capable of excreting infectious oocysts, and thus the presence of domestic cats should be better quantified.

Moisture and temperature can also affect the viability of *T. gondii* oocysts in the environment (Dubey 2010). Rainfall data for the southern Hawke's Bay region show increased rainfall 4 to 6 months before the 2017 sampling took place (Appendix Figure A1), but it is not known if this influenced the observed seroprevalence levels in this study.

Seasonality can also influence *T. gondii* transmission, with a study in Sweden showing that most sheep became infected in autumn (Lundén et al. 1994). Although the sampling in this study occurred at about the same time in spring each year, the scope and success of the predator control varied across seasons for different sites and sampling years. The effect this had on the observed seroprevalence levels in sheep is not known, but the influence of season might be worth considering, particularly as it relates to trapping effort. Other risk factors that are known to influence *T. gondii* seroprevalence in sheep include elevation, farm size, the age of sheep (all were 1-year-olds in this study), and whether sheep use surface water for drinking (Dubey 2010). All of the factors discussed here should be considered when planning future toxoplasmosis investigations.

Due to the ability of the *T. gondii* parasite to persist for long durations in the absence of cat definitive hosts, in either the environment or within intermediate host populations, it is likely necessary to reduce cat numbers for a longer period of time before any changes in infection levels in sheep can be observed. Specifically, a response may not be detected until at least 1-2 years after feral cat numbers are knocked down and maintained at low levels. The sampling of sheep would also then need to continue long enough to take into account any lag effect that may be occurring between the removal of feral cats and any subsequent infections observed in sheep.

6 Recommendations

General

- Continue to assess the influence of feral cat control on toxoplasmosis infection in sheep.
- Maintain feral cat numbers at low levels for a longer period of time following initial reductions.
- Incorporate control and monitoring of rodents, particularly mice, into future toxoplasmosis management strategies.

Pest control

- Increase efforts to remove feral cats from the landscape by:
  - improving the current cat control over the entire C2C treatment area, and/or
  - targeting cat control in and around the sheep sampling sites.
- Improve the capture rate of feral cats by utilising traps and techniques designed specifically for feral cats (e.g. leg-hold trapping).
- Rodents, especially mice, should also be targeted, instead of relying on by-catch using current methods.

Pest monitoring

- In addition to maintaining high-quality feral cat trapping records, continue to independently monitor changes in the relative abundance of feral cats in response to trapping efforts (e.g. via camera traps).
- Obtain a better understanding of domestic, stray, and farm cat numbers and how these compare with those of feral cats, particularly near areas grazed by sheep.
- Monitor changes in the relative abundance of rats and mice in response to trapping efforts (e.g. via tracking tunnels).

Toxoplasmosis sampling of sheep

- Continue to measure *T. gondii* seroprevalence levels in sheep by:
  - maintaining sampling for multiple years following any any reductions in cat numbers
  - sampling 1-year-old ewes
  - sampling from unvaccinated flocks only
  - using an antibody titre cut-off of 1:64 when determining seropositivity.
- Increase the number of treatment and non-treatment sites (to be decided using a power analysis).

Toxoplasmosis infection surveys in rodents

- Collect data on *T. gondii* infection status in rodent intermediate hosts through trapping and molecular analysis of tissue samples.
- Identify and compare *T. gondii* strain types between:
  - Rodents (using fresh tissue samples)
  - Sheep (using fresh tissue samples from dead animals)
  - Cats (using fresh faeces from the environment or collected from necropsies; faeces is necessary to determine strains that are actively being shed)

7 Acknowledgements

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Appendix

Rainfall data

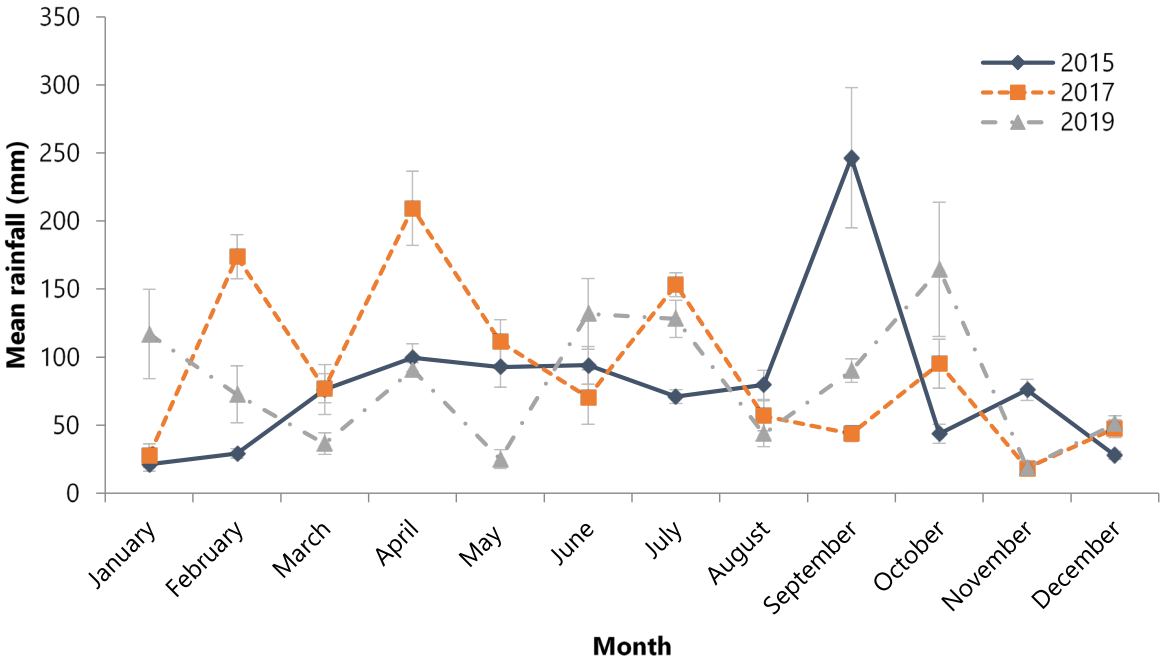

Figure A1. Monthly rainfall data for 2015, 2017, and 2019, averaged from five sites in the southern Hawke's Bay region: Mangaorapa, Waipoapoa, Ben Nevis, Wallingford and Maraetotara. Error bars represent ± 1 standard error of the mean. Data provided by Hawke's Bay Regional Council.

Antibody titres

Additional explanation of laboratory testing and antibody titres

Antibodies are produced by an animal's immune system when a pathogen is detected. The latex agglutination test (LAT) was used here to quantify the antibody response to the parasite that causes toxoplasmosis, *Toxoplasma gondii*. Blood sera were tested at a range of dilutions, doubling from 1:16 to 1:2048, resulting in an assigned antibody titre for each sample. This titre, which measures the level of antibodies in a host, is defined as the highest dilution at which there was a visible reaction.

For example, if blood serum from a single sheep reacted at a dilution of 1:128, then again at 1:256, but not at 1:512, the titre assigned to that sheep sample would be 1:256. Since no dilutions were tested above 1:2048, samples that reacted at this dilution are assigned a titre value of ≥1:2048. For samples that did not react at 1:16, no titre was given and they were given a designation of 'negative' by the laboratory. Therefore in this study, one of nine possible titre designations was assigned to each sample analysed (Table A1).

However, not every serum sample that is assigned a titre is necessarily considered seropositive. Often a sensitivity threshold, or cut-off titre, is used when determining

whether a sample is seropositive or not. So, if a cut-off titre of 1:64 is chosen, all samples with titre values below 1:64 (e.g. 1:16, 1:32) are considered negative. The criteria for choosing a cut-off titre is not well defined, often varying by study, location, and test (Dubey 2010). A cut-off titre of 1:64 was used for this study because it is considered the most appropriate titre for interpreting toxoplasmosis prevalence in sheep (Zaki 1995; Pita Gondim et al. 1999; Dempster et al. 2011; Amina et al. 2015; B. Vaatstra, Gribbles Veterinary, pers. comm.).

Table A1. List of all potential titre designations for this study, including examples of seropositivity for a 1:64 cut-off titre scenario (as in this study)

Titre designation given by laboratory	Considered seropositive in this study
Negative	No
1:16	No
1:32	No
1:64	Yes
1:128	Yes
1:256	Yes
1:512	Yes
1:1024	Yes
≥1:2048	Yes

Titre distribution results

The percentage distribution of *T. gondii* titres in this study shows the most frequent antibody titres were 1:16 (223/556; 40.1%) and ≥1:2048 (153/556; 27.5%) (Figure A2). The most frequent antibody titres were 1:16 (223/556; 40.1%) and ≥1:2048 (153/556; 27.5%). Interestingly, the second most common antibody titre identified here, after 1:16, was the highest titre of ≥1:2048. In generally, high titres (≥1:512) observed in sheep suggest recent exposure or infection, and can indicate acute toxoplasmosis, although it can also be a result of reactivation of latent toxoplasmosis in immunosuppressed individuals (Amina et al. 2015). Titres of 1:128 and 1:256 can indicate exposure in the last few months or even years, while lower titres can indicate very old infections or light infections (B. Vaatstra, Gribbles Veterinary, pers. comm.).

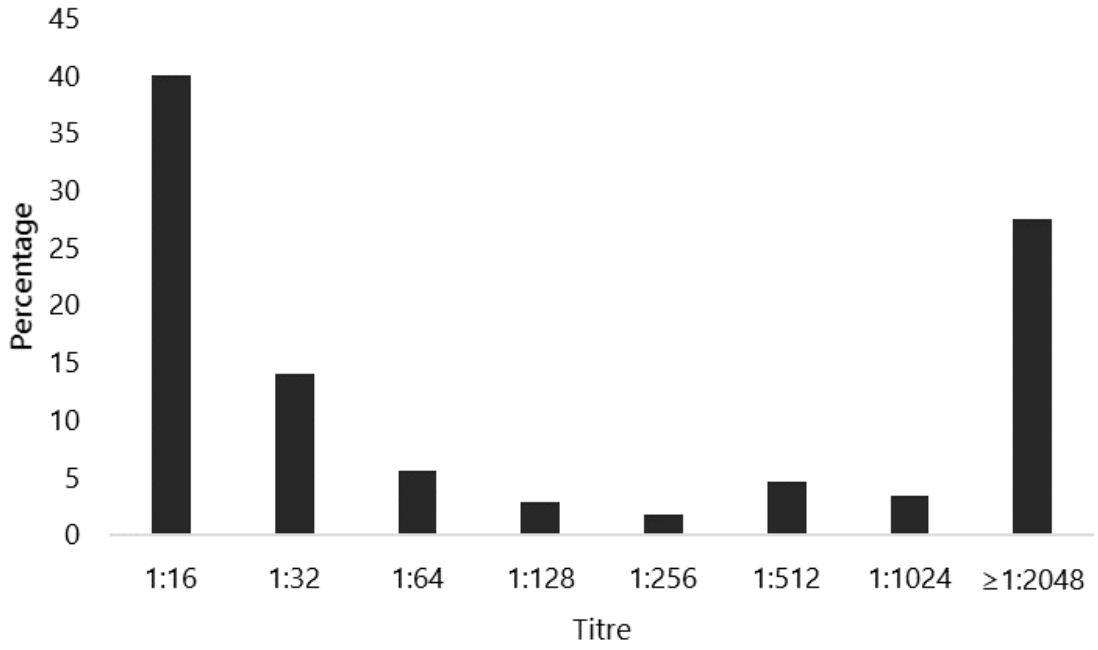

Figure A2. Percentage distribution of *Toxoplasma gondii* titres identified from 556 domestic sheep in and adjacent to the Cape to City (C2C) treatment area, including 2015, 2017, and 2019.

Toxoplasma gondii in sheep (results using a 1:16 cut-off titre)

To maintain continuity with the previous surveys in this study (Landcare Research 2017), seroprevalence results and statistical analysis are presented again using a 1:16 cut-off titre to determine seropositivity.

Methods (using a 1:16 cut-off titre)

Results presented here are using a cut-off titre of 1:16. This was to maintain continuity with the previous surveys in this study (Landcare Research 2017). However, caution should be taken when interpreting LAT titres in sheep that are less than 1:64 for *T. gondii*, as these low titre levels (e.g. 1:16 or 1:32) do not necessarily indicate a clinically significant infection and could be a result of false-positive reactions (Trees et al. 1989; Dempster et al. 2011). Site labels have been changed in this report from those used in previous surveys (Table A2). All other methods are the same as presented above.

Table A2. List of sheep sampling sites and corresponding labels used in previous progress report (Landcare Research 2017).

Site ID	Site category	Previous label
L1	Limited treatment	Farm 1
T1	Treatment	Farm 2
T2	Treatment	Farm 3
NT1	Non-treatment	Farm 4
NT2	Non-treatment	Farm 5
NT3	Non-treatment	Farm 6

Results (using a 1:16 cut-off titre)

When considering a cut-off titre of 1:16, 53.3% (556/1044) of all sheep sera tested in this study were seropositive for *T. gondii*, with values ranging between 21.7% and 93.3% (Table A3).

Table A3. Seroprevalence in domestic sheep (using a cut-off titre of 1:16) at six sites in and adjacent to the Cape to City predator control treatment area. T1 – T2 = treatment sites; L1 = limited treatment site; NT1, NT2 & NT3 = non-treatment sites. Year 2015 was pre-treatment.

Sampling year	Site	No. seropositive	Total sampled	% seropositive
2015	T1	18	60	30.0
	T2	30	60	50.0
	L1	19	60	31.7
	NT1	14	60	23.3
	NT2	13	60	21.7
	NT3	48	60	80.0
2017	T1	29	60	48.3
	T2	35	60	58.3
	L1	52	60	86.7
	NT1	42	60	70.0
	NT2	35	60	58.3
	NT3	56	60	93.3
2019	T1	23	57	40.4
	T2	37	59	62.7
	L1	26	60	43.3
	NT1	29	60	48.3
	NT2	16	29	55.2
	NT3	34	59	57.6
Total		**556**	**1,044**	**53.3**

The results from the statistical model, using a 1:16 titre cut-off, suggest that the only significant difference in observed *T. gondii* seroprevalence was between the years 2015 and 2017 for the non-treatment sites (outside C2C), but that same effect was not evident for the treatment sites (inside C2C) (Table A4; Figure A3). However, since this treatment effect was influenced by a large presence of low titres, any interpretation with in regard to clinically significant infections in sheep should be treated with caution.

Table A4. Statistical results from the model (using a cut-off titre of 1:16). An asterisk represents statistical significance with $p \leq 0.05$.

	Estimate	Std. error	z value	Pr(>|z|)	
(Intercept)	–0.361	0.380	–0.952	0.341	
Year1	1.522	0.242	6.300	<0.001	*
Year2	0.407	0.239	1.705	0.088	
Treatment1	–0.058	0.598	–0.098	0.922	
Year1:Treatment1	–0.999	0.359	–2.781	0.005	*
Year2:Treatment1	0.078	0.359	0.218	0.827	

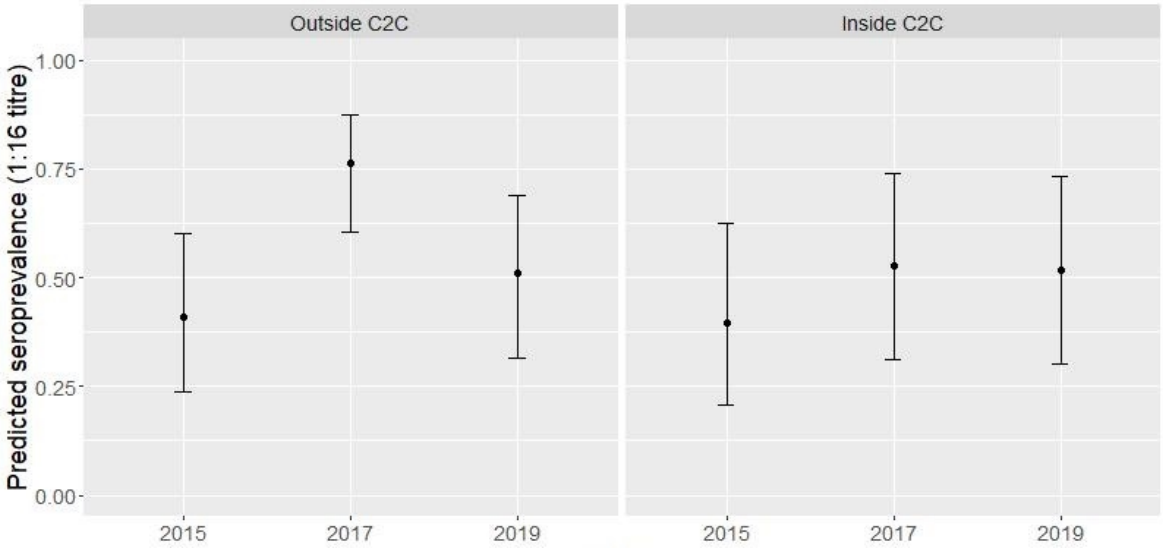

Figure A3. Predicted seroprevalence results for *Toxoplasma gondii* in sheep at treatment sites (inside C2C) and non-treatment sites (outside C2C). Year 2015 was pre-treatment. A cut-off titre of 1:16 was used to determine seropositivity. Error bars represent 95% confidence intervals, obtained by bootstrapping.

Conclusions (using a 1:16 cut-off titre)

Comparison of results between treatment and non-treatment sites varied slightly depending on the cut-off titre used to determine seropositivity. However, based on current literature, we recommend using an antibody titre cut-off of 1:64 when determining seropositivity.