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SHORT REPORT
A case-control study to identify risk factors for acute salmonellosis in New Zealand dairy herds, 2011–2012

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SUMMARY

In late 2011 the New Zealand Ministry for Primary Industries reported an increase in confirmed laboratory diagnoses of salmonellosis in dairy herds. To identify risk factors for herd-level outbreaks of salmonellosis we conducted a case-control study of New Zealand dairy herds in 2011–2012. In a multivariable analysis, use of continuous feed troughs [adjusted odds ratio (aOR) 6·2, 95% confidence interval (CI) 2·0–20], use of pelletized magnesium supplements (aOR 10, 95% CI 3·3–33) and use of palm kernel meal as a supplementary feed (aOR 8·7, 95% CI 2·5–30) were positively associated with a herd-level outbreak of salmonellosis between 1 July 2011 and 31 January 2012. We conclude that supplementary feeds used on dairy farms (regardless of type) need to be stored and handled appropriately to reduce the likelihood of bacterial contamination, particularly from birds and rodents. Magnesium supplementation in the pelletized form played a role in triggering outbreaks of acute salmonellosis in New Zealand dairy herds in 2011–2012.

Key words: Epidemiology, *Salmonella*.

INTRODUCTION

Salmonellosis is a bacterial disease of all animal species, including humans. In cattle salmonellosis can vary from an asymptomatic carrier state through to clinical disease characterized by acute onset of fever, severe diarrhoea, and toxaemia [1]. Infected animals excrete organisms in large numbers and infect other animals, directly or indirectly by contamination of the environment, particularly via feed and water supplies. In general terms, outbreaks of salmonellosis in dairy herds are characterized by acute onset of diarrhoea and debility affecting a significant proportion of the herd over a relatively short period of time.

Most cases of salmonellosis in humans in New Zealand are reported to be foodborne; however,

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Table 1. *Acute salmonellosis in New Zealand dairy herds, July 2011 to January 2012. Timeline showing start and end dates of the three studies described in the text*

Date	Details
December 2011	MPI cross-sectional study started ($n = 1337$ herds).
	Taranaki case-control study completed (16 case herds, 16 control herds; time-frame of interest 1 July 2011 to 31 November 2011).
January 2012	MPI cross-sectional study completed.
	Leading brand of pelletized magnesium withdrawn from the New Zealand market.
April 2012	National case-control study started (46 case herds, 79 control herds; time-frame of interest 1 July 2011 to 31 January 2012).
June 2012	National case-control study completed.

MPI, Ministry for Primary Industries

notification and hospitalization data from 1997 show that the incidence of disease is higher in rural areas compared to urban areas [2]. This observation is broadly supportive of the hypothesis that there is a link between the presence of salmonellosis in farmed livestock and incident cases of disease in humans. If this is so, epidemiological investigations of salmonellosis in domestic animal populations are important, not only because they lead to interventions which reduce the likelihood of productivity losses in farmed livestock, but also because reducing the risk of disease in farmed livestock should also reduce the risk of disease in humans.

The portal of infection for salmonellosis is almost always the mouth and the severity of disease in an individual or group of animals depends on the number of salmonellae in the environment as well as conditions of temperature and dryness that determine bacterial survival time. Except in the newborn, infection with *Salmonella* spp. is not usually a sufficient cause of clinical disease. The response to infection varies depending on the size of the challenge dose and the immunological status of the host, itself dependent on previous exposure to infection and the presence of stressors.

On 19 December 2011 the New Zealand Ministry for Primary Industries (MPI) reported that the National Animal Health Information Surveillance programme had detected a change in confirmed laboratory diagnoses of salmonellosis in dairy herds, indicated by an increase in the incidence of uncommonly reported cattle *Salmonella* serotypes and an increase in laboratory case counts for *Salmonella* spp. [3]. To deal with what appeared to be an emerging infectious disease problem, a liaison group was formed in December 2011 comprised of representatives from MPI, the Dairy Companies Association of New Zealand, dairy veterinarians, the New Zealand Veterinary Association and Massey University. The mandate of this group was to coordinate activities related to learning more about the epidemiology of the disease in New Zealand dairy cattle and the development of evidence-based control strategies. This paper provides a description of the main investigatory activity performed by the liaison group, a national case-control study to identify herd-level risk factors for acute salmonellosis.

METHODS

A description of the timing and details of the three studies (the MPI cross-sectional study, the Taranaki case-control study and the national case-control study) conducted as part of this overall investigation are provided in Table 1. This paper provides a detailed description of the national case-control study of risk factors for salmonellosis in New Zealand dairy herds. In the context of this study a dairy herd is defined as a collection of dairy cattle kept at a single geographical location under a common system of management (but not necessarily with a common owner).

Data for the national case-control study were collected between 1 April 2012 and 30 June 2012 (inclusive). The target population comprised the 10 532 dairy herds located in both the North and South Islands of New Zealand that supplied fresh milk to the Fonterra Dairy Co-operative for the milking season 1 July 2011 to 30 June 2012. In New Zealand in 2012 there were 11 735 dairy herds [4] supplying fresh milk to one of eight dairy companies. Ninety percent of dairy farms producing milk for human consumption supplied milk to the Fonterra Dairy Co-operative.

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Case herds in the national case-control study were identified from responses to a cross-sectional study performed by MPI between December 2011 and January 2012 [5] (Table 1). The eligible population for the MPI cross-sectional study comprised commercial dairy herd managers that supplied milk for human consumption to the Fonterra Dairy Co-operative in December 2011. The MPI cross-sectional study was administered as a web-based questionnaire, with 1337 responses from a total population of 10 532 Fonterra suppliers, a response rate of 13%.

Case herds for the cross-sectional study were those where respondents to the MPI cross-sectional study reported clinical signs consistent with the case definition of acute salmonellosis [6] and where the date of onset of clinical signs in the index animal (hereafter referred to as 'the date of onset') occurred between 1 July 2011 and 31 January 2012 (inclusive). For the purpose of this study the signs of a herd-level outbreak of salmonellosis included acute onset of diarrhoea and debility affecting >5% of the milking herd over a 10- to 14-day period. Affected animals were defined as those with initially high fever (rectal temperature 40–41 °C) that subsides with the onset of diarrhoea which is severe and accompanied occasionally by dysentery and tenesmus. The crude incidence mortality risk in affected herds was <2%. The laboratory criteria for diagnosis included isolation of *Salmonella* serotypes Typhimurium, Mbandaka, and/or Bovismorbificans from faecal samples retrieved from clinical cases.

For the national case-control study a probable case was a herd where there were clinical signs consistent with those listed above. A confirmed case herd was one that met all of the above criteria as well as the laboratory criteria for diagnosis. In total, 62 herds of the 1337 herds included in the MPI cross-sectional study met the criteria to be classified as probable case herds. Of this group, managers of 46 herds agreed to take part in the study. Three herds were excluded because the date of onset fell outside of the 1 July 2011 to 31 January 2012 case recruitment time-frame. Of the remaining 43 probable case herds, 38 had laboratory confirmation of their diagnosis.

Sample size calculations were performed to determine the number of control herds to recruit to meet the objectives of the national case-control study. A sample size of 40 case herds and 80 control herds was estimated using the Power and Sample Size Program v. 3.0 [7]. These numbers were based on a case-control ratio of 1:2, with an alpha of 0·05 and 80% power to detect an odds ratio (OR) of at least 3·25 for each of the exposures under investigation, assuming the prevalence of exposure among controls was 0·3.

Two sets of control herds were selected for the national case-control study. The first was a set of population-based controls [8] selected from a sampling frame comprised of all dairy herds in the North and South Islands of New Zealand that supplied fresh milk to the Fonterra Dairy Co-operative for the milking season starting on 1 July 2011. The second set of controls was selected at random from the *Salmonella*-negative herds identified in the MPI cross-sectional study. Because it was of interest to rule out the presence of spatial clustering of case herds, the population-based control herds were selected so that their geographical distribution matched the spatial distribution of the population of Fonterra dairy herds using Generalized Random Tessellation Stratified methods [9]. Assuming the response rate of herd managers of control herds to a mail-out questionnaire would be in the order of 20% and to ensure that there would be least two controls for every case, a total of 411 population-based controls were selected from the 10 532 herds that supplied Fonterra Dairy Co-operative in December 2011. A total of 55 control herds were selected from the 1275 *Salmonella*-negative herds identified in the MPI cross-sectional study.

A questionnaire containing 64 questions requesting details of herd demographics, nutritional management (amount and type of feeds offered, including mineral supplements and the storage of feed ingredients) and effluent management was developed (Table 2). The questionnaire was modified from a pilot case-control study that had been conducted in Taranaki, a provincial centre of the North Island of New Zealand, in December 2011 [10] (Table 1). The time-frame of interest for questions that related to the use of supplementary feeds and effluent management was October 2011. In New Zealand, dairy herds are typically managed so that cows calve as a single group during late winter (July and August). October, about 60–90 days after the planned start of calving date (i.e. the date on which the first cow in the herd is expected to calve for a given milking year), corresponds to the time when cows are in full milk and fed to capacity. It was reasoned that asking questions about how herds were managed at this important stage of the production cycle would minimize the impact of recall bias due to the unavoidable delay between the timing of the exposures that were being asked about and administration of the questionnaire. Our rationale

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Table 2. *Acute salmonellosis in New Zealand dairy herds, July 2011 to January 2012. Details of questions about the herd, use of supplementary feeds and water and effluent management*

Question	Details
Herd	Number of cows calved in July, August and September 2011; number of cows in milk October 2011; expected milk solids yield per cow for the 2011–2012 season*; are calves reared on farm; are heifers reared on farm; farm area; number of full-time staff working on farm on 1 October 2011; number of cows in milk on 1 October 2011; any introductions (cows or bulls) into the herd from 1 October 2011 to 15 December 2011; are cats kept on farm; are dogs kept on farm; use of rodent control.
Supplementary feeds	Are supplementary feeds routinely used; in what year did you start routinely using supplementary feeds; details of ration fed in the first 2 weeks of October 2011; details of different methods for delivering supplementary feed to stock (continuous troughs, individual troughs, feed pad, feed bins); was the herd supplemented with magnesium at any time throughout the lactation; what type of magnesium supplementation was used (magnesium oxide, magnesium sulphate, magnesium chloride); what was the physical form of magnesium supplementation used (pellets, loose mix, intra-ruminal bolus, in-line water delivery); do you mix your own supplementary feeds; do you weigh out ingredients; where are feed ingredients stored after mixing.
Water and effluent	Source of water for stock; was strip grazing used in the first 2 weeks of October 2011; do you routinely spread effluent on pasture; how long is effluent left to stand before it is spread on pasture; how often is effluent spread on pasture; what method do you use to spread effluent on pasture (slurry tanker, travelling irrigator, stationary irrigator).

* Expected average milk solids (i.e. kilograms of fat plus protein) yield per cow for the 2011–2012 season.

for restricting the time-frame over which outbreaks occurred for case herds (1 July 2011 to 31 January 2012) was driven by a requirement to reduce the impact of recall bias. We were confident that herd managers were able to accurately recall the content of the ration fed to their herds at peak lactation for the current milking season (i.e. October 2011). We had less confidence that they were able to recall ration content details for previous milking years.

Administration of the nationwide case-control questionnaire was conducted by the Fonterra Service Team, a group of eight individuals whose routine tasks are to manage milk quality and compliance issues on farm. Questionnaires were mailed out by the Service Team to the managers of case and control herds during the second week of April 2012 with a consent form and a completed self-addressed, pre-paid envelope for replies. At 3 and 6 weeks after mail-out, herd managers that had not returned a completed questionnaire were contacted by telephone to encourage a response. Ethics approval for this study was not required because all study participants were Fonterra suppliers and information collected fell under their Fonterra supplier contract.

Data were double-entered onto a microcomputer and statistical analyses performed using R v. 2.15.1 [11]. Bivariate (i.e. univariable) analyses were undertaken to select, for multivariable modelling, explanatory variables associated with a herd being *Salmonella* positive. The association between each of the continuously distributed exposure variables and herd case-control status was tested using the Student's t test. The association between each of the categorical exposure variables and herd case-control status was tested using the χ^2 test and ORs. All exposure variables associated with a herd being *Salmonella* positive at an alpha level of <0·2 at the bivariate level were entered into a binary logistic regression model. A backward elimination process was used to select explanatory variables associated with a herd being *Salmonella* positive. The significance of each explanatory variable in the model was tested using the Wald test. Those that were not statistically significant were removed from the model one at a time, beginning with the least significant, until the estimated regression coefficients for all the variables retained were significant at an alpha level of <0·05. The results of the final model are reported in terms of adjusted odds ratios (aORs) for each explanatory variable. Assuming a causal relationship between a given exposure and salmonellosis, an aOR [and its 95% confidence interval (CI)] of >1 indicates that, after adjusting for other variables in the model, exposure to the explanatory variable increased the risk of a herd being

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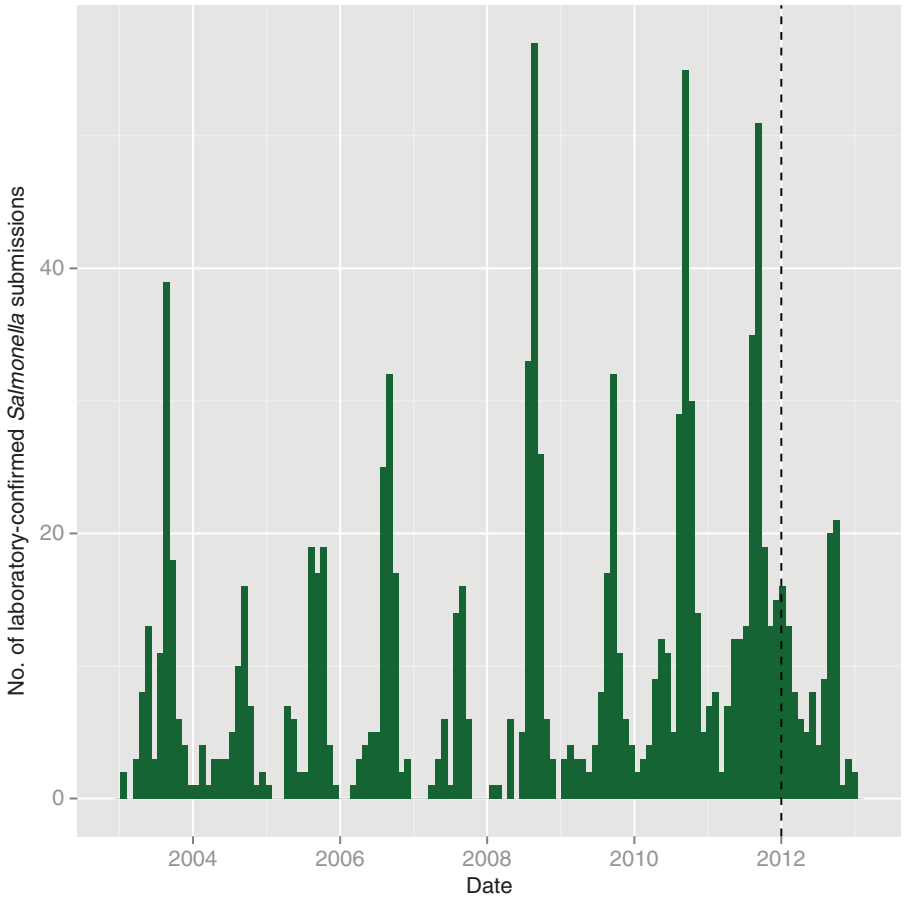

Fig. 1. Frequency histogram showing the number of laboratory-confirmed *Salmonella* submissions from cattle, New Zealand, January 2003 to December 2013. The date of withdrawal of the leading brand of pelletized magnesium is indicated by the vertical dashed line.

Salmonella positive. An adjusted odds ratio (and its 95% CI) of <1 indicates that exposure to the explanatory variable was protective, and an OR of 1 indicates that the variable was not associated with *Salmonella* risk.

The contribution of each of the explanatory variables in the final model on the risk of salmonellosis in the population was quantified using the population attributable fraction (PAF). The PAF is the proportional reduction in outcome event incidence avoided by eliminating exposure to an aetiological agent or completely preventing the effects of exposure, assuming the aetiological agent is causative and assuming no bias and sampling error in the study population [12]. Because the population-based controls in this study represented a random sample of the population from which the cases were obtained, the prevalence of exposure in the controls was used to calculate the PAF [13].

A receiver-operating characteristic (ROC) curve was constructed on the basis of the *Salmonella* status of herds predicted by the model. The area under the ROC curve, which ranges from zero to 1, provided a measure of the model's ability to discriminate between *Salmonella*-positive and *Salmonella*-negative herds. The greater the area under the ROC curve the better the model's discriminatory power.

RESULTS

A frequency histogram showing monthly counts of laboratory-confirmed *Salmonella* submissions as a function of calendar time, July 2003 to December 2013 as recorded by the New Zealand MPI National Animal Health Information Surveillance programme is shown in Figure 1 (J. Watts, personal communication).

Questionnaires were returned from 46 of the 62 probable case herds selected from the MPI cross-sectional study, a response rate of 74%. A total of 79 questionnaires were returned from of the 411 population-based controls, a response rate of 19%.

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For the 55 control herds selected from those that participated in the MPI cross-sectional study 41 questionnaires were returned, a response rate of 74%.

Separate analyses were performed using the population-based controls and the controls selected from the cross-sectional survey. Results and inferences drawn from the datasets using details from the population-based and cross-sectional controls were similar. In the remainder of this paper results are reported for the population-based control dataset.

The date of onset fell outside the 1 July 2011 to 31 January 2012 case recruitment time-frame for three case herds; these were excluded. Data from 43 case and 79 population-based control herds were available for analysis, a ratio of 1:1·8. For case herds the median onset date was 4 October 2011. The range of onset date was from 1 July 2011 to 15 December 2011. Of the 43 case herds, 38 had laboratory confirmation of their diagnosis. All case herds were located in the North Island. For control herds 64 were in the North Island and 15 were in the South Island. The spatial distribution of case herds in the North Island was similar (by inspection) to that of controls.

Descriptive statistics of key herd-level characteristics, stratified by case-control status are presented in Table 3. Median herd size, the number of full-time equivalent (FTE) staff/100 cows, and stocking rate (expressed as the number of cows per hectare) were all numerically greater in case herds compared to controls, but the differences in groups were not significant at an alpha level of 0·05. Expected average total lactation milk solids yield per cow, as estimated by questionnaire respondents, was significantly greater in case herds compared to controls (t test statistic 2·35, D.F. = 116, $P = 0·02$).

At the bivariate level use of palm kernel meal, use of starch concentrates apart from palm kernel meal (e.g. wheat, biscuit meal, kibbled maize) and use of magnesium supplementation in a pelletized form were significantly (alpha level of 0·05) associated with case herds. The odds of using continuous troughs for feeding, weighing out feed ingredients (as opposed to estimating feed quantities by eye), use of a travelling irrigator to spread effluent, leaving effluent to stand for <50 days prior to spreading on pasture, rearing of calves on farm, introducing animals into the herd between 1 October 2011 and 15 December 2011 and an expected milk solids yield of >400 kg/cow per lactation were all greater in case herds compared to controls. The odds of feeding supplements on grass and strip grazing were less in case herds compared to controls.

Table 3. *Acute salmonellosis in New Zealand dairy herds, July 2011 to January 2012. Descriptive statistics of key herd-level characteristics, stratified by case and control status*

Variable	n	Mean (S.D.)	Median (Q1, Q3)
Herd size			
Cases	43	419 (352)	344 (241, 446)
Controls†	79	364 (263)	305 (200, 470)
Total	122	383 (297)	320 (205, 470)
Expected MS yield (kg)‡			
Cases	43	404 (99)	400 (362, 450)
Controls†	79	364 (82)	372 (347, 400)
Total	122	378 (90)	386 (350, 415)
FTEs/100 cows§			
Cases	43	0·77 (1·09)	0·59 (0·46, 0·73)
Controls†	79	0·62 (0·31)	0·58 (0·47, 0·75)
Total	122	0·67 (0·69)	0·58 (0·46, 0·74)
Stocking rate (no. of cows/hectare)			
Cases	43	2·89 (0·65)	2·86 (2·51, 3·29)
Controls†	79	2·77 (0·55)	2·76 (2·46, 3·11)
Total	122	2·81 (0·58)	2·77 (2·47, 3·16)

MS, Milk solids; FTE, full-time equivalent.
* Number of cows that calved following the 2011 planned start of calving (as defined in the text).
† Population-based controls.
‡ Expected average milk solids (i.e. kilograms of fat plus protein) yield per cow for the 2011–2012 season.
§ Number of full-time equivalent staff per 100 milking cows.

In the multivariable analysis, three variables were statistically significant and remained in the final model: use of continuous troughs, use of magnesium supplementation in a pelletized form, and use of palm kernel meal (Table 4). The interaction between use of palm kernel meal and use of pelletized magnesium supplements was tested and found not to be significant (Wald $P = 0·78$). Independent of the other risk factors included in the model, the odds of using continuous troughs was 6·2 (95% CI 2·0–20) times greater in case herds compared to controls. The odds of using magnesium supplementation in a pelletized form was 10 (95% CI 3·3–33) times greater in case herds compared to controls. The odds of using palm kernel meal was 8·7 (95% CI 2·5–30) times greater in case herds compared to controls. The PAFs were 0·23 (95% CI 0·11–0·29) for continuous troughs, 0·22 (95% CI 0·08–0·31) for pelletized magnesium and 0·62 (95% CI 0·41–0·70) for palm kernel meal. The relatively large PAF for palm kernel meal was due to the relatively high proportion of controls (38 of 79, 48%) reporting use of this supplement.

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Table 4. *Acute salmonellosis in New Zealand dairy herds, July 2011 to January 2012. Regression coefficients and their standard errors from the final logistic regression model of herd-level salmonellosis risk*

Exposure	Cases	Controls*	Coefficient (S.E.)	*P* value	aOR (95% CI)
Intercept	43	79	−3·4161 (0·6579)	<0·01	
Palm kernel meal					
No	7	41	Reference		1·0
Yes	36	38	2·1653 (0·6331)	<0·01	8·7 (2·5–30)
Pelletized magnesium					
No	15	66	Reference		1·0
Yes	28	10	2·3332 (0·5863)	<0·01	10 (3·3–33)†
Continuous troughs					
No	18	63	Reference		1·0
Yes	25	10	1·8249 (0·5876)	<0·01	6·2 (2·0–20)

aOR, Adjusted odds ratio; CI, confidence interval; S.E., standard error.
* Population-based controls.
† Interpretation: the odds of using pelletized magnesium supplementation in case herds was 10 (95% CI 3·3–33) times that of control herds.

The area under the ROC curve for the predictions from our multivariable model was 0·87, indicating that the multivariable model had satisfactory ability to discriminate between case and control herds on the basis of the values of each of the explanatory variables.

DISCUSSION

A number of independent sources provided evidence that the incidence of salmonellosis in New Zealand dairy cattle had increased between 2009 and 2011. Since 2009 data from the diagnostic laboratories contracted to the MPI to serotype *Salmonella* isolates showed an increase in the number of laboratory submissions (Fig. 1) and an increase in the incidence of unusual *Salmonella* serotypes from cattle. Over the same time period, the MPI received a number of reports from veterinarians regarding outbreaks of salmonellosis in adult dairy cattle where high morbidity and low mortality was a clinical feature [14]. Several case studies describing these outbreaks were subsequently reported in the veterinary literature [15, 16].

Throughout the 2011–2012 milking season, four dairy veterinarians in the Taranaki region reported 16 laboratory-confirmed (herd) outbreaks of salmonellosis among a client base of ~1600 dairy herds. Prior to 2009, each veterinarian estimated that they diagnosed, on average, a single outbreak of acute salmonellosis every 10 years. Assuming that there had been little change in the size of the dairy herd population in this area of New Zealand, the incidence rate of acute salmonellosis for the 2011–2012 season was estimated to be 10 (95% CI 5·9–16) cases/1000 herd-years at risk compared to 0·2 (95% CI 0·1–0·6) cases/1000 herd-years at risk for the 10 years prior to 2009. The presence of similar indicators of a change in disease frequency from three independent sources (routinely collected surveillance data from diagnostic laboratories, investigations performed by the state veterinary service, and anecdotal reports from private veterinary practitioners) provided sufficient evidence to conclude that in 2011 the frequency of salmonellosis in New Zealand dairy herds had changed sufficiently to warrant further investigation [17].

On New Zealand dairy farms troughs are typically installed in the milking parlour, allowing cows to be fed supplements at the time of milking. With continuous troughs individual cows have access to the rations of cows adjacent to them in the milking parlour whereas with individual feed troughs (as the name suggests) no such access exists. Compared to controls, case herds were more likely to use continuous feed troughs (aOR 6·2, 95% CI 2·0–20, Table 4). With continuous troughs it is likely that the amount of supplementary feed consumed by individual cows will vary, with dominant cows consuming more than their allotted daily feed allowance and submissive cows consuming less. Fluctuations in supplementary feed intake is likely to influence the balance of rumen microflora, allowing *Salmonella* to multiply and trigger clinical disease. A second explanation is that continuous feed troughs increased the likelihood of disease transmission arising from contact with saliva from *Salmonella*-positive cows. In a study comparing dairy herds with

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low and high within-herd incidence risks of salmonellosis in Victoria, Australia in 1993 Morton [18] showed that the odds of using continuous troughs was greater in herds with a high incidence of salmonellosis cases compared to those with a low incidence. The biological plausibility of the association reported in our study and the consistency of our findings with those of Morton [18] indicate that systems that provide better control over individual cow feed intakes (such as individual troughs) should assist in reducing the risk of acute salmonellosis on dairy farms. An additional benefit of this approach is that it is likely to reduce the likelihood of other, feed related problems in intensively managed dairy herds such as clinical and subclinical ruminal acidosis [1].

In New Zealand, dairy cattle are typically managed to calve as a single group in the spring so that the timing of peak milk production coincides with the time of maximum pasture growth. The uptake of soil magnesium by rapidly growing pasture can be poor throughout the spring (September–November) making it necessary for herd managers to provide magnesium supplementation. Ninety-three percent (113 of 122) of those that took part in the national case-control study stated that they routinely used magnesium in the form of prills (pellets), as a loose mix or in the drinking water at some stage throughout the lactation. We found no significant association between the use of magnesium in the form of prills, powder or in the drinking water and *Salmonella* risk. In the multivariable model the odds of using pelletized magnesium supplements were 10 (95% CI 3·3–33) times greater in case herds compared to controls (Table 4). Similar findings were reported by Morton [18]; however, in that study magnesium oxide (in a granulated form) was the only form of magnesium supplementation used and a marked dose-response effect was identified, with high *Salmonella*-incidence herds being more likely to have inclusion rates of $\geqslant$20 g/cow per day compared to low *Salmonella*-incidence herds.

The strong association between a herd being *Salmonella* positive and the use of pelletized magnesium supplementation, as opposed to magnesium supplementation in other forms, is a novel finding. Selection bias might be one explanation for our findings if pelletized magnesium users were overrepresented in the case herds that responded to the MPI cross-sectional study relative to the general population of affected herds. This situation could have arisen if herd managers believed that by responding to the cross-sectional study they might have been eligible for some form of compensation. The marked difference in invitations to take part in the national case-control study from managers of case (74%) and control (19%) herds is consistent with this hypothesis. Our argument against selection bias as being the only explanation for our findings is that in a pilot case-control study conducted in Taranaki in December 2011 using 16 case herds identified by the four veterinary practitioners mentioned earlier and 32 controls [10] a similar, strong association between herd *Salmonella* status and pelletized magnesium use was also found. The key issue with the Taranaki study was that the practitioners were from four veterinary practices that serviced the majority of dairy herds in the region and, as a result, one can be reasonably confident that the 16 cases of salmonellosis represented all incident cases of disease that occurred in the region during the period 1 July and 1 December 2011.

Bias arising from differential misclassification of exposure status was a possible non-causal explanation for our findings because herd managers of case herds were likely to have a different level of recall of past exposures compared to managers of control herds [19]. To reduce the impact of this bias our approach was to focus questioning on an important time of the year [20] for New Zealand dairy farmers, the onset of peak lactation. We reasoned that recall of the feeding regimen at peak lactation was likely to be better compared to the only alternative, which was to ask specific questions about ration components in the month (say) before the date of onset. It should be noted that some case herds with an onset date before October 2011 had used pelletized magnesium earlier during lactation but had stopped using it by 1 October 2011. The impact of this on our results was to bias the association between pelletized magnesium use and *Salmonella* risk towards the null, assuming there was no little or no carryover effect of feeding pelletized magnesium on *Salmonella* risk once feeding has ceased. This being the case, it is possible that the true association between pelletized magnesium use and *Salmonella* risk may have been actually greater than that reported in this study.

Failure of managers of control herds to report a herd-level outbreak of salmonellosis was a second potential misclassification bias in this study. Our assessment is that the managers of control herds were unlikely to have failed to mention that their herds had experienced an outbreak of salmonellosis between 1 July 2011 and 31 January 2012; first, because the

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clinical signs of a herd outbreak are distinctive [6], and second, because the reason the case-control study was being performed was relatively well known in the dairy farming community and it was clearly explained to those that participated in the case-control study that the purpose of the investigation was to identify risk factors for herd outbreaks of salmonellosis.

Confounding was a third, non-causal explanation for our findings. Herd production level was a contender here with high-producing herds having a greater risk of being *Salmonella* positive, high-producing herds being more likely to be pelletized magnesium users, with the physiological mechanism of each of these effects on the risk of disease operating on two separate causal pathways. Inclusion of a term in the multivariable model to account for herd production produced no significant change in the strength of association between pelletized magnesium use and *Salmonella* risk, ruling out herd production level as an important confounder. In addition, if the association between pelletized magnesium use and *Salmonella* risk was due to high herd productivity then either the association between pelletized magnesium and productivity must have been very strong or the association between productivity and salmonellosis must have been very strong and therefore relatively easy to identify [21]. We found no evidence to support either of these scenarios in this study.

A final (albeit weak) argument for concluding that pelletized magnesium was a component cause of herd outbreaks of salmonellosis in New Zealand in 2011–2012 was the substantial reduction in *Salmonella* isolate frequency following withdrawal of the leading brand of pelletized magnesium in January 2012 in response to the findings reported in the pilot case-control study performed in Taranaki in December 2011 [10] (Fig. 1). For the 6-month period from 1 July 2011 to 1 January 2012 the number of *Salmonella* isolates in cattle was 148. For the 6-month period from 1 July 2012 to 1 January 2013 there were 58 cattle isolates (J. Watts, personal communication), a 0·61 reduction in *Salmonella* isolate frequency. To the best of our knowledge, there were no substantial changes in the way New Zealand dairy cattle were fed or managed over this period (e.g. introduction or elimination of concentrate feeds) or the way diagnostic veterinary laboratories retrieved or processed samples submitted for *Salmonella* testing. Assuming the reduction in *Salmonella* isolate frequency is a suitable proxy measure of the actual number of herd-level outbreaks of acute salmonellosis throughout the country, we note that this reduction in *Salmonella* isolate frequency is greater than the 0·22 (95% CI 0·08–0·31) reduction in *Salmonella* isolate frequency expected using the PAF estimates from the case-control study. Several explanations exist for this, including greater awareness of risk factors for salmonellosis in dairy herd managers in 2012 leading to more widespread application of preventive measures such as vaccination and improved management of supplementary feeds to reduce contamination by wildlife and rodents.

The precise physiological mechanism by which pelletized magnesium increases the risk of salmonellosis is, at the present time, unknown. The pH of the rumen contents has been shown to affect the number of salmonellae surviving passage through the rumen into the abomasum and small intestine. A high rumen volatile fatty-acid content and low pH, such as that which occurs when an animal is on full feed, provides unfavourable conditions for salmonellae to pass through the forestomachs [22]. Magnesium oxide and lime flour are rumen alkalinizing agents and it has been shown that as rumen pH increases salmonellae grow more vigorously [22, 23]. The leading brand of pelletized magnesium was known to be poorly soluble in the digestive tract and we speculate that this characteristic of the supplement may have influenced growth of salmonellae within the gastrointestinal tract even though the absolute quantity of magnesium fed might have been well within recommended daily requirements.

The odds of using palm kernel meal as a supplement in case herds were 8·7 (95% CI 2·5–30, Table 4) times that of control herds. This finding could be the result of one of two scenarios. The first is that palm kernel meal is a vehicle by which *Salmonella* organisms are introduced into previously uninfected herds. The second scenario is that palm kernel meal may be a proxy variable representing more intensively managed herds and the risk of disease was greater in more intensively managed herds rather than entirely due to palm kernel meal use alone. It should be noted that one of the features of the most commonly used pelletized magnesium supplement was that mixing it with palm kernel meal improved its flow through feed delivery equipment on farm. An interaction term was included in the multivariable model to test the hypothesis that the use of palm kernel meal and pelletized magnesium supplementation increased the risk of salmonellosis beyond that expected from addition of the estimated risks arising from the two factors working alone. The interaction term was not significant at the alpha level of 0·05 (Wald $P = 0·78$) and inclusion of the term provided little improvement to overall model fit.

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Palm kernel meal has been widely used on New Zealand dairy farms since 2007 and since that time there was an increase in the number of laboratory-confirmed *Salmonella* diagnoses in New Zealand compared to previous years (Fig. 1). Of note is the strong seasonal peak in laboratory confirmations, consistent with peak lactation in spring calving dairy herds and the increase in laboratory confirmations since 2010–2011, particularly at times of the year not associated with calving. Based on the findings presented in this study, a plausible inference is that the increase in *Salmonella* confirmations since 2010–2011 was due to widespread use of pelletized magnesium supplementation on dairy farms (the leading brand of pelletized magnesium was launched onto the market in New Zealand in 2009). Given the relatively strong association between use of palm kernel meal as a supplementary feed and *Salmonella* risk our second inference is that palm kernel meal is a likely vehicle for *Salmonella* transmission in dairy herds. Assuming there is little or no *Salmonella* contamination when it is delivered on farm by a feed supplier, we conclude that palm kernel meal needs to be stored and handled appropriately to reduce the likelihood of contamination, particularly from birds and rodents. This recommendation would extend to all supplementary feeds used on New Zealand dairy farms, not just palm kernel meal.

CONCLUSION

A case-control study designed to identify herd-level risk factors for acute salmonellosis in New Zealand dairy herds was conducted between April and June 2012. Case herds were more likely to use continuous feed troughs, more likely to use pelletized magnesium supplements and more likely to use palm kernel meal as a supplementary feed compared to control herds. We conclude that supplementary feeds (i.e. concentrates and mineral supplements) and their method of delivery were risk factors for acute salmonellosis in New Zealand dairy farms in 2011–2012. Supplementary feeds used on dairy farms (regardless of type) need to be stored and handled appropriately to reduce the likelihood of bacterial contamination, particularly from birds and rodents. This recommendation applies to all involved in provision of supplementary feeds to dairy cattle: those sourcing commodity feeds offshore, feed transporters, feed compounder as well as dairy herd managers. Magnesium supplementation in the pelletized form played a role in triggering outbreaks of acute salmonellosis in New Zealand dairy herds in 2011–2012.

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DECLARATION OF INTEREST

None.

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