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Changes in ovine Johne's disease prevalence following vaccination with Gudair™ for an extended period

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Abstract

This project was conducted to evaluate the effectiveness of Gudair™ vaccine in reducing ovine Johne's disease (OJD) prevalence in vaccinating flocks over more than one decade. Six biennial faecal samplings were conducted in 12 enrolled flocks that commenced vaccinating lambs in or prior to 2002; three samplings were done in a previous project (OJD.033) and three in the current project. At each sampling, faecal pools created by collecting one pellet per sheep, from each of four age groups (3, 4, 5 and 6 year-old sheep) were selected from each flock. Actual number and sizes of pools varied. Samples were cultured using pooled faecal culture and the sheep level OJD prevalence was calculated and compared between years. The proportion of positive pools and the animal level prevalence of shedding significantly reduced over time but faecal shedding persisted and was still present in 3 of the 8 flocks (37.5%) that remained in the study at the last sampling conducted in 2013-14. The findings of this project will enable sheep producers make better informed decisions on managing OJD in their farms, and in evaluating the risks of purchasing and trading vaccinated sheep.

Executive Summary

Ovine Johne's disease (OJD) is a debilitating disease of sheep caused by *Mycobacterium avium* subsps. *paratuberculosis* (Mptb) that causes chronic diarrhoea and emaciation in infected animals and significant economic losses to Australian producers. Animals are usually infected as lambs from contaminated pastures or milk although the clinical signs and pathology of the disease are often expressed much later when the animals are adults. Vaccination with Gudair™ has become a key strategy to control the disease since registration was achieved in Australia in 2002. However, there is limited quantitative information on the effectiveness of the vaccine over an extended period of time, both in Australia and in other sheep producing countries.

A project (OJD.033) was funded by the sheep industry to evaluate effectiveness of the Gudair™ vaccine. In this project, 12 flocks of varying prevalence were enrolled and sampled three times biennially to estimate changes in OJD prevalence over 6 years. This project determined a significant decrease in shedding rates of Mptb following the introduction of vaccination in the majority of flocks in the study. However shedding of Mptb was detectable in 10 of the 11 flocks that remained in the study at the last sampling conducted in 2008. The current project was developed to continue the studies in the enrolled flocks for a further six years, determining changes in OJD prevalence over an extended period of time that exceeded a decade. This is the longest and most comprehensive paratuberculosis vaccine efficacy study ever attempted. In this report we present results of changes in OJD prevalence and Mptb shedding in these 12 flocks from the time they were first enrolled in 2003-04 in the previous OJD.003 project until the final sampling in the current project in 2013-14.

The design of the current project was similar to the previous project (OJD.033). We continued to sample the enrolled flocks for another three biennial samplings. At each sampling, we aimed to select seven faecal pools of 50 sheep each (collecting one pellet per sheep) from each of four age groups (3, 4, 5 and 6 year-old sheep) from each flock, although the actual number and sizes of pools varied due to logistical issues including variations in availability of suitable animals from each age cohort. The desired pool size was reduced from 50 to 25 and the numbers of pools increased from 7 to14 towards later stages of the project as the OJD prevalence decreased in the enrolled flocks. Samples were cultured using pooled faecal culture and the sheep level OJD prevalence was calculated and compared over time. Descriptive and statistical analyses were conducted to evaluate changes in the probability of a pool to be positive and the OJD prevalence over time.

The proportion of positive pools significantly declined over time from 50.3% at the first sampling in 2003-04 to only 3.1% at the last sampling, suggestive of a 30 fold reduction in the odds of a pool to be positive ($p<0.001$). Similarly, the average animal level prevalence dropped from 7.64% at the first sampling to just 0.12% at the last sampling. However, 7 of the 10 flocks remaining in the project at the second last sampling conducted in 2011-12, and 3 of the 8 flocks at the last sampling in 2013-14 still had sheep with detectable shedding of Mptb after more than a decade of vaccination.

The results confirm that Gudair™ vaccination is effective in reducing OJD prevalence and Mptb shedding, but due to persistence of low-level shedding in vaccinates, sheep producers are advised to continue to vaccinate sheep for an extended period of time. Unfortunately,

recommendations on a preferred time for cessation of vaccination cannot be determined from the study as Mptb could still be detected in some 40% of the flocks at the last sampling, about 12 years after the commencement of vaccination. The study also indicates that sheep producers should carefully evaluate the risk of introduction of Mptb to their farms when purchasing vaccinated re-stocker sheep, particularly from known or suspected OJD infected properties.

The effectiveness of the vaccine was variable between flocks. Further epidemiological studies should be conducted to evaluate the reasons for continuous presence of Mptb shedding on some farms, whereas on several other farms it has eliminated (or at least reduced to undetectable levels) during the same period of time. Identification of management and biosecurity measures that can be used in conjunction with vaccination to eliminate infection (or rapidly reduce Mptb shedding) would be invaluable for farmers. Investigations are also required to understand what would happen at the cessation of vaccination. For example, is it likely that the putative negative or low prevalence farms continue to stay that way, or would the prevalence of shedding of Mptb increase as soon as vaccination ceased? Findings from such studies would complement the findings from this and previous projects and enable sheep producers to make more informed decisions for managing OJD in their flocks.

Table of Contents

1 Background

Ovine Johne's disease (OJD) is a fatal enteric infection of sheep caused by *Mycobacterium avium* subsps. *paratuberculosis* (Mptb) that has proven difficult to both diagnose and control. Production and financial losses through wasting and mortality due to OJD have been significant in sheep in Australia (Bush et al., 2008). In addition, speculation of a potential zoonotic link with Crohn's disease of humans, and hence food safety issues, has further enhanced interest in controlling the disease.

A National Ovine Johne's Disease Control and Evaluation Program commenced in 1998 and funded a research project (OJD.009) that demonstrated the efficacy of vaccinating lambs with Gudair^{TM}, a killed whole cell vaccine imported from Spain, for controlling OJD in high prevalence sheep flocks (Reddacliff et al., 2006). Vaccination reduced the prevalence of mortality by 90%, delayed the onset of faecal shedding of Mptb by 12 months, and reduced the prevalence of shedders by 90% compared to the unvaccinated lambs. This study led to the registration of Gudair™ which became established as the key strategy in Australia to control the disease and manage the risk of transmission of OJD both within and between flocks. However importantly, a small proportion of vaccinates went on to develop OJD, shed infectious organisms at high levels and eventually die of the disease (Reddacliff et al., 2006).

Whilst the original research was conducted in high prevalence flocks, many flocks with lower OJD prevalence also commenced vaccination following registration of Gudair™, both as a precaution against increased OJD associated mortalities and for a period, improved access to markets for sale of vaccinated re-stocker sheep through the risk-based trading Assurance Based Credit point scheme for OJD. When the vaccine became widely available, it was suggested that effectiveness of the vaccine might be superior in flocks with lower OJD prevalence, where later drops of vaccinated lambs were exposed to sheep of a much lower risk of developing OJD than in the original study. Objective evidence for this suggestion was unavailable. Disease modelling suggested that the prevalence of mortalities and shedding would fall rapidly after the commencement of a vaccination control program but that this depended on OJD prevalence at the time vaccination commenced. Validation of this work by field research was required.

The issue of the risk of OJD occurring in vaccinates as possibly being dependent on the disease prevalence at the time of commencing a vaccination control program, has become increasingly important as OJD has continued to spread in Australia. As the effectiveness of vaccination will become more evident over an extended period following vaccination of a number of successive lamb crops, it became apparent that a longitudinal study on the shedding rates of vaccinates in flocks of varying prevalence at the time of initiation of the vaccination program, was required .

A project (OJD 033) was, therefore, funded to evaluate effectiveness of the vaccine on 12 farms of varying prevalence (H=high, M=medium, L=low) in the central tablelands of NSW. Analysis of this study conducted between 2003 and March 2009 reported significant decline in shedding rates on most of the farms, but low rates of shedding (range 0.13% to 1.29%) persisted on 10 of the 11 properties at completion of the sampling in 2008 when all sheep in the flocks were vaccinates. The response to Gudair™ vaccination appeared to be highly variable between flocks in these early stages of implementing the vaccination program.

These findings were presented to industry in February 2008 and a recommendation was made that this study should be extended to determine the rate of decline in shedding and the degree of shedding when all animals in these flocks were 2nd generation vaccinates, that is, the progeny of lambs born when the entire flock consisted of approved vaccinates. As a result, the current project (P.PSH.0565) was funded to assess the long-term effectiveness of Gudair™ vaccine for the reduction in bacterial shedding in flocks of initial low, medium or high OJD prevalence.

In this report, we present changes in the prevalence of shedding over more than a decade in the enrolled flocks of variable prevalence after they commenced vaccinating 1-4 month old lambs in 2002, i.e. since the commencement of the previous project (OJD.033). This approach has been taken to provide a complete picture of the changes in OJD prevalence in these flocks because results from three samplings in this project alone would only provide partial information without considering the changes in these flocks for the six years prior to the commencement of this project.

It was anticipated that the outcomes from this study would assist sheep producers and their advisors as well as disease control authorities, in assessing both the risk of cessation of vaccination in infected flocks and the risk of purchasing vaccinated re-stocker sheep.

2 Project objectives

This project was funded to examine the extended changes over time in the prevalence of *Mptb* shedding following the commencement of a Gudair™ vaccination program in 2002 in flocks varying in initial OJD prevalence. The 11 flocks of initial high (3), medium (4) or low (4) OJD prevalence at the commencement of the vaccination program were to be assessed for an additional 3 rounds of PFC sampling at 2 yearly intervals. It was anticipated that by September 2015, this and the previous project (OJD.033) will have measured changes in the prevalence of shedding in enrolled OJD infected flocks on 6 occasions since the commencement of vaccination in 2002, providing estimates of the rate of decline and risk of shedding from vaccinated sheep in flocks of varying initial OJD prevalence.

3 Methodology

3.1 Selection of sheep flocks

The reference population for the study were OJD infected sheep flocks in New South Wales with a range of prevalence levels. The study population included flocks that met the following selection criteria: (a) high level of willingness to participate in the study and managerial ability of the flock owner; (b) self-replacing Merino flock lambing >500 ewes per year; (c) an OJD positive diagnosis with a continuous vaccination program that commenced with lambs in 2002 or earlier; and (d) vaccination of only lambs in the first year.

Potential flocks were initially identified from official databases and local animal health staff. Flock owners were contacted to confirm their suitability and interest in participating in the project. Available data on previous OJD testing and OJD-related mortalities were used to classify the flocks into three crude prevalence categories as follows: (a) High: >5% mortalities, >4/7 positive pools; (b) Medium: <5% mortalities, 2-4/7 positive pools; and (c) Low: mortalities not recognized, <2/7 positive pools.

3.2 Faecal sample collection

In the previous project (OJD.033), three biennial faecal samplings were conducted at the enrolled flocks. At each sampling, the aim was to collect seven pools of 50 sheep (one pellet per sheep) from each of the four age groups (3, 4, 5 and 6 year-old sheep) from each enrolled flock, providing samples from 350 sheep from each age group. This was the sample size required to achieve a sensitivity of 98% for detecting flocks with ≥2% prevalence (Sergeant et al., 2002). Although largely successful in achieving these sample size objectives, the pool numbers and sizes collected did vary on occasions.

Faecal sample collection was performed by the local district livestock research officer and involved collecting one faecal pellet per rectum from each selected sheep. One member of the research team (JE) attended most of the sample collections which helped in quality control. Pellets from 50 sheep were pooled in a sterile plastic container to constitute one pool and stored at –80°C until cultured.

The same approach was used in the current project. However, the desired pool size was reduced from 50 to 25 and the number of pools increased from 7 to14 towards later stages of the project as the OJD prevalence decreased in the enrolled flocks (see Supplementary Tables 1 to 3). Thus, we were successful in conducting six biennial samplings in total, although some flocks dropped out of the project due to reasons beyond the control of the project team.

3.3 Pooled faecal culture

Pooled faecal samples were cultured initially using a modified BACTEC radiometric method (Whittington et al., 2000). However, this method was replaced by culture in M7H9C medium, a newly validated method from project P.PSH.0576 (Whittington et al., 2013, Plain et al., 2015) in the second half of the study.

3.4 Statistical analyses

Unless indicated otherwise, all statistical analyses were conducted using the SAS statistical program (release 9.3 © 2002–2008, SAS Institute Inc., Cary, NC, USA) while the graphics were prepared using the R statistical package (version 3.2.2, R Development Core Team, 2015, http://www.r-project.org).

Animal level prevalence of sheep shedding Mptb in faeces was estimated, based on the total number of pools, the number of pools positive and the number of pellets constituting a pool using SAS GENMOD procedure (Williams and Moffitt, 2001). Prevalence calculations assumed perfect test sensitivity and specificity but allowed for variable pool size (Williams and Moffitt, 2001).

Descriptive analyses included estimation of number and proportions of pools positive for each flock and age group at each sampling. Similarly, prevalence estimates were summarised for each flock, age group and pre-vaccination status. In addition to measuring changes in prevalence in flocks over time, we also evaluated changes in prevalence in specific cohorts within flocks. Since sampling was conducted every two years, the 3-year-old (4-year-old) sheep were again sampled when they were 5-year-olds (6-year-olds) (Table 1). Descriptive analyses were conducted to evaluate changes in prevalence in these cohorts.

Generalised linear mixed models with pool status as a binary outcome and flock as a random effect were fitted employing the SAS GLIMMIX procedure to estimate and compare odds of a pool to be positive. Three variables – pre-vaccination prevalence, age and log pool size – were tested by including in the model and retained if significant. Probabilities predicted by the model were calculated and presented.

In a second analysis, changes in prevalence over time in different age groups were determined by the linear mixed model approach using the SAS MIXED procedure, by including flock as a random effect to account for clustering of observations within a flock. Model assumptions were evaluated using residual diagnostics and the prevalence was log transformed to satisfy the assumptions of normality and homoscedasticity. Predicted means from the model were exponentiated to calculate geometric means for presentation.

Similar linear mixed model analyses were conducted to evaluate changes in prevalence in cohorts of sheep followed over two consecutive samplings within flocks. Effect of time (pre and post), cohort (1 to 5 representing sampling years – see Table 1), and age group ('3- to 5-year-old' or '4- to 6-year-old sheep') on log prevalence was evaluated by including flock as a random effect.

All P-values reported in the manuscript are two sided. A P-value of <0.05 was considered significant.

Table 1. A schematic representation of the follow up of five cohorts for a flock in the study. Since sheep were sampled every two years, 3- and 4-year-old sheep were again sampled when they were 5- and 6-year-old, respectively. Note that the 5- and 6-year-old sheep in the first sampling and 3- and 4-year-old sheep from the last sampling (shaded grey) were only sampled once. Also note that not all five cohorts could be sampled from all flocks.

Cohort	2003-04	2005-06	2007-08	2009-10	2011-12	2013-14
1	3-year-old	5-year-old				
	4-year-old	6-year-old				
2	5-year-old	3-year-old	5-year-old			
	6-year-old	4-year-old	6-year-old			
3			3-year-old	5-year-old		
			4-year-old	6-year-old		
4				3-year-old	5-year-old	
				4-year-old	6-year-old	
5					3-year-old	5-year-old
					4-year-old	6-year-old
						3-year-old
						4-year-old

Note: All sheep at the first sampling and 5- and 6-year-olds at the second sampling were not vaccinated.

4 Results

4.1 Descriptive results

4.1.1 Number and proportions of pools positive

Twelve flocks that met the selection criteria and ranged across the spectrum of OJD prevalence from low to high were enrolled in the study. Consideration of mortality data and previous testing resulted in designation of three high prevalence farms (H1 to H3), four medium prevalence farms (M1 to M4) and five low prevalence farms (L1 to L5). Prevalence data collected at the first sampling confirmed that selected flocks did represent a range of flock prevalences.

Due to unforeseen circumstances, it was not possible for all 12 flocks to remain enrolled for the duration of the two projects. We were successful in collecting samples on all six occasions from eight flocks, on five occasions from two flocks, on three and two occasions from one flock each. The numbers of positive pools and the total number of pools collected for each flock and for each age group are presented in Table 2. The proportions of positive pools over five samplings by pre-vaccination prevalence status are displayed in Figs 1 and 2.

In general, the proportions of positive pools appeared to decline over time, but there was a wide variation over time in different flocks and age groups. Seven of the 10 flocks had at least one pool positive at the second last sampling (2011-2012), with two low prevalence flocks having 10/42 (23.8%) and 12/56 (21.4%) pools positive (Table 2). At the last sampling in 2013-2014, faecal shedding was only detected in 3 of the 8 flocks that remained in the study, although they still represented 37.5% of the sampled flocks at that time.

Table 2. The numbers of positive and total pools collected for each flock over six samplings from 2003-2004 to 2007-2008 for project OJD.033 and from 2009-2010 to 2013-2014 for the current project (P.PSH.056). Details of the numbers of positive and total pools from each age group in a flock are presented in Supplementary Table 1 in the Appendix.

Flock number[A]	**Number of positive/total pools**					
	2003-2004	**2005-2006**	**2007-2008**	**2009-2010**	**2011-2012**	**2013-2014**
H1	17/23	10/25	4/25	3/28	2/56	0/54[B]
H2	19/23	15/27	3/20	1/14	0/36	0/38
H3	15/28	19/28	10/21	7/28	6/37	-
M1	21/28	7/28	2/25	3/17	2/30	1/27
M2	3/28	4/28	0/28	0/123	0/128	-
M3	8/20	5/14	2/16	1/34	0/22	0/22
M4	17/21	5/16	6/19	-	-	-
L1	17/26	10/26	4/21	5/29	1/55	0/53
L2	6/28	6/24	10/28	5/28	12/56	6/56
L3	0/21	4/19	**-**	**-**	**-**	**-**
L4	13/24	5/17	1/17	4/13	1/19	0/18
L5	11/22	13/23	11/37	11/31	10/42	3/53
Total	**147/292**	**103/275**	**53/257**	**41/345**	**34/481**	**10/321**

[A]H=High pre-vaccination prevalence; M=Medium pre-vaccination prevalence; L= Low pre-vaccination prevalence.
[B]Due to logistical issues, the last sampling from this flock was collected in February 2015.

4.1.2 Prevalence estimates

The prevalence estimates from the PFC culture data for each age group for each year calculated using the Williams and Moffitt, 2001 approach are presented in Table 3 and Figs 3-4. Although the expected decrease in rate of excretion was not consistently observed in all flocks, there was an obvious trend of decreasing average prevalence over time (Fig 5).

Table 3. Prevalence estimates for each age group for each year calculated using the Williams and Moffitt (2001) approach.

Age/Year	Num	Min	Q1	Median	Q3	Max	Mean	SD
3-year-old								
2003-2004	12	0.00%	0.67%	1.15%	3.04%	82.52%	11.12%	24.87%
2005-2006	12	0.00%	0.00%	0.31%	0.68%	1.98%	0.48%	0.57%
2007-2008	11	0.00%	0.00%	0.31%	0.91%	3.82%	0.66%	1.11%
2009-2010	10	0.00%	0.00%	0.44%	1.00%	1.98%	0.56%	0.65%
2011-2012	9	0.00%	0.00%	0.00%	0.96%	1.75%	0.44%	0.69%
2013-2014	8	0.00%	0.00%	0.00%	0.00%	0.61%	0.08%	0.22%
4-year-old								
2003-2004	12	0.00%	0.00%	1.93%	3.82%	38.09%	4.77%	10.62%
2005-2006	12	0.00%	0.00%	0.00%	0.92%	1.68%	0.41%	0.60%
2007-2008	11	0.00%	0.00%	0.67%	1.11%	1.42%	0.57%	0.58%
2009-2010	10	0.00%	0.29%	0.67%	0.94%	2.32%	0.83%	0.83%
2011-2012	10	0.00%	0.00%	0.15%	0.53%	1.34%	0.35%	0.48%
2013-2014	8	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
5-year-old								
2003-2004	12	0.00%	0.15%	1.39%	3.15%	61.15%	6.38%	17.31%
2005-2006	12	0.94%	1.72%	3.13%	3.82%	45.33%	9.08%	15.36%
2007-2008	11	0.00%	0.00%	0.33%	0.49%	1.68%	0.42%	0.47%
2009-2010	10	0.00%	0.00%	0.00%	0.31%	4.32%	0.56%	1.34%
2011-2012	10	0.00%	0.00%	0.15%	0.96%	2.30%	0.54%	0.76%
2013-2014	8	0.00%	0.00%	0.00%	0.33%	1.01%	0.21%	0.36%
6-year-old								
2003-2004	11	0.00%	0.81%	2.63%	4.19%	36.82%	8.35%	14.14%
2005-2006	10	0.00%	0.81%	1.64%	1.95%	38.09%	5.08%	11.65%
2007-2008	8	0.00%	0.00%	0.29%	0.68%	1.68%	0.45%	0.59%
2009-2010	10	0.00%	0.00%	0.00%	0.31%	0.85%	0.18%	0.28%
2011-2012	9	0.00%	0.00%	0.00%	0.30%	0.96%	0.24%	0.35%
2013-2014	7	0.00%	0.00%	0.00%	0.45%	0.96%	0.20%	0.37%

Num: Number of flocks; Min: Minimum; Q1: First quartile; Q3: Third quartile;
Max: Maximum; SD: Standard deviation.

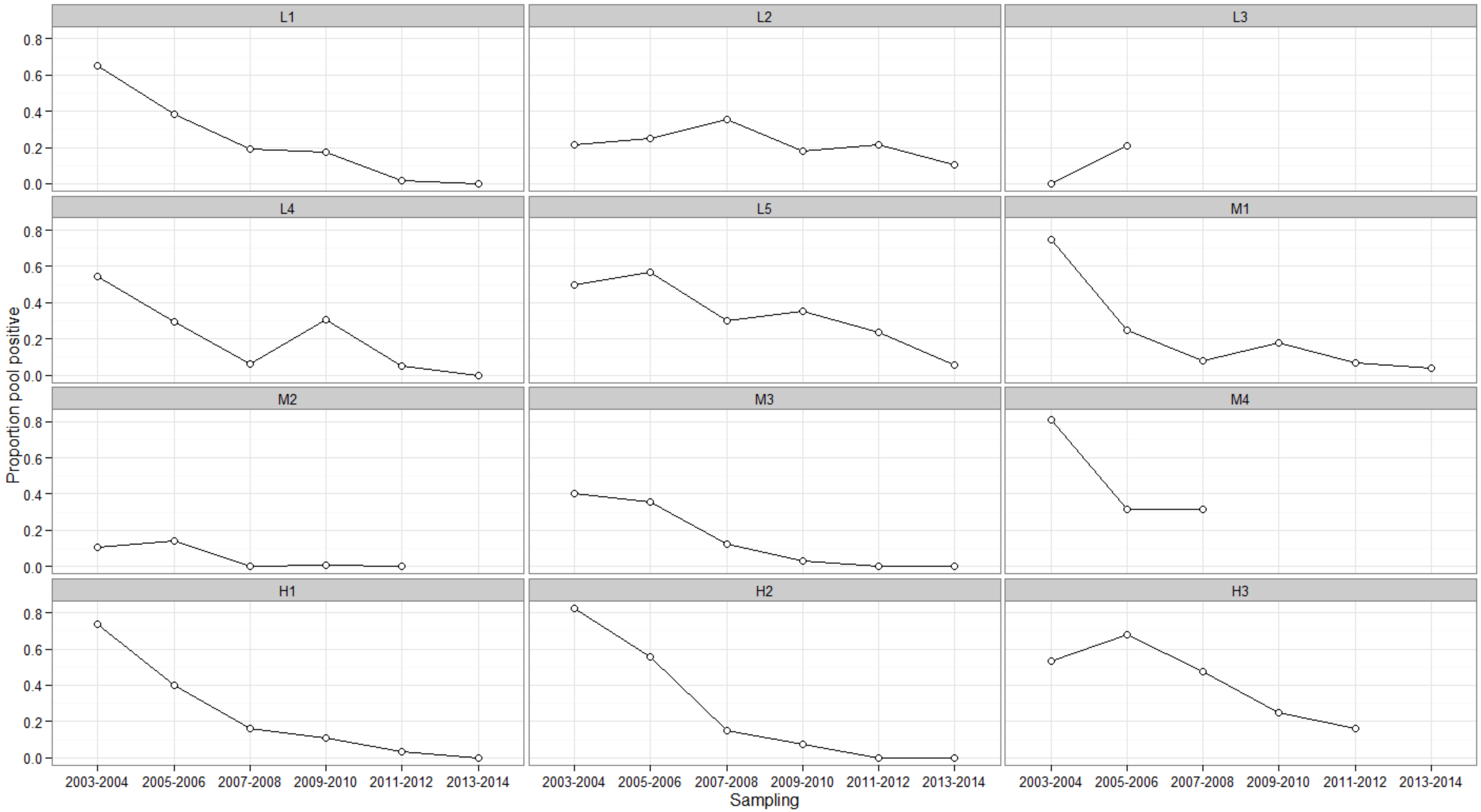


Fig 1. The proportions of positive pools over six samplings for low (L), medium (M) and high (H) pre-vaccination prevalence flocks enrolled in the study. Pre-vaccination status was determined based on previous OJD testing and OJD-related mortalities.

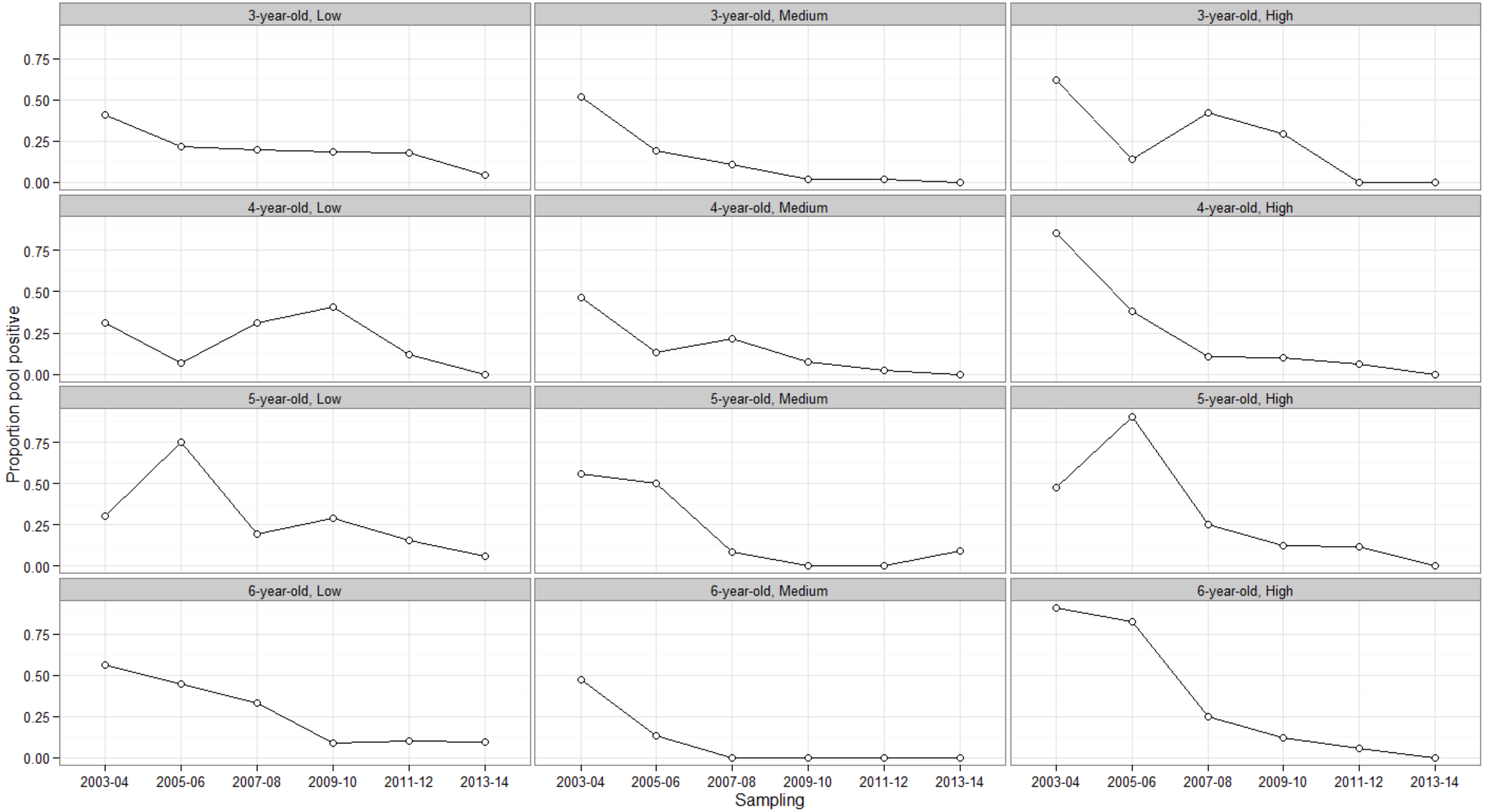


Fig 2. The proportions of positive pools in flocks classified by pre-vaccination prevalence status (low, medium and high) and age (3-, 4-, 5- and 6-year-olds) over six samplings. Pre-vaccination status was determined based on previous OJD testing and OJD-related mortalities.

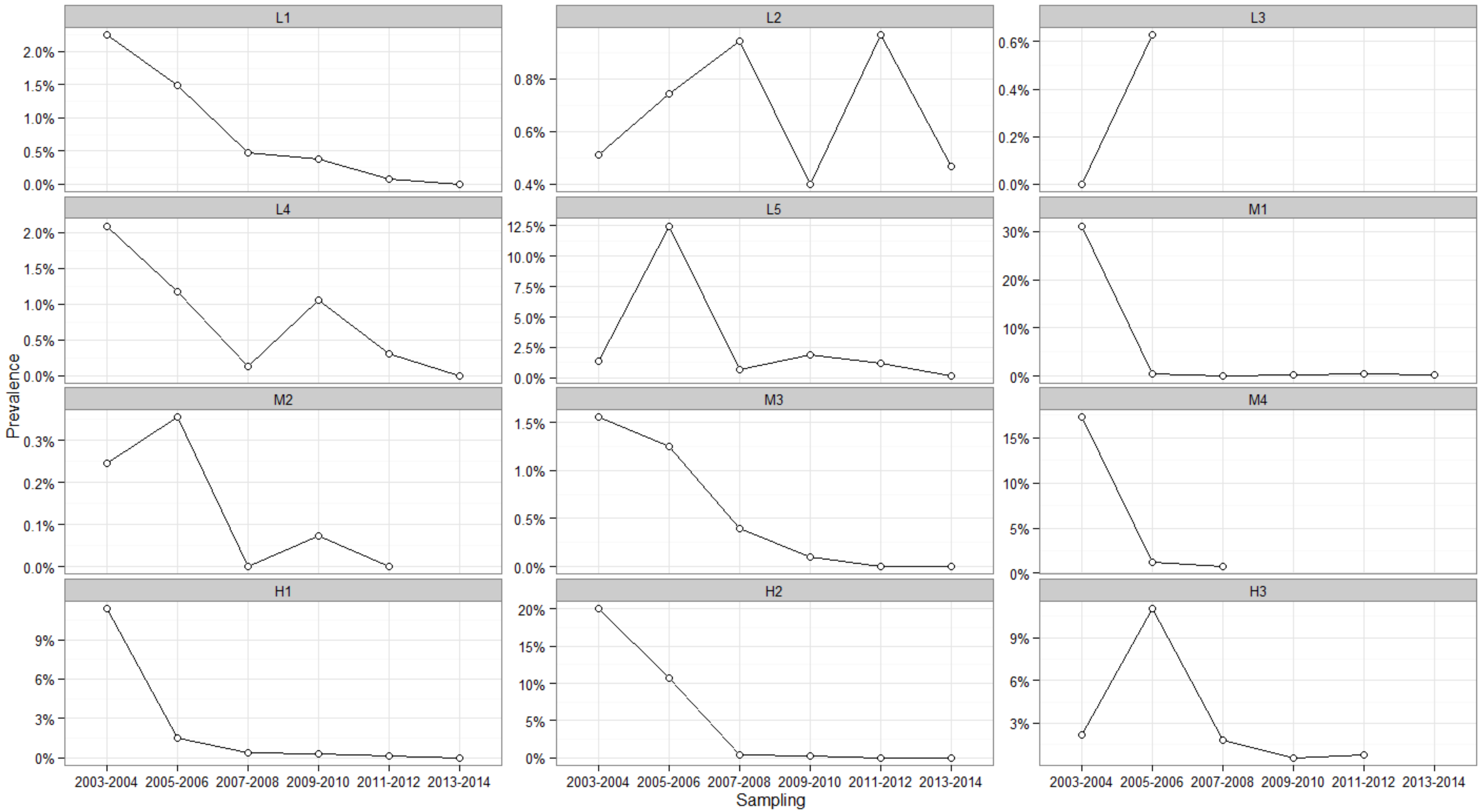


Fig 3. Animal level prevalence for each flock estimated using the Williams and Moffitt (2001) approach in flocks with low (L), medium (M) and high (H) pre-vaccination prevalence over six samplings. Pre-vaccination status was determined based on previous OJD testing and OJD-related mortalities. Different *y-axis* scales were used for different plots to highlight the trends in prevalence over time. Plots with consistent *y-axis* scales are presented in the Appendix (Supplementary Fig 1).

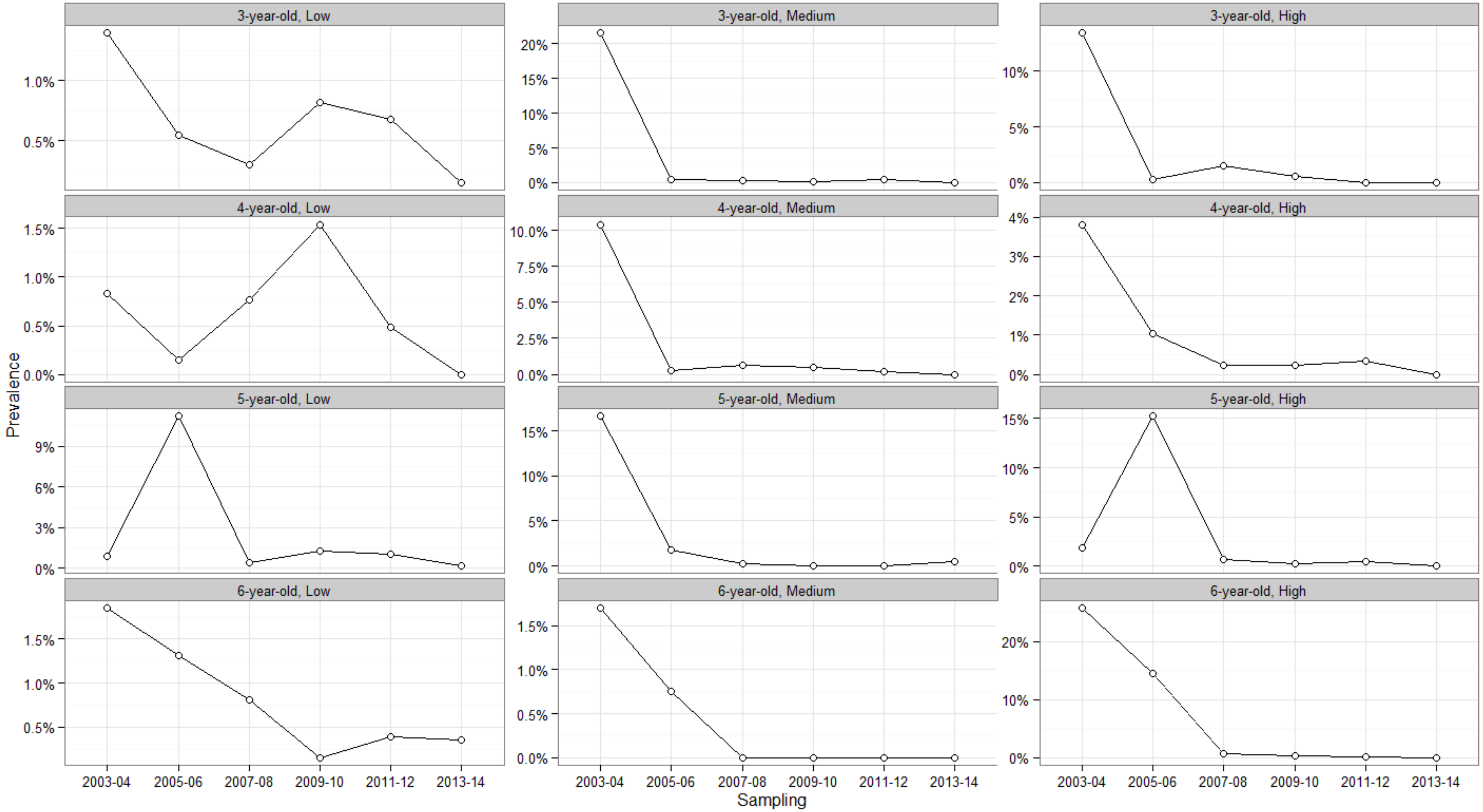


Fig 4. Animal level prevalence estimated using the Williams and Moffitt (2001) approach classified by pre-vaccination prevalence status (low, medium and high) and age groups (3-, 4-, 5- and 6-year-old) over six samplings. Pre-vaccination status was determined based on previous OJD testing and OJD-related mortalities. Different *y-axis* scales were used for different plots to highlight the trend in prevalence overtime. Plots with consistent *y-axis* scales are presented in the Appendix (Supplementary Fig 2).

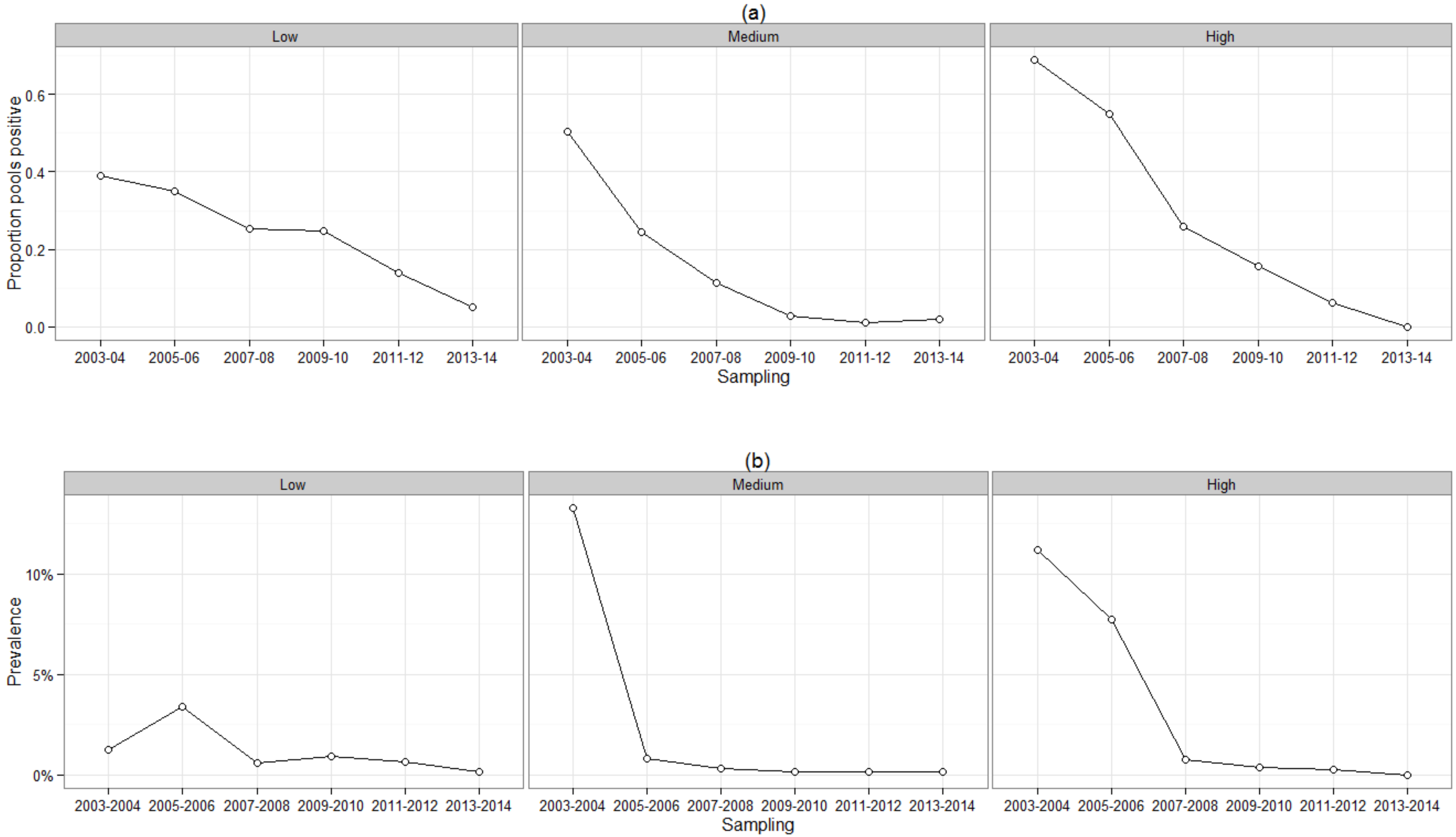
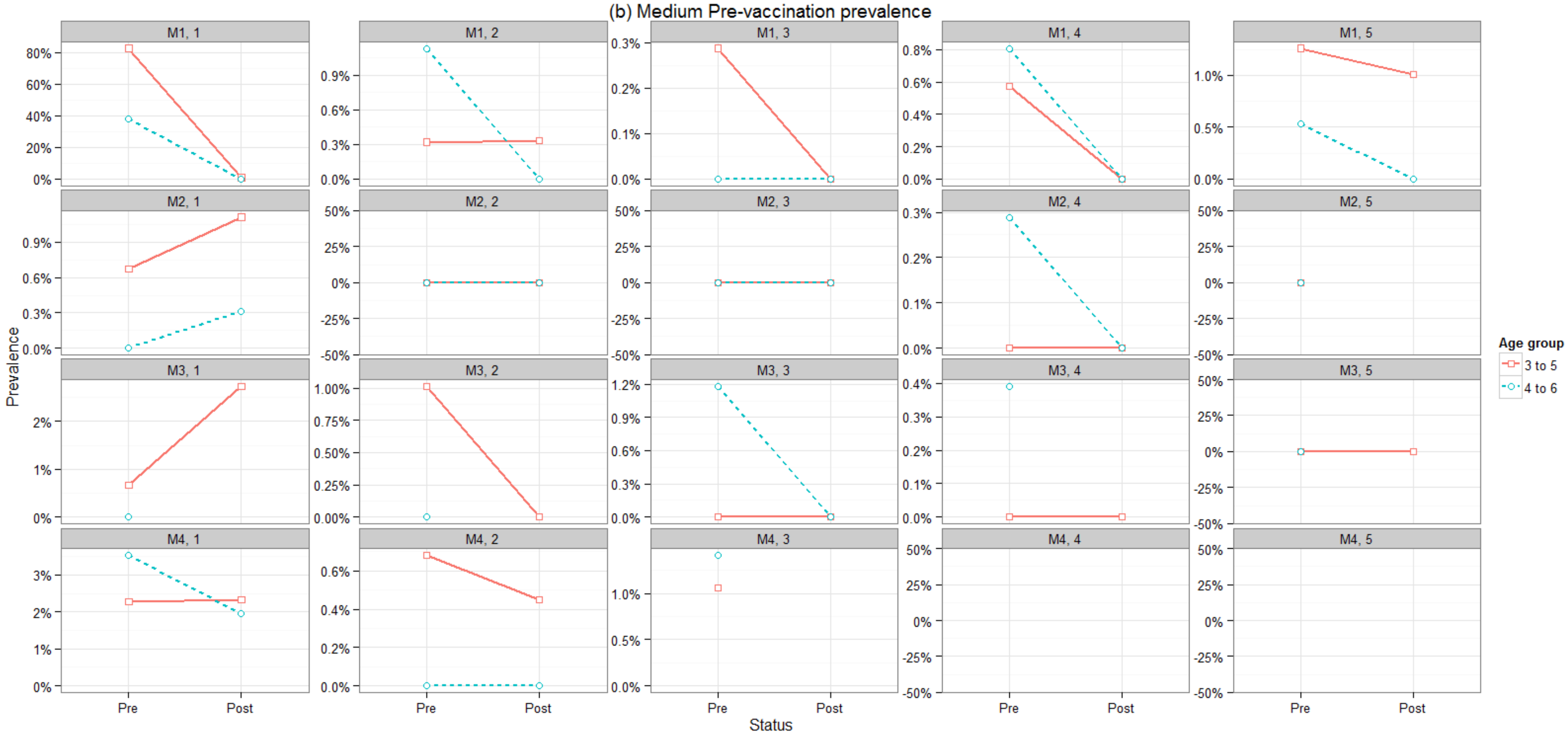


Fig 5. Overall trends in the proportions of positive pools (a) and animal level prevalence (b) in enrolled flocks with low, medium and high pre-vaccination prevalence over six samplings. Pre-vaccination status was determined based on previous OJD testing and OJD-related mortalities.

4.1.3 Changes in prevalence within cohorts

In addition to measuring changes in prevalence in flocks over time, we also evaluated changes in prevalence in specific cohorts within flocks. Results presented in Fig 6 do not show any consistent pattern. While prevalence reduced in many cohorts, it remained steady in others and actually increased in some other cohorts. Overall results presented in Fig 7 suggest that the prevalence reduced over a block of two years in most cohorts although the rates of change varied.


(a) Low Pre-vaccination prevalence
L1, 1
L1, 2
L1, 3
L1, 4
L1, 5
L2, 1
L2, 2
L2, 3
L2, 4
L2, 5
L3, 1
L3, 2
L3, 3
L3, 4
L3, 5
L4, 1
L4, 2
L4, 3
L4, 4
L4, 5
L5, 1
L5, 2
L5, 3
L5, 4
L5, 5
Prevalence
Status
Pre
Post
Age group
3 to 5
4 to 6


(b) Medium Pre-vaccination prevalence
M1, 1
M1, 2
M1, 3
M1, 4
M1, 5
M2, 1
M2, 2
M2, 3
M2, 4
M2, 5
M3, 1
M3, 2
M3, 3
M3, 4
M3, 5
M4, 1
M4, 2
M4, 3
M4, 4
M4, 5
Prevalence
Status
Pre
Post
Age group
3 to 5
4 to 6

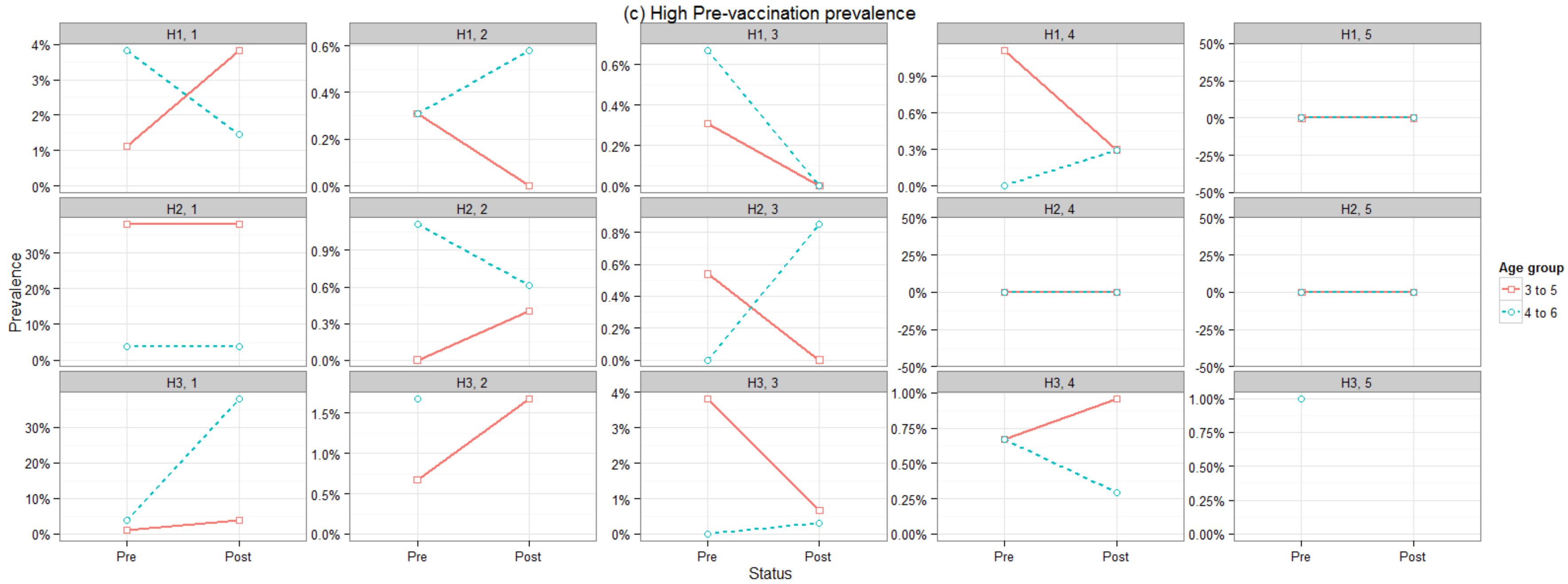


Fig 6. Changes in OJD prevalence in specific cohorts within flocks. Since sheep were sampled every two year, the 3- and 4-year-old sheep were again sampled as 5- and 6-year-old sheep after two years. These plots show changes in prevalence in these sheep in low (a), medium (b) and high (c) pre-vaccination prevalence flocks. Each plot in a row represents one of the five cohorts in a flock; see Table 1 for the details of codes used for various cohorts. Each plot has been plotted with a different *y-axis* scale to highlight the trends. Plots with the same *y-axis* scale are presented in the Appendix (Supplementary Fig 3).

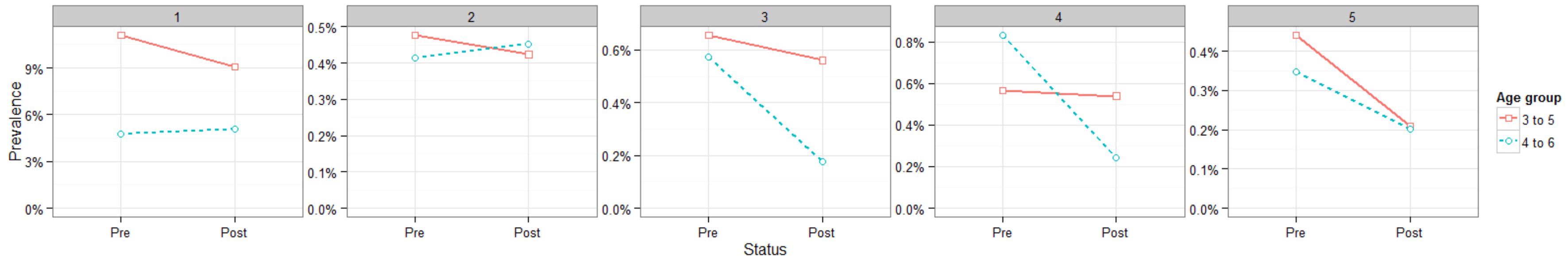


Fig 7. Overall changes in OJD prevalence in 3- and 4-year-old cohorts followed for six samplings. Since sheep were sampled every two year, the 3-year-old (4-year-old) cohorts were again sampled as 5-year-old (6-year-old) cohorts after two years from the same flocks. Five plots presented represent five cohorts as described in Table 1. Each plot has been plotted with a different *y-axis* scale to highlight the trends. Plots with the same *y-axis* scale are presented in the Appendix (Supplementary Fig 4).

4.2 Statistical modelling results

4.2.1 Generalised linear mixed model

Results of the final generalized linear mixed model are presented in Table 4 and Fig 6. Pre-vaccination prevalence was not significant when added to the model (*P*=0.52), indicating that the change in prevalence over time in different age groups was not different according to the pre-vaccination prevalence status. The interaction between age and year could not be tested as the model with the interaction term did not converge. The final model had three terms: log of pool size (*P* = 0.043), age group (*P* = 0.01) and sampling year (*P* <0.001). Odds ratios plotted in Fig 6 suggest that there was about 30 times reduction in the odds of a pool to be positive at the final sampling compared to the first sampling.

Table 4. Final generalised linear mixed model for the binary outcome *pool status* (positive/negative) built to evaluate change in probability of a pool to be positive over time based on 1,971 observations of faecal pools collected from sheep flocks over six samplings from 2003 to 2014 in enrolled flocks.

Variable	Categories	b	SE	Odds ratio	95% CI	P-value
Intercept		-2.54	1.17	-	-	
Log pool size		0.59	0.29	1.81	1.02, 3.21	0.043
Year						<0.001
	2003-2004	0.00	-	-	-	
	2005-2006	-0.60	0.18	0.55	0.38, 0.79	
	2007-2008	-1.60	0.21	0.20	0.13, 0.30	
	2009-2010	-1.76	0.23	0.17	0.11, 0.27	
	2011-2012	-2.25	0.31	0.11	0.06, 0.19	
	2013-2014	-3.39	0.41	0.03	0.02, 0.08	
Age						0.01
	3-year-old	0.00	-	-	-	
	4-year-old	0.04	0.18	1.04	0.73, 1.49	
	5-year-old	0.52	0.18	1.68	1.18, 2.38	
	6-year-old	0.36	0.20	1.43	0.97, 2.11	

b: Parameter estimate; SE: Standard error; CI: Confidence interval.
Covariance parameter estimate for the flock random effect: Estimate (SE) = 0.78 (0.38).

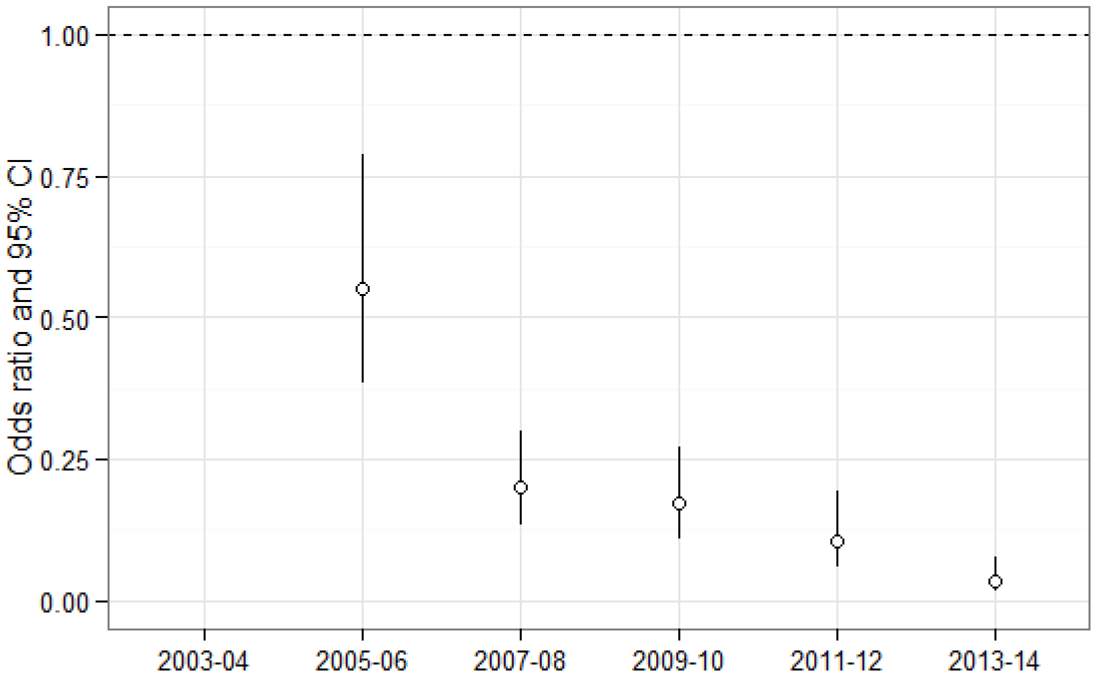


Fig 6. Odds ratios and confidence intervals with 2003-04 as a reference year, based on the generalised linear mixed model presented in Table 3 above.

4.2.2 Linear mixed model

Results of the final linear mixed model are presented in Table 5 and Fig 7. There was a significant interaction between age and year indicating that there were significant differences in prevalence over the years and in different age groups (P<0.001). However, the variable 'pre-vaccination prevalence' was not significant when added to the model (P=0.57). Intra-class correlation for flocks was calculated from the variance components to be 0.25 indicating a substantial clustering due to flocks. Exponentiated predicted means for prevalence presented in Fig 7 suggest that compared to the commencement of the study in 2003-04, the average prevalence substantially reduced in all age groups in subsequent years.

Table 5. Final linear mixed model for the outcome *log prevalence* built to evaluate change in prevalence over time based on 243 observations from the faecal samples collected from enrolled sheep flocks over six samplings from 2003 to 2014.

Variable	Categories1	Categories2	b	SE	LCL	UCL	P-value
Intercept			-3.96	0.39	-4.81	-3.10	
Year							<0.001
	2003-2004		0.00	-	-	-	
	2005-2006		-1.66	0.47	-2.60	-0.72	
	2007-2008		-1.74	0.49	-2.70	-0.78	
	2009-2010		-1.69	0.50	-2.67	-0.70	
	2011-2012		-2.07	0.51	-3.09	-1.06	
	2013-2014		-2.83	0.53	-3.88	-1.77	
Age							0.19
	3-year-old		0.00	-	-	-	
	4-year-old		-0.62	0.47	-1.56	0.31	
	5-year-old		-0.59	0.47	-1.53	0.34	
	6-year-old		0.22	0.49	-0.74	1.18	
Year*Age							<0.001
	2003-2004	3-year-old	0.00	-	-	-	
	2003-2004	4-year-old	0.00	-	-	-	
	2003-2004	5-year-old	0.00	-	-	-	
	2003-2004	6-year-old	0.00	-	-	-	
	2005-2006	3-year-old	0.00	-	-	-	
	2005-2006	4-year-old	0.28	0.67	-1.04	1.60	
	2005-2006	5-year-old	2.95	0.67	1.63	4.27	
	2005-2006	6-year-old	1.13	0.70	-0.24	2.50	
	2007-2008	3-year-old	0.00	-	-	-	
	2007-2008	4-year-old	0.63	0.69	-0.72	1.98	
	2007-2008	5-year-old	0.58	0.69	-0.77	1.94	
	2007-2008	6-year-old	-0.43	0.73	-1.86	1.00	
	2009-2010	3-year-old	0.00	-	-	-	
	2009-2010	4-year-old	1.08	0.70	-0.30	2.47	
	2009-2010	5-year-old	0.13	0.70	-1.26	1.52	
	2009-2010	6-year-old	-0.90	0.71	-2.30	0.50	
	2011-2012	3-year-old	0.00	-	-	-	
	2011-2012	4-year-old	0.64	0.71	-0.77	2.05	
	2011-2012	5-year-old	0.79	0.71	-0.62	2.20	
	2011-2012	6-year-old	-0.43	0.73	-1.88	1.01	
	2013-2014	3-year-old	0.00	-	-	-	
	2013-2014	4-year-old	0.38	0.75	-1.10	1.85	
	2013-2014	5-year-old	1.02	0.75	-0.46	2.49	
	2013-2014	6-year-old	0.04	0.77	-1.48	1.56	

b: Parameter estimate; SE: Standard error; LCL and UCL: 95% Lower and upper confidence limits.

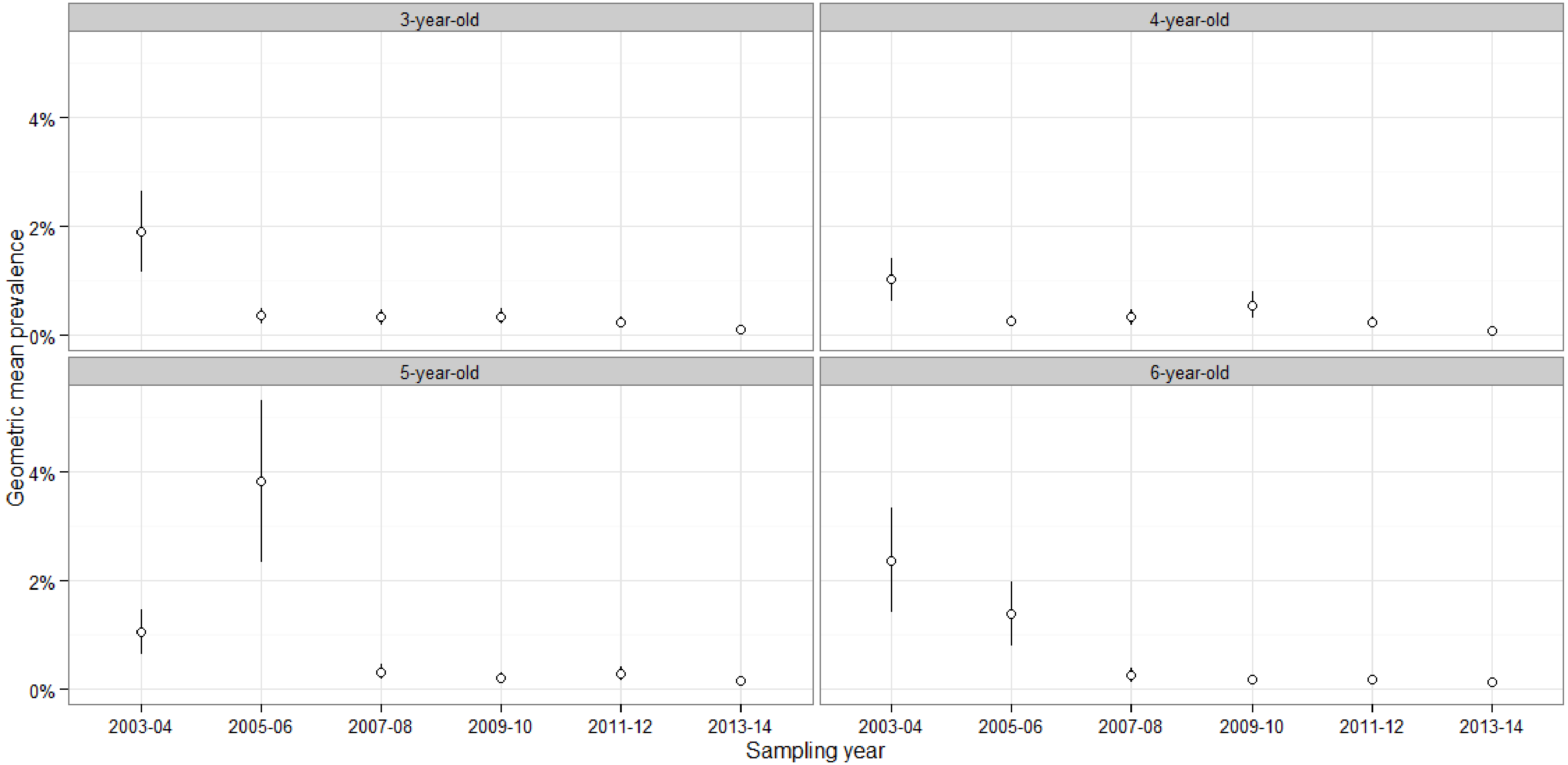


Fig 7. Geometric mean prevalence (± standard error) in different age groups over time based on the results of a linear mixed model presented in Table 4, built to evaluate changes in OJD prevalence in enrolled flocks over time.

4.2.3 Changes in cohort prevalence

Cohort (coded 1 to 5 representing sampling years – see Table 1) was the only significant variable in linear mixed models built to evaluate changes in cohort prevalence over time. Surprisingly, time (pre vs post) was not significant suggesting that prevalence was not significantly different in cohorts followed over two years. Similarly, there were no significant differences in trends in prevalence whether a 3-year-old or a 4-year-old age group was followed. None of the three first order interaction terms tested was significant.

Table 6. Final linear mixed model for the outcome *log prevalence* built to evaluate change in prevalence in cohorts, each followed for two years, based on 204 observations from the faecal samples collected from enrolled sheep flocks over six samplings from 2003 to 2014. See Table 1 for the definition of cohorts' categories.

Variable	Categories	b	SE	LCL	UCL	P-value
Intercept		-4.01	0.25	-4.56	-3.46	
Cohort						<0.001
	1	0.00	-	-	-	
	2	-1.77	0.24	-2.24	-1.29	
	3	-1.91	0.24	-2.39	-1.43	
	4	-1.69	0.25	-2.18	-1.20	
	5	-2.16	0.26	-2.67	-1.65	

b: Parameter estimate; SE: Standard error; LCL and UCL: 95% Lower and upper confidence limits.

5 Discussion

Analysis of the pooled faecal culture results from this longitudinal observational study on 12 OJD-infected farms over more than a 10 year period provides important information on the impact of Gudair™ vaccination on the prevalence of OJD as determined by faecal shedding of Mptb. A previous study had identified that the onset of shedding of Mptb in vaccinates was delayed by 12 months and the prevalence of shedders amongst vaccinates was reduced by 90% (Reddacliff et al., 2006). However, that study did identify high levels of excretion by vaccinates on some occasions, the presence of multibacillary lesions of OJD in the few vaccinates that died, plus persistence of subclinical infection in a significant proportion of vaccinates. Findings from another study of shedding rates in 2-year-old vaccinates on seven farms confirmed that shedding persisted in vaccinates although at a declining rate (Eppleston et al., 2005). These observations led to the conclusion that there is a risk that some vaccinated sheep will spread the disease and implied that sustained vaccine use will be necessary to avoid recrudescence of infection and clinical disease in infected flocks (Reddacliff et al., 2006).

The current study was designed to explore this risk and address the issue of vaccine effectiveness in flocks of varying OJD prevalence, as determined by rates of shedding of Mptb in sheep of different ages (3-, 4-, 5- and 6-year-olds) following their vaccination with

Gudair™ as lambs. The data confirm as expected, that vaccination significantly reduced the prevalence of shedding over time. Proportion of positive pools reduced from 50.3% at the first sampling to only 3.1% at the last sampling suggestive of a 30 fold reduction in odds of a pool to be positive. Similarly, the average animal level prevalence dropped from 7.64% at the first sampling to 0.12% at the last sampling. The results are consistent with the previous studies undertaken to evaluate effectiveness of the vaccine.

However, at the third sampling (i.e. about six years after commencing vaccination), 10 of the 11 flocks still in the trial, had detectable shedding. Similarly, at the second last sampling, 7 of the 10 flocks had detectable shedding although the number of positive flocks reduced to 3 of the 8 at the last sampling. This is consistent with a cross-sectional study in the same region as this study that found the response to vaccination is variable between flocks. In that study the prevalence of OJD was estimated in 37 flocks vaccinating for at least 5 years and it was found that 30 of these flocks were still shedding Mptb and that shedding occurred independent of the estimated OJD prevalence at the time vaccination commenced (Dhand et al., 2013). The results suggest that clinical disease may recur if the program of annual lamb vaccinations is discontinued and that there is still some risk of transferring the disease if vaccinated sheep are introduced into uninfected flocks.

Interestingly, the pre-vaccination prevalence status was not significant in any of the statistical models. This finding should be interpreted with caution as the pre-vaccination prevalence status was a subjective measure based on prior testing and producer observed and thus presumptive-OJD attributable mortalities in flocks. On the other hand, age groups were significant in both the models, suggesting that the probability of a pool to be positive as well as the animal level disease prevalence, did vary by age group. Faecal samples collected from sheep of 5- and 6-year-old age groups had 1.68 and 1.43 times the odds of a pool to be positive, respectively, compared to 3-year-old sheep (Table 4). This is expected as OJD is a chronic disease and sheep are more likely to shed Mptb in faeces as they become older. Secondly, older sheep would also have had a greater chance to get infected by having lived on contaminated paddocks over longer periods of time.

Interesting patterns in animal level OJD prevalence emerged when cohorts of sheep within flocks were followed for two successive samplings (see Fig 6). Despite vaccination, prevalence increased in some cohorts over a period of two years suggesting that either the vaccination had not been effective in those cohorts or more likely, that these flocks were in the ascending phase of the outbreak. Regardless, it suggests that the effectiveness of vaccination in reducing disease prevalence is highly variable between flocks, at least in the short to medium term. The reasons for such variation are not clear, but could be related to variations in management practices, including the period since commencement of vaccination, the proportions of the flock vaccinated, introduction of infected sheep, presence of super-shedder sheep in the flock, variable levels of biosecurity etc. As 12 flocks is an insufficient number to analyse risk factors that are associated with different patterns of Mptb shedding, further epidemiological studies would be required to understand this issue.

Overall, the results suggest that the Gudair ™ vaccine is effective in reducing prevalence and shedding of Mptb in faeces over an extended period of time to a level that in some flocks, the organisms are no longer detectable. However, given that Mptb could be detected in 7 of the 10 flocks (70%) at the second last sampling and 3 of the 8 flocks (37.5%) at the last sampling, it is apparent that not all flocks respond in a similar manner to the use of this vaccine and that 100% efficacy cannot be assumed. The results from other studies including the original Gudair vaccine validation trial (OJD.009) and a current MLA project (P.PSH.0576) indicate that at least some of the sheep that shed Mptb despite vaccination are super-shedders. These sheep present the greatest risk of all to spread the infection and further research is recommended to quantify this risk and determine the circumstances under which it presents.

5.1 Success in achieving objectives

This study has successfully met the objectives described. The project examined the use of Gudair™ to elicit changes over time in the prevalence of Mptb shedding following the commencement of vaccination in flocks varying in initial OJD prevalence. Of the 11 flocks that remained in the study at the end of the previous project (OJD 033), 11, 10 and 8 flocks were sampled at the fourth, fifth and the sixth sampling, respectively. One flock could not be sampled at the fifth sampling as the farm was sold while two that dropped out the last sampling had changed enterprises. By December 2015, the project had measured changes in the prevalence of shedding in the enrolled flocks remaining in the study as per the agreement in the project contract.

The study demonstrated no clear differences in pattern of response to vaccination among high, medium and low prevalence flocks. As expected, the project provided data on the likely infectivity of sheep over time following the commencement of a vaccination program and is very useful in validating computer models.

It was considered that this information may assist decisions on the risks of trading vaccinated sheep, the timing of cessation of vaccination and the removal of a risk status from a flock. These findings provide further support to previous conclusions that there is a risk that some vaccinated sheep will spread the disease and that sustained vaccine use will be necessary to avoid recrudescence of OJD in infected flocks. The findings are an important reminder of the implications from previous studies on the risk of shedding from vaccinates and suggest that continued use of the vaccination in OJD infected flocks is warranted at this stage.

6 Conclusions/recommendations

This study suggests that vaccination with Gudair™ is effective in reducing the prevalence of shedding of Mptb but the response to vaccination is variable between flocks and that shedding is persistence for many years. The risks of disease spread or recrudescence that is present even 10-12 years after the commencement of vaccination is a concern. It appears that in the study area, annual vaccination of lambs with Gudair™ for as long as 10-12 years may not be able to completely prevent the spread of OJD associated with the sale of vaccinated sheep. The results from other studies including the original Gudair vaccine validation trial (OJD.009) and a current MLA project (P.PSH.0576) indicate that at least some of the sheep that shed Mptb despite vaccination are super-shedders. These sheep present the greatest risk of all to spread the infection and further research is recommended to quantify this risk and determine the circumstances under which it presents.

The study also generated some new research questions warranting further investigation. The finding of variable effectiveness of the vaccine in vaccinating flocks suggests that further investigations should be done to understand why shedding of Mptb is persisting in some flocks and not in others. This could be done by conducting an epidemiological study but it would require enrolling a greater number of flocks vaccinating for more than 5-6 years. Prevalence of shedding in enrolled flocks could be estimated based on culture or PCR and information about management and biosecurity practices could be obtained using a questionnaire. Data could then be analysed to identify and quantify factors associated with presence of shedding. This will enable identification of management and/or biosecurity practices that could be implemented by producers in conjunction with vaccination for an effective control of OJD on their farms.

Further, this study was conducted in vaccinating flocks but didn't provide information about changes in prevalence at cessation of vaccination. Given that Mptb could still be detected in vaccinating flocks more than a decade after commencement of the vaccine, it would be interesting to know whether the disease resurfaces in flocks that cease vaccinating. It would also be useful to know what proportion of farmers that commenced vaccination are continuing with it and whether this proportion varies from place to place, and if so, is it related to initial area prevalence. Similarly, information about the proportion of producers only vaccinating ewe lambs would be useful in modelling the effectiveness of the vaccine in the future.

Although the vaccine is not fully effective, it is a major suppressant of OJD infection in vaccinating flocks and an important strategic component of disease control programs. While vaccination is recommended by disease control authorities, not all producers vaccinate their sheep and not all continue to vaccinate all of their sheep. To develop an OJD control program that creates effective change management for paratuberculosis, it is considered important to understand the drivers and the barriers to continued vaccination (Windsor, 2014). Studies conducted in Australia and overseas have suggested that social and behavioural factors play an important role in implementation of disease control programs (Windsor, 2015). Understanding of such factors would enable disease control authorities in adapting the program to the needs of the farmers.

7 Key messages

- Gudair™ vaccination is effective in reducing OJD prevalence and Mptb faecal shedding over an extended period of time. The producers should continue to use this vaccine to manage OJD in their flocks.

- Many sheep producers enrolled in the project were able to eliminate infection or reduce Mptb shedding levels to below detectable limits over 11-12 years. Other producers can also achieve these goals after an extended period of vaccination.

- Cessation of vaccination cannot be recommended since 3 of the 8 flocks were not able to clear infection even 10-12 years after the commencement of vaccination. Clinical disease may recur if the program of annual lamb vaccinations is discontinued.

- There is still some risk of transferring the disease if vaccinated sheep are introduced into uninfected flocks. Therefore, sheep producers should carefully evaluate the risk of introduction of Mptb to their farms when purchasing vaccinated re-stocker sheep, particularly from known or suspected OJD infected properties.

- Sheep in some 40% of the flocks were still shedding Mptb in their faeces after more than a decade of vaccination, suggesting that the effectiveness of the vaccine varies in different flocks and depends on other managemental and biosecurity practices. Further studies are required to objectively identify these practices which can be used by sheep producers in conjunction with the vaccine to reduce OJD prevalence in their flocks.

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9 Appendix

Supplementary Table 1. The numbers of positive and total pools collected for each flock and for each age group over six samplings. See Table 2 for a summary of the results.

Flock number[A]	**Age group**	**Number of positive/total pools**					
		2003-2004	**2005-2006**	**2007-2008**	**2009-2010**	**2011-2012**	**2013-2014**
H1							
	3	3/7	1/7	1/7	3/7	0/14	0/14
	4	6/7	1/7	2/7	0/7	0/14	0/14
	5	6/7	6/7	0/7	0/7	1/14	0/12
	6	2/2	2/4	1/4	0/7	1/14	0/14
	Total	*17/23*	*10/25*	*4/25*	*3/28*	*2/56*	*0/54* [B]
H2							
	3	7/7	0/7	1/5	0/3	0/11	0/14
	4	6/7	3/7	0/5	0/6	0/11	0/8
	5	4/7	7/7	1/6	0/2	0/6	0/8
	6	2/2	5/6	1/4	1/3	0/8	0/8
	Total	*19/23*	*15/27*	*3/20*	*1/14*	*0/36*	*0/38*
H3							
	3	3/7	2/7	6/7	2/7	-	-
	4	6/7	4/7	0/7	2/7	2/9	-
	5	0/7	6/7	4/7	2/7	3/14	-
	6	6/7	7/7	-	1/7	1/14	-
	Total	*15/28*	*19/28*	*10/21*	*7/28*	*6/37*	-
M1							
	3	5/5	1/7	1/8	1/4	1/4	0/6
	4	7/7	3/7	0/5	2/6	1/8	0/10
	5	3/7	3/8	1/7	0/5	0/10	1/5
	6	6/9	0/6	0/5	0/2	0/8	0/6
	Total	*21/28*	*7/28*	*2/25*	*3/17*	*2/30*	*1/27*
M2							
	3	2/7	0/7	0/7	0/35	0/35	-
	4	0/7	0/7	0/7	0/35	0/35	-
	5	0/7	3/7	0/7	0/35	0/35	-
	6	1/7	1/7	0/7	0/18	0/23	-
	Total	*3/28*	*4/28*	*0/28*	*0/123*	*0/128*	-
M3							
	3	2/7	2/5	0/7	0/7	0/8	0/9
	4	0/6	0/5	2/5	1/11	0/7	0/7
	5	6/7	3/4	0/4	0/10	0/7[C]	0/6
	6	-	-	-	0/6	-	-
	Total	*8/20*	*5/14*	*2/16*	*1/34*	*0/22*	*0/22*

Flock number[A]	**Age group**	**Number of positive/total pools**					
		2003-2004	**2005-2006**	**2007-2008**	**2009-2010**	**2011-2012**	**2013-2014**
M4							
	3	4/6	2/7	2/5	-	-	-
	4	5/6	0/4	3/6	-	-	-
	5	6/6	2/3	1/6	-	-	-
	6	2/3	1/2	0/2	-	-	-
	Total	*17/21*	*5/16*	*6/19*	-	-	-
L1							
	3	4/7	1/7	0/7	0/7	0/14	0/14
	4	4/7	0/7	3/7	3/8	1/14	1/14
	5	5/7	6/7	1/7	1/7	0/13	0/13
	6	4/5	3/5	-	1/7	0/14	0/14
	Total	*17/26*	*10/26*	*4/21*	*5/29*	*1/55*	*0/53*
L2							
	3	1/7	0/6	1/7	1/7	3/14	2/14
	4	1/7	0/6	3/7	2/7	4/14	0/14
	5	1/7	4/6	2/7	1/7	2/14	1/14
	6	3/7	2/6	4/7	1/7	3/14	3/14
	Total	*6/28*	*6/24*	*10/28*	*5/28*	*12/56*	*6/56*
L3							
	3	0/6	1/5	-	-	-	-
	4	0/5	0/6	-	-	-	-
	5	0/7	2/5	-	-	-	-
	6	0/3	1/3	-	-	-	-
	Total	*0/21*	*4/19*	-	-	-	-
L4							
	3	6/7	0/6	0/2	2/4	0/3	0/4
	4	0/4	0/5	0/6	2/3	0/8	0/4
	5	1/6	5/6	1/5	0/2	1/4	0/4
	6	6/7	-	0/4	0/4	0/4	0/6
	Total	*13/24*	*5/17*	*1/17*	*4/13*	*1/19*	*0/18*
L5							
	3	3/7	5/8	5/14	2/9	5/14	0/10
	4	4/6	2/7	4/12	4/9	1/14	0/10
	5	3/6	4/4	1/7	5/8	3/7	2/23
	6	1/3	2/4	1/4	0/5	1/7	1/10
	Total	*11/22*	*13/23*	*11/37*	*11/31*	*10/42*	*3/53*
Grand Total		**147/292**	**103/275**	**53/257**	**41/345**	**34/481**	**10/321**

[A]H=High pre-vaccination prevalence; M=Medium pre-vaccination prevalence; L= Low pre-vaccination prevalence; [B]Due to logistical issues, the last sampling from this flock was collected in February 2015.

[C]Sheep sampled from this flock on this occasion were of mixed age of 5 to 6 year old but were considered to be 5 year old for all analytical purposes.

Supplementary Table 2. Details of the numbers and sizes of faecal pools collected from the enrolled flocks from six samplings. See Supplementary Table 3 for a summary.

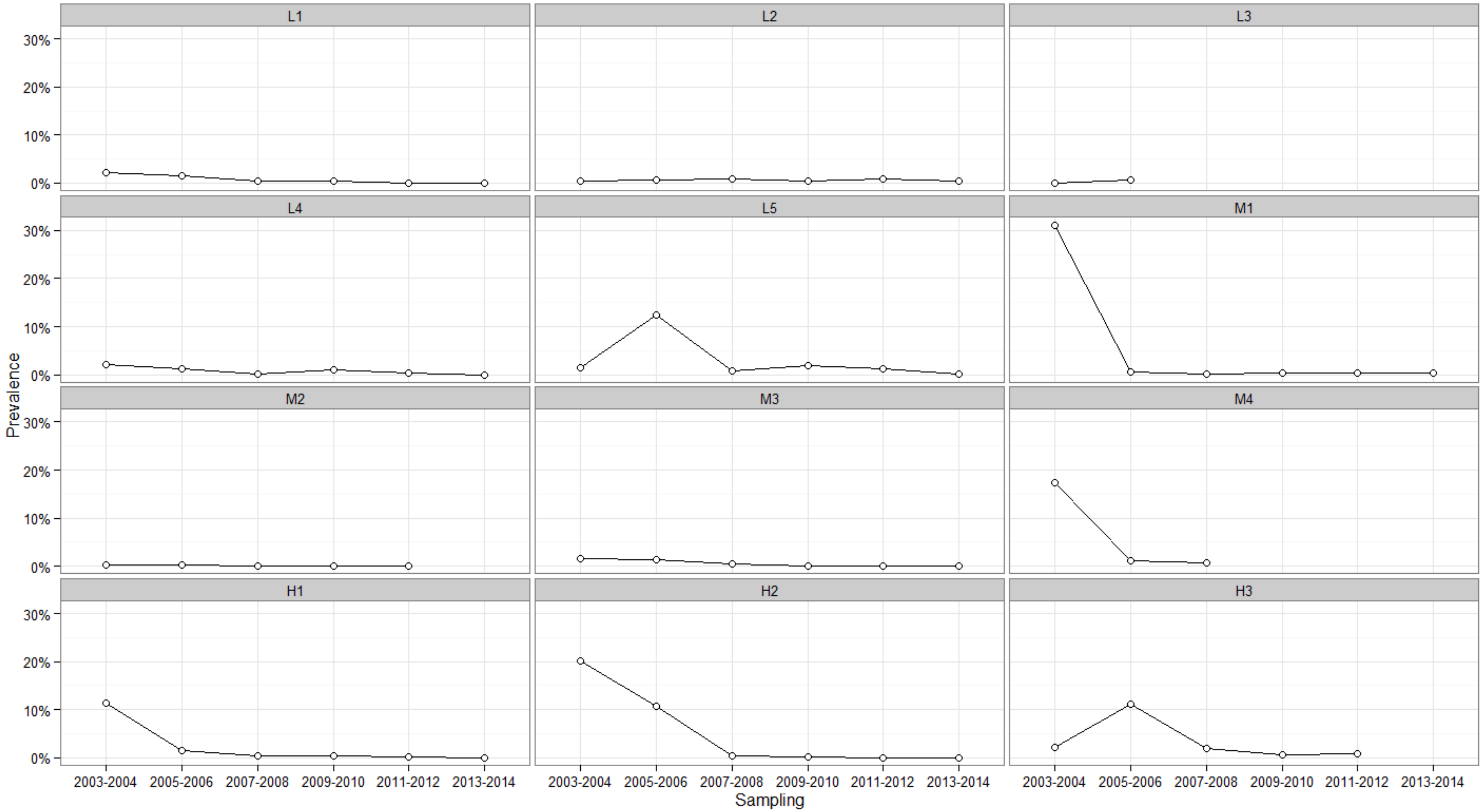
Flock ID	Sampling	Pool size	Pool numbers
H1	1	50	23
H1	2	42	1
H1	2	48	1
H1	2	50	23
H1	3	48	1
H1	3	50	24
H1	4	50	28
H1	5	25	56
H1	6	14	1
H1	6	25	53
H2	1	50	23
H2	2	25	1
H2	2	50	26
H2	3	11	1
H2	3	23	1
H2	3	39	1
H2	3	50	17
H2	4	36	1
H2	4	39	1
H2	4	42	1
H2	4	43	1
H2	4	50	10
H2	5	11	1
H2	5	25	35
H2	6	12	1
H2	6	14	1
H2	6	18	1
H2	6	20	1
H2	6	25	34
H3	1	50	28
H3	2	50	28
H3	3	45	1
H3	3	50	20
H3	4	50	28
H3	5	25	37
L1	1	50	26
L1	2	50	26
L1	3	50	21
L1	4	50	29
L1	5	25	55
L1	6	25	53
L2	1	50	28
L2	2	50	24
L2	3	50	28
L2	4	50	28
L2	5	25	56
L2	6	25	56
L3	1	16	1
L3	1	27	1
L3	1	50	19
L3	2	13	1
L3	2	25	1
L3	2	50	17
L4	1	15	1
L4	1	50	23
L4	2	14	1
L4	2	44	1
L4	2	50	15
L4	3	28	1
L4	3	38	1
L4	3	44	1
L4	3	50	14
L4	4	12	1
L4	4	19	1
L4	4	42	1
L4	4	43	1
L4	4	46	1
L4	4	50	8
L4	5	10	1
L4	5	20	1
L4	5	25	17
L4	6	11	3
L4	6	13	3
L4	6	14	2
L4	6	20	1
L4	6	25	9
L5	1	34	1
L5	1	50	21

Flock ID	Sampling	Pool size	Pool numbers
L5	2	28	1
L5	2	30	1
L5	2	37	1
L5	2	38	1
L5	2	39	1
L5	2	50	18
L5	3	11	1
L5	3	14	1
L5	3	29	1
L5	3	38	1
L5	3	47	1
L5	3	48	1
L5	3	50	31
L5	4	10	1
L5	4	12	1
L5	4	25	29
L5	5	13	1
L5	5	25	41
L5	6	9	1
L5	6	14	1
L5	6	24	1
L5	6	25	50
M1	1	9	1
M1	1	12	1
M1	1	13	1
M1	1	33	1
M1	1	45	1
M1	1	50	23
M1	2	9	1
M1	2	41	1
M1	2	42	1
M1	2	45	1
M1	2	50	24
M1	3	18	1
M1	3	24	1
M1	3	26	1
M1	3	28	1
M1	3	50	21
M1	4	30	1
M1	4	50	16
M1	5	17	1
M1	5	25	29
M1	6	7	1

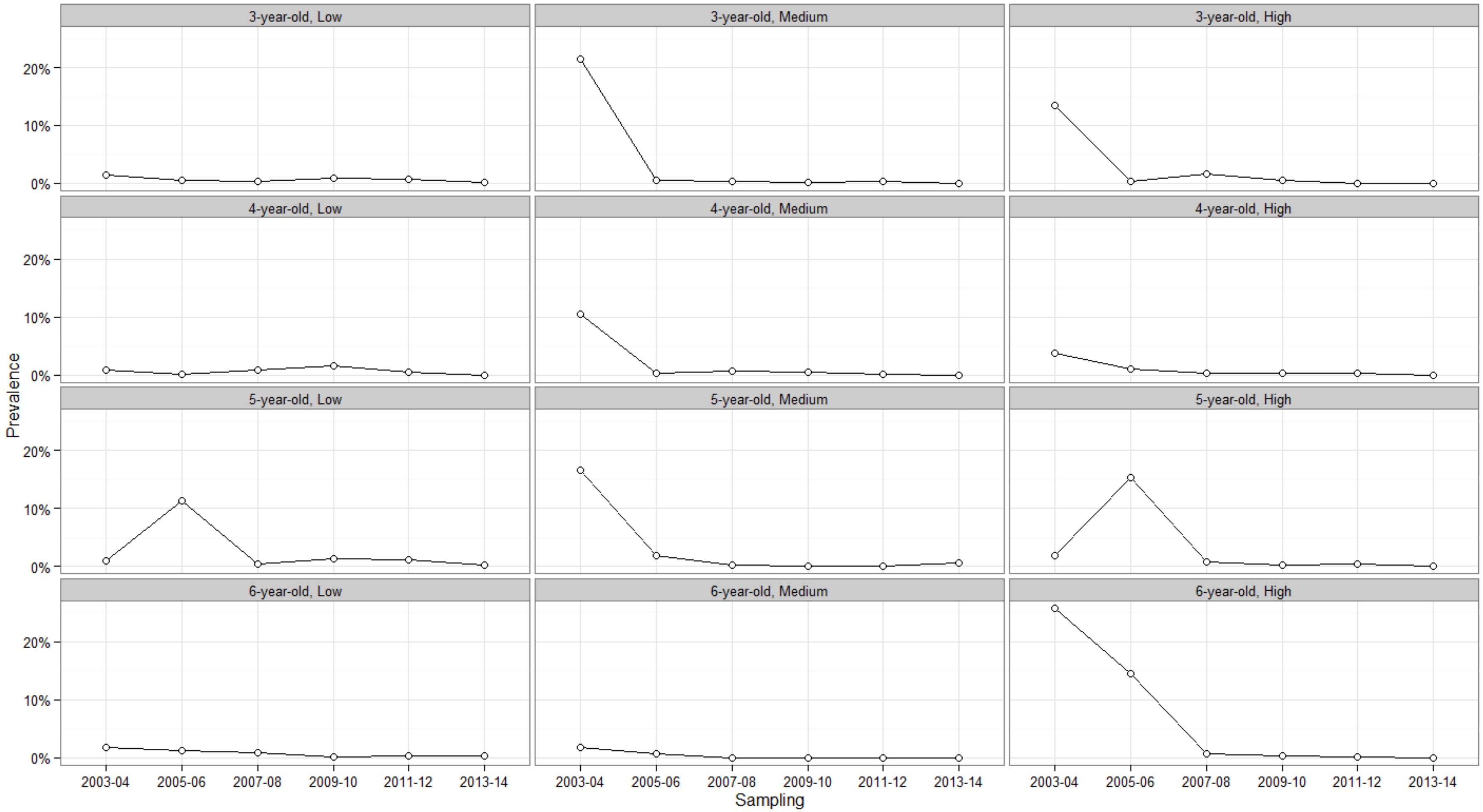
Flock ID	Sampling	Pool size	Pool numbers
M1	6	9	1
M1	6	12	1
M1	6	18	2
M1	6	25	22
M2	1	50	28
M2	2	50	28
M2	3	50	28
M2	4	10	123
M2	5	10	128
M3	1	30	1
M3	1	50	19
M3	2	50	14
M3	3	17	1
M3	3	23	1
M3	3	40	1
M3	3	50	13
M3	4	15	1
M3	4	16	1
M3	4	17	1
M3	4	25	31
M3	5	25	22
M3	6	8	1
M3	6	25	21
M4	1	24	1
M4	1	30	1
M4	1	35	1
M4	1	48	1
M4	1	50	17
M4	2	7	1
M4	2	21	1
M4	2	34	1
M4	2	41	1
M4	2	45	1
M4	2	50	11
M4	3	13	1
M4	3	30	1
M4	3	34	1
M4	3	44	1
M4	3	47	1
M4	3	50	14

Supplementary Table 3. Summary of the numbers and sizes of faecal pools collected from the enrolled flocks in six samplings. See Supplementary Table 2 for the details.

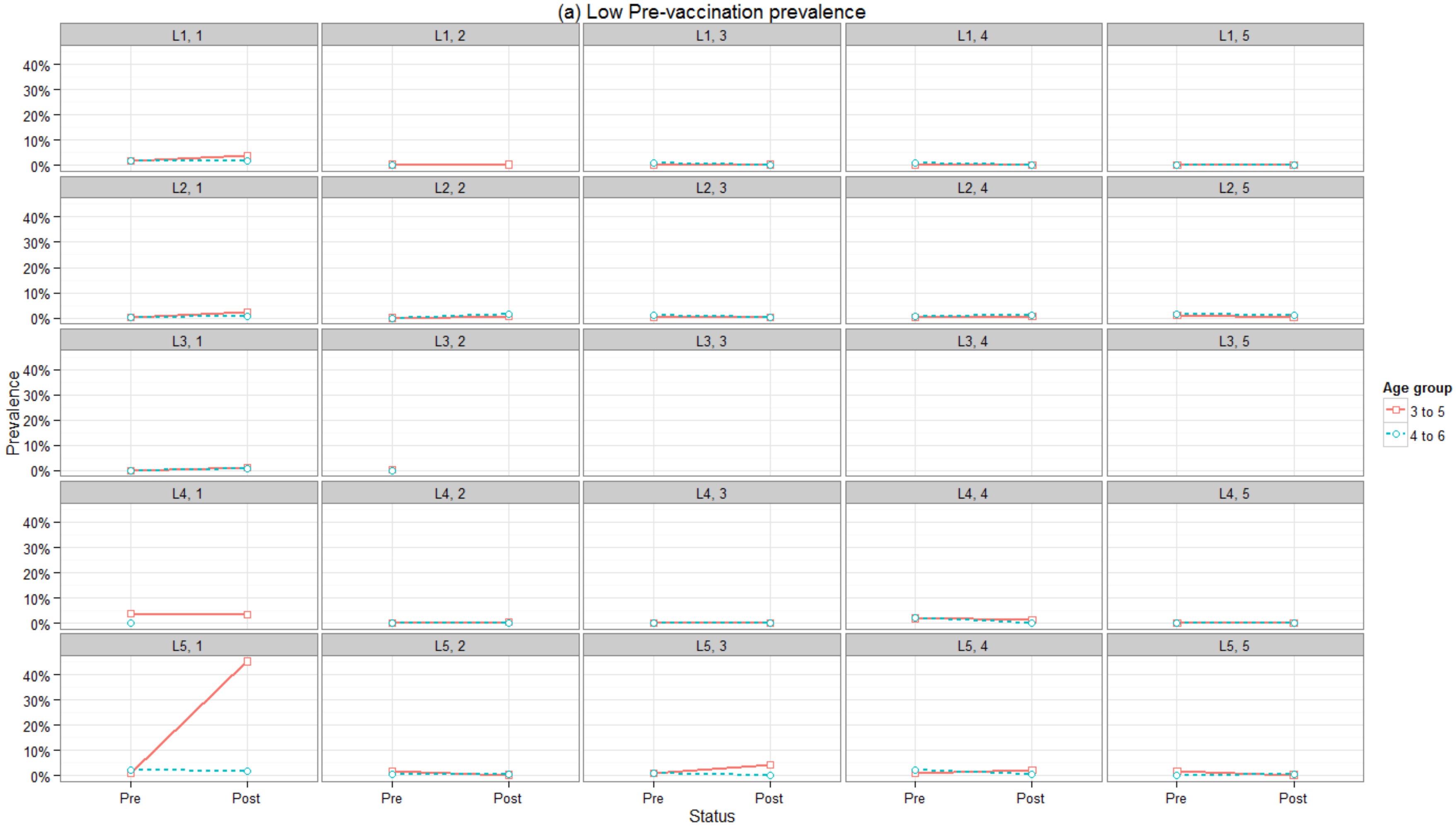
Pool size	Sampling						Total
	1	2	3	4	5	6	
7		1				1	2
8						1	1
9	1	1				2	4
10				124	129		253
11			2		1	3	6
12	1			2		2	5
13	1	1	1		1	3	7
14		1	1			5	7
15	1			1			2
16	1			1			2
17			1	1	1		3
18			1			3	4
19				1			1
20					1	2	3
21		1					1
23			2				2
24	1		1			1	3
25		2		60	348	298	708
26			1				1
27	1						1
28		1	2				3
29			1				1
30	2	1	1	1			5
33	1						1
34	1	1	1				3
35	1						1
36				1			1
37		1					1
38		1	2				3
39		1	1	1			3
40			1				1
41		2					2
42		2		2			4
43				2			2
44		1	2				3
45	1	2	1				4
46				1			1
47			2				2
48	1	1	2				4
50	278	254	231	147			910
Total	**292**	**275**	**257**	**345**	**481**	**321**	**1971**

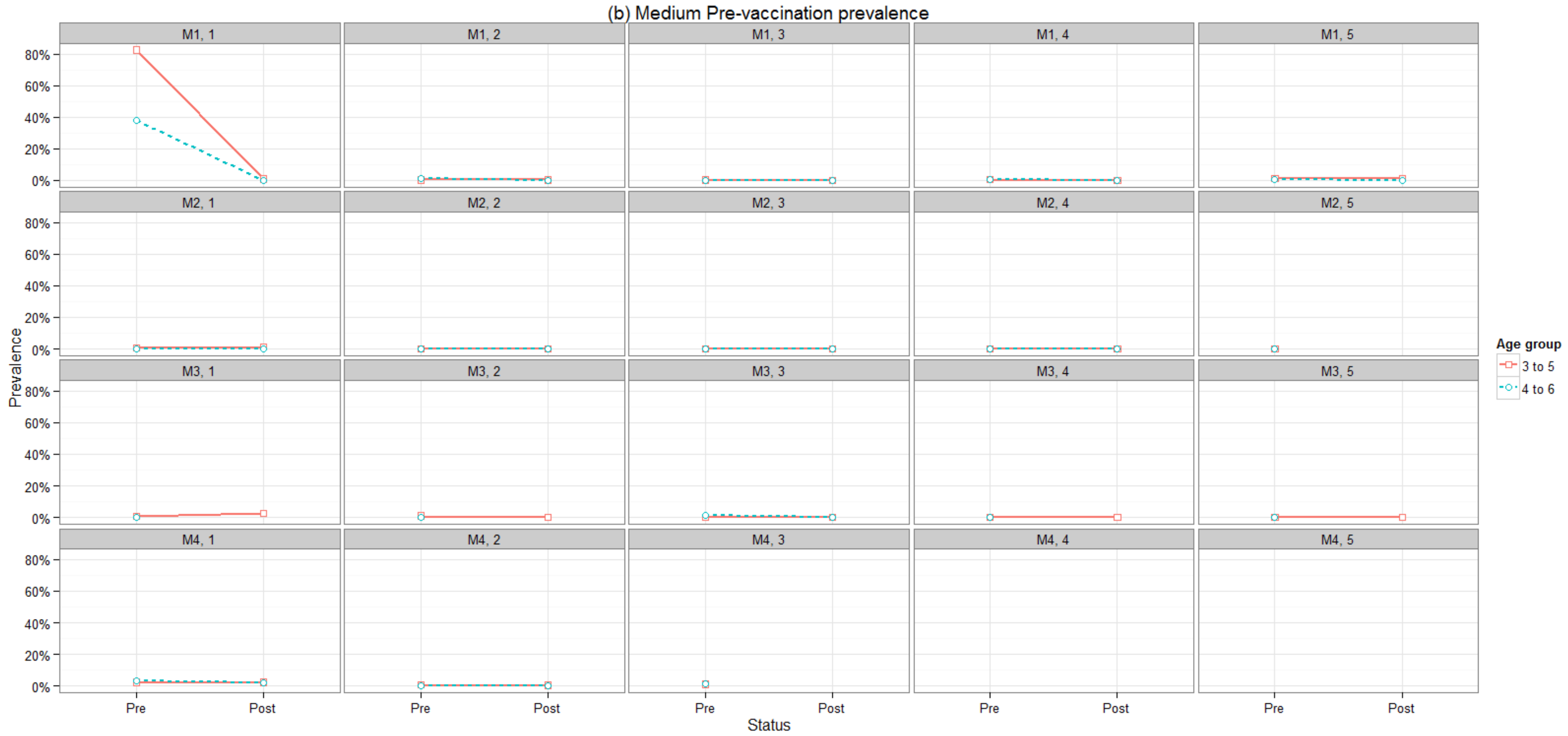


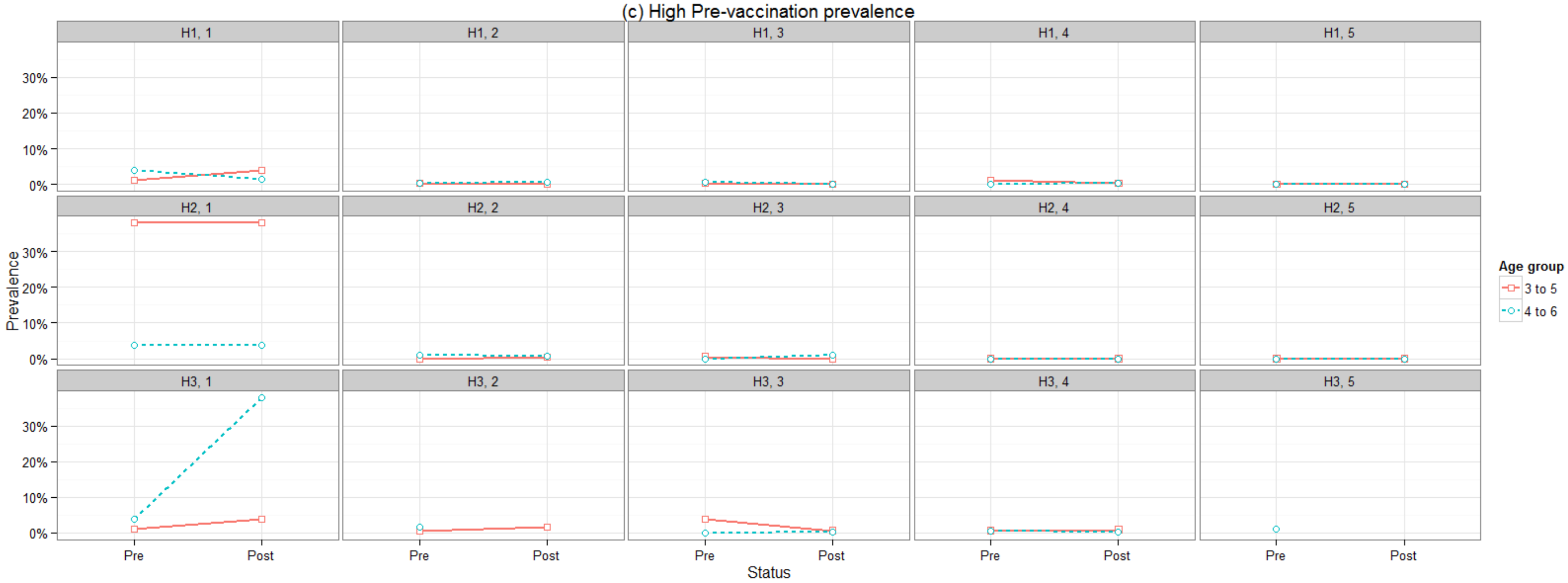
Supplementary Fig 1. Animal level prevalence for each flock estimated using the Williams and Moffitt (2001) approach in flocks with low (L), medium (M) and high (H) pre-vaccination prevalence over six samplings. Pre-vaccination status was determined based on previous OJD testing and OJD-related mortalities. Same *y-axis* scales were used for different plots in this figure. Plots with different *y-axis* scales are presented in the main body of the report to highlight the trends (Fig 3).



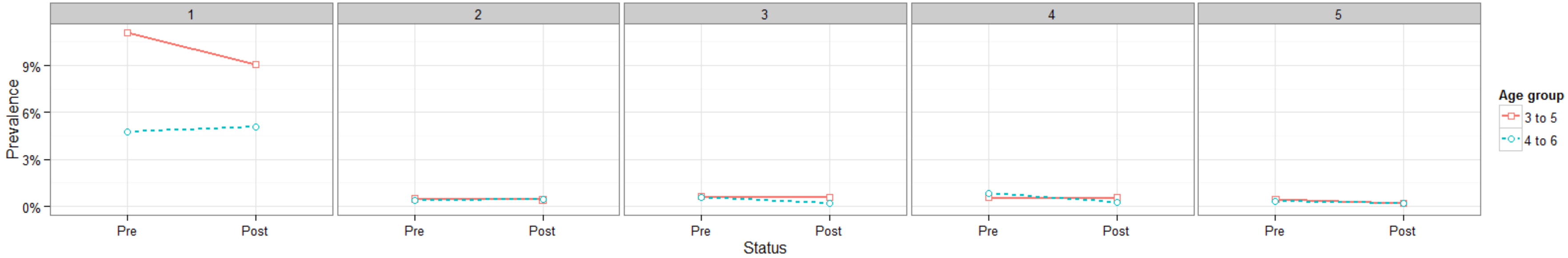
Supplementary Fig 2. Animal level prevalence estimated using the Williams and Moffitt (2001) approach classified by pre-vaccination prevalence status (low, medium and high) and age groups (3-, 4-, 5- and 6-year-old) over six samplings. Pre-vaccination status was determined based on previous OJD testing and OJD-related mortalities. Same *y-axis* scales were used for different plots in this figure. Plots with different *y-axis* scales are presented in the main body of the report to highlight the trends (Fig 4).


(a) Low Pre-vaccination prevalence
L1, 1
L1, 2
L1, 3
L1, 4
L1, 5
L2, 1
L2, 2
L2, 3
L2, 4
L2, 5
L3, 1
L3, 2
L3, 3
L3, 4
L3, 5
L4, 1
L4, 2
L4, 3
L4, 4
L4, 5
L5, 1
L5, 2
L5, 3
L5, 4
L5, 5
Prevalence
40%
30%
20%
10%
0%
Pre
Post
Status
Age group
3 to 5
4 to 6


(b) Medium Pre-vaccination prevalence
M1, 1
M1, 2
M1, 3
M1, 4
M1, 5
M2, 1
M2, 2
M2, 3
M2, 4
M2, 5
M3, 1
M3, 2
M3, 3
M3, 4
M3, 5
M4, 1
M4, 2
M4, 3
M4, 4
M4, 5
Prevalence
80%
60%
40%
20%
0%
Pre
Post
Status
Age group
3 to 5
4 to 6



Supplementary Fig 3. Changes in OJD prevalence in specific cohorts within flocks. Since sheep were sampled every two year, the 3- and 4-year-old sheep were again sampled as 5- and 6-year-old sheep after two years. These plots show changes in prevalence in these sheep in low (a), medium (b) and high (c) pre-vaccination prevalence flocks. Each plot in a row represents one of the five cohorts in a flock; see Table 1 for the details of codes used for various cohorts. Same *y-axis* scales were used for different plots in this figure. Plots with different *y-axis* scales are presented in the main body of the report to highlight the trends (Fig 6).



Supplementary Fig 4. Overall changes in OJD prevalence in 3- and 4-year-old cohorts followed for six samplings. Since sheep were sampled every two year, the 3-year-old (4-year-old) cohorts were again sampled as 5-year-old (6-year-old) cohorts after two years from the same flocks. Five plots presented represent five cohorts as described in Table 1. Same *y-axis* scales were used for different plots in this figure. Plots with different *y-axis* scales are presented in the main body of the report to highlight the trends (Fig 7)