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Temporal trends in bulk milk antibodies to *Salmonella*, *Neospora caninum* and *Leptospira interrogans* serovar *hardjo* in Irish dairy herds

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Abstract

Bulk milk samples were collected from 309 Irish dairy herds at four time points during 2009 and tested for antibodies to *Salmonella* spp., *N. caninum* and *L. hardjo*, three abortifacient agents in Irish dairy herds. Of the 309 study herds, 49% vaccinated for *Salmonella* and 76% vaccinated for *L. hardjo*. In unvaccinated herds, the overall prevalence of antibody positive herds was 49% for *Salmonella*, 19% for *N. caninum* and 86% for *L. hardjo*. There was no association between both testing positive for and incidence of *Salmonella* or *L. hardjo* on sample date and calving season. A significant association was found between sample date and both testing positive for [p = <0.0001 OR = 2.41 (95% CI 1.54–3.80)] and incidence [p = 0.001 OR = 3.10 (95% CI 1.72–5.57)] of *N. caninum*. No association with region of Ireland was found for either testing positive for or incidence of *N. caninum* or *L. hardjo*. There was, however, a tendency towards a higher incidence of *Salmonella* in regions of Ireland with higher cattle densities.

Keywords: *Salmonella*; *N. caninum*; *L. hardjo*; Bulk milk; Herd prevalence; Ireland

Introduction

Salmonella spp, *N. caninum* and *L. hardjo* are three causes of infectious bovine abortion (Cabell, 2007), have a worldwide distribution, and all three have been isolated in cases of bovine abortion in Ireland (O'Reilly and Egan, 1988; McNamee et al., 1996). Disease due to *Salmonella*, *N. caninum*, or *L. hardjo* on a dairy farm can result in serious economic losses. *Salmonella* has been shown to cost between Dfl.5000 (€2,300) and Dfl.18000 (€8,200) per herd in the Netherlands (Visser et al., 1997). Chi et al. (2002) documented an annual loss of CDN$ 2305 (€1,566) in a 50 cow dairy herd from *N. caninum*, and Bennett (1993) reported losses of £6000 (€7,000) in a 100-cow dairy herd in the United Kingdom due to an outbreak of *L. hardjo*. There are little recent published data on the prevalence of *Salmonella*, *N. caninum*, and *L. hardjo* in Ireland. The prevalence of a combination of these abortifacient agents on Irish dairy farms and their seasonal pattern has never been reported. Examination of the seasonal pattern of these diseases is important in the context of the Irish dairying system as the majority of Irish dairy herds calve in spring time to co-incide with the grass growing season. This maximises the amount of milk produced from grazed grass thereby increasing farm efficiency (Dillon et al., 1995). The aims of this study therefore were to, (i) determine the herd-level prevalence and incidence of *Salmonella*, *N. caninum* and *L. hardjo* among Irish dairy herds using bulk milk samples, and (ii) examine the association between both testing positive for and incidence of exposure to these pathogens with sampling date, calving season and region.

Materials and methods

Herd selection

Herds were recruited from HerdPlus®, a breeding information decision support tool for farmers co-ordinated by the Irish Cattle Breeding Federation (ICBF). In 2009, HerdPlus® contained records from 3, 500 dairy herds representing 18% of the national dairy herd. A total of 500 randomly selected herds were invited to participate in this study and HerdPlus® herds were selected within strata of herd size (31 to 65 cows, 66 to 99 cows and greater than 99 cows) and geographical location (county; n = 26). Of these 500 herds, 312 volunteered to participate in the study.

Sample collection and laboratory analysis

Four bulk milk samples were submitted by study farmers during 2009 on the 23rd March, 8th June, 31st August, and 2nd November. Participants were issued with a standardised sampling kit. A mobile phone text message was sent to each participant on the day before and on the morning of sampling to ensure co-ordination of the sampling process. In excess of 98% of the samples arrived in the laboratory one day after sample collection, with remaining samples arriving one or two days later. Samples were tested in commercial laboratories for antibodies to, (i) *Salmonella* using a Lipopolysacharide (LPS) Enzyme Linked Immunosorbant Assay (ELISA) [detects a minimum within herd prevalence of 10% with sensitivity (Se) 63.2% and specificity (Sp) 99.7%] developed by GD Animal Health Services, (Netherlands) using a percent positivity (pp) cut-off of 0.34 (Veling et al., 2001), (ii) *Neospora caninum* using an indirect ELISA [detects a minimum within herd prevalence of 15% with Se 99% and Sp 96% (Svanova, Sweden)] using a pp cut-off of 0.20 (Chanlun et al., 2002) and (iii) *L. hardjo* using an indirect ELISA test [detects a minimum within herd prevalence of 5% with Se 93.4–99.4% (Se 96.4%) and Sp 95.2–98.2% (Sp 96.7%), (Ceditest, Celtic Diagnostics, Ireland)] using a pp cut-off value of 0.40 (Lewis et al., 2009). The vaccination protocol applied in each study herd in 2009 was recorded by questionnaire. No vaccination data were available for three herds and these herds were removed from the study. Calving data from HerdPlus® was used to determine the calving season of participating herds.

Data and statistical analysis

Herds were classified as 'all-spring-calving' if at least 85% of the herd calved between January and June and as 'not-all-spring-calving' if cows calved at other times of the year. A chi-square analysis was performed to examine how representative the study population was of the Irish national dairy farm population (CSO, 2008). A chisquare analysis was also performed to determine the differences in geographic location (region) and herd size (31–65 cows, 66–99 cows, and >99 cows) between responders and non-responders. Based on Irish Central Statistic Office procedures (CSO, 2008) counties (n = 26) were combined into seven geographical regions to determine the location of study herds. The location of study herds and the kernel density of the dairy population in the Republic of Ireland during 2008 are shown in Fig. 1. The map was created using ESRI Arcview 3.2 (Redlands, California, USA). Dairy population density was based upon the herd type classification on the Department of Agriculture, Food and the Marine's Animal Health Computer System (AHCS) for 2008. The location of study herds was attributed to the centroid of the largest fragment of land for each herd according to the Land Parcel Identification System (LPIS) for 2008.

The apparent prevalence (Ap) of *Salmonella*, *N. caninum* and *L. hardjo* in unvaccinated herds at each of the four sample time points was calculated. A herd was assigned a score of zero if the bulk milk reading was negative and a score of one if the bulk milk reading was positive. The true prevalence (Tp) at each sampling time point was derived using the Rogan-Gladen estimator as implemented in the survey toolbox version 1.04 [www.ausvet.com.au (Cameron, 1999)]. The incidence (a herd recording a negative result at the previous sampling time point that became positive) of *Salmonella*, *N. caninum* and *L.*

hardjo in unvaccinated herds was calculated for the periods March to June, June to August and August to November. A herd was assigned a score of zero if no incidence occurred and a score of one in cases of a test positive result.

Generalised estimating equations [PROC GENMOD (SAS, Version 9.1, USA)] were used to examine the association between both testing positive for, and incidence of, exposure with sample date, calving season, and region in unvaccinated herds. A binomial distribution of the data was assumed and a logit link function was used. Herd was included as a repeated measure and an exchangeable correlation structure was used. An association between overall test positive result and incidence of each pathogen and test positive result and incidence of the other pathogens in the study herds was determined using logistic regression [PROC GENMOD (SAS, Version 9.1, USA)]. The overall test positive result and incidence of the four samplings were the dependent variables, being zero if all four samples were negative and one if any of the four samples was positive.

Results

A total of 269 herds were classified as 'all spring-calving' and 40 as 'not-all-spring-calving'. A total of 49% (151/309) of herds vaccinated for *Salmonella* and 76% (235/309) for *L. hardjo*. There is no vaccine for *N. caninum* available in Ireland. Study participants were shown to represent the national population of Irish dairy farmers in terms of geographical location ($p = 0.76$). However, a significant difference ($p < 0.0001$) between participant herd size (average herd size 99 cows, range 28–540 cows) and the herd size of the national dairy farmer population (average herd size 60 cows) was found. There was no difference ($p = 0.81$) in geographic location between responders and non-responders; however, there was a significant difference (p = 0.0015) in herd size with a higher proportion of larger herds amongst responders than non-responders.

Prevalence

Over the study period the herd level prevalence in unvaccinated herds of *Salmonella* was 49% (78/158 herds), of *N. caninum* was 19% (60/309 herds) and of *L. hardjo* was 86% (64/74 herds). The Ap of *Salmonella* in unvaccinated herds was 32%, 36%, 37% and 35%, of *N. caninum* was 7%, 5%, 3% and 12%, and of *L. hardjo* was 81%, 84%, 84% and 84% in March, June, August and November respectively. The Tp of *Salmonella*, *N. caninum* and *L. hardjo* in March, June, August and November is shown in Fig. 2 and in Table 1.

Test positive associations

There was no association between region (p = 0.07), sample date (p = 0.36) or calving season (p = 0.81) and testing positive for *Salmonella*. There was a significant association between sample date [p = <0.0001 OR (odds ratio) = 2.41 (95% CI. 1.54–3.80)] and testing positive for *N. caninum* with the highest number of positive herds reported at the final sampling time point in November. The exchangeable working correlation was 0.20. No association was found between calving season (p = 0.17) and testing positive for *N. caninum*. No association was found between region and testing positive for *N. caninum*. There was no association between sample date (p = 0.39) and testing positive for *L. hardjo*.

There were no associations detected between an overall test positive result for one pathogen and an overall test positive result for another pathogen. No other test positive related associations were detected.

Incidence

The incidence (95% CI) of *Salmonella*, *N. caninum* and *L. hardjo* between each sample time point is shown in Fig. 3. There was a significant association between sample date [p = 0.001 OR = 3.10 (95% CI 1.72–5.57)] and incidence of *N. caninum* with the highest incidence occurring between the third and final sampling time points. The exchangeable working correlation was −0.04. There was no association between the overall incidence of *L. hardjo* (p = 0.53) and the overall incidence of *N. caninum*. There was, however, a significant association (p = 0.002) between overall incidence of *L. hardjo* and overall incidence of *Salmonella* in study herds. No other incidence related associations could be detected.

Discussion

The objectives of this study were firstly to estimate the herd-level prevalence and incidence of *Salmonella*, *N. caninum* and *L. hardjo* in Ireland using bulk milk diagnostics, and secondly to examine the associations between both herds testing positive for and incidence of each pathogen with sample date, herd calving-season and region. HerdPlus® herds were used to ensure access to the calving data necessary to accurately classify herds as 'all-spring-calving' or 'not-all-spring-calving' and may have introduced bias into the study. There are approximately 18,500 dairy herds in Ireland with an average herd size of 60 cows per herd and an average milk yield of 4681 l per cow (CSO, 2010). Participating herds were found to be representative (p = 0.76) of the national population in terms of geographical location, but did differ significantly in terms of herd size (p < 0.01). This was a result of a larger number of herds with a large herd size (>99 cows) volunteering to participate in the study. The recruitment of HerdPlus® herds may also have introduced bias toward more progressive herds as the rate of vaccine usage reported here was higher than reported by Sayers and Mee (2009). This increased vaccine use also reduced the number of herds that were available to examine prevalence and incidence associations, especially for *L. hardjo*.

Sampling strategy and methods

The diagnostic assays used in the current study all have high Sp and multiple bulk milk samples were used and interpreted in parallel for overall apparent prevalence. Parallel interpretation is likely to have led to increased diagnostic Se with a reduction in Sp. Due to the highly likely covariance and conditional dependence between samples we were unable to calculate an overall herd Se and Sp for the tests used and therefore we were unable to calculate a Tp. However, test kit Se and Sp were used to calculate the Tp at each sample time point. Furthermore, changes in management practices including the addition or removal of positive animals and differences in the biology of the infections, e.g. animals only demonstrating a positive antibody response to *N. caninum* infection in late lactation would make the calculation of an overall Se and Sp difficult. The ELISA used in the current study to detect antibodies to *N. caninum* had a design prevalence of 15%. In infected herds, the within herd prevalence of *N. caninum* may possibly be lower than the design prevalence. Therefore, an underestimation of the prevalence of *N. caninum* may have been possible. The *Salmonella* and *L. hardjo* ELISA's are designed to detect a within herd prevalence of greater than 10% and 5%, respectively. Due to the efficient spread of both *Salmonella* and *L. hardjo* within herds the prevalence of these diseases in infected herds would be expected to be higher than the design prevalence of the ELISA tests. Therefore, the ELISA kit design prevalence was unlikely to contribute to an underestimation of the herd prevalence of either *Salmonella* or *L. hardjo*. However, the low Se of the *Salmonella* ELISA used in the study may have led to false negative results being recorded, hence the large difference between Ap and Tp. This will also have had the potential to impact strongly on the reported incidence of *Salmonella* exposure.

Prevalence and incidence

The overall prevalence of *Salmonella* (i.e. 49%), *N. caninum* (i.e. 19%) and *L. hardjo* (i.e. 86%) reported in this study are higher than that documented in other European studies (Table 2). However, the prevalence of *L. hardjo* amongst study herds is similar to that found in previous studies in Ireland and Scotland (Table 2). The higher prevalence of exposure in Irish dairy herds may be as a result of differences in herd size and management practices (Leonard et al., 2004). An association between *Salmonella* prevalence and region was reported by Carrique-Mas et al. (2010) who reported a higher prevalence of *Salmonella* in areas of England with higher numbers of dairy cows. Similarly, in the current study there was a tendency ($p = 0.07$) towards a higher odds of testing positive for *Salmonella* in areas with higher cattle densities. Leonard et al. (2004) reported a significant association between region and prevalence of *L. hardjo* in Irish dairy herds. However, the association between both testing positive for and incidence of *L. hardjo* with region could not be examined in the current study. As *N. caninum* infection is not spread directly from cow to cow (Dubey et al., 2007), both inter and intra-herd spread is likely to be less affected by cattle densities within regions. Therefore, the lack of association between both testing positive for ($p = 0.88$) and incidence ($p = 0.43$) of *N. caninum* with region was an expected result. There was a significant association ($p = 0.002$) between incidence of *Salmonella* and incidence of *L. hardjo* in study herds. However, due to the small number of incidence amongst unvaccinated *L. hardjo* herds no definite conclusions can be drawn as to why this occurred. There was no association between incidence of *N. caninum* and incidence *L. hardjo* or *Salmonella*, which agrees with Bartels et al. (1999), who found that outbreaks of *N. caninum* have the potential to occur on farms in the absence of infection with *L. hardjo* or *Salmonella*.

Temporal trends

A previous study has documented an increase in outbreaks of *Salmonella* in the late summer/autumn period (Carrique-Mas et al., 2010). However, this finding was not replicated in this study where no association between both testing positive for and incidence of *Salmonella* with sample date was detected. The low Se of the *Salmonella* ELISA may have resulted in a lower apparent prevalence and may have also influenced the incidence figures observed. Animals infected with *N. caninum* often only demonstrate a detectable antibody rise in late lactation. This corresponds to the reported increased risk of abortions associated with *N. caninum* in month's five to eight of pregnancy (Dubey et al., 2007). Therefore, the increase in both the number of herds testing positive for and incidence of *N. caninum* at the final sampling time point was an expected result especially for herds containing spring calving animals which would be in late lactation and in mid to late gestation at this time point. Due to the small number of herds unvaccinated for *L. hardjo* it is difficult to draw definite conclusions for the occurrence of the lack of association between both testing positive for and incidence of *L. hardjo* and sample date. An increase in the incidence of *L. hardjo* in late lactation would be expected as herds would be in the third trimester of gestation, a time with an increased risk of abortions associated with *L. hardjo* (Rushbridge et al., 2004). However, an association between calving season and incidence of *L. Hardjo* was not detected in the current study. As abortions due to infection with *L. hardjo* infection can occur one to three months after initial infection (Anderson, 1997), the lack of association between both testing positive for and incidence of L. *hardjo* with sample date reported in the current study would therefore be expected.

Conclusions

The results demonstrate a high prevalence of *Salmonella*, *N. caninum* and *L. hardjo* compared with international findings and suggests the need for suitable control programmes. The usefulness of bulk milk testing in estimating herd exposure status was highlighted in this study. Importantly the study highlights the benefits of taking multiple samples over an entire lactation in order to increase the overall sensitivity when determining herd exposure status.

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Table 1. True prevalence (Tp) and 95% confidence interval (CI) of *Salmonella*, *N. caninum*, and *L. hardjo* in 'all-spring-calving' and in 'not-all-spring-calving' Irish dairy herds at each sampling time point

	Overall		All spring calving		Not all spring calving	
Sample date	Tp (%)	95% CI (%)	Tp (%)	95% CI (%)	Tp (%)	95% CI (%)
Salmonella						
	(n=158)		(n=134)		(n=24)	
March	50.4	45.9 - 54.9	50.4	45.5 - 55.2	52.0	40.4 - 63.5
June	56.8	52.2 - 61.4	56.8	51.8 - 61.7	58.3	46.5 – 70.2
August	58.3	53.7 - 63.0	58.3	53.3 - 63.4	66.3	54.2 - 78.4
November	55.2	50.6 – 59.7	58.8	53.8 - 63.9	32.9	22.9 - 42.9
Neospora caninum						
	(n=309)		(n=269)		(n=40)	
March	3.2	1.7 – 4.6	4.2	2.5 – 5.9	1.1	0 - 4.5
June	1.1	0 - 2.3	0	0 - 1.2	9.5	4.1 – 14.8
August	0	0 - 1	0	0 - 1	4.2	0 - 8.5
November	8.4	6.6 - 10.3	7.4	5.5 – 9.3	14.7	8.6 - 20.8
L. Hardjo						
	(n=74)		(n=62)		(n=12)	
March	83.5	78.8 - 88.1	79.2	73.8 – 84.6	100	100 - 100
June	86.7	82.4 - 91.0	83.5	78.4 – 88.5	100	100 - 100
August	86.7	82.4 - 91.0	83.0	77.9 – 88.1	100	100 - 100
November	86.7	82.4 - 91.0	83.0	77.9 – 88.1	100	100 - 100

Table 2. Prevalence of *Salmonella*, *N. caninum*, and *L. hardjo* in dairy herds in European studies using bulk milk tank testing.

Country	Prevalence	Reference
Salmonella		
Denmark	15% - 20%	Weederkoop et al., 2001
Denmark	11%	Nielsen, 2009
N. Caninum		
Czech Republic	1.01%	Hurkova et al., 2003
Sweden	8.3%	Frossling et al., 2008
Norway	0.7%	Klevar et al., 2010
L. Hardjo		
England	50% - 60%	Pritchard, 1999
Ireland	79%	Leonard et al., 2004
Scotland	67%	Lewis et al., 2009

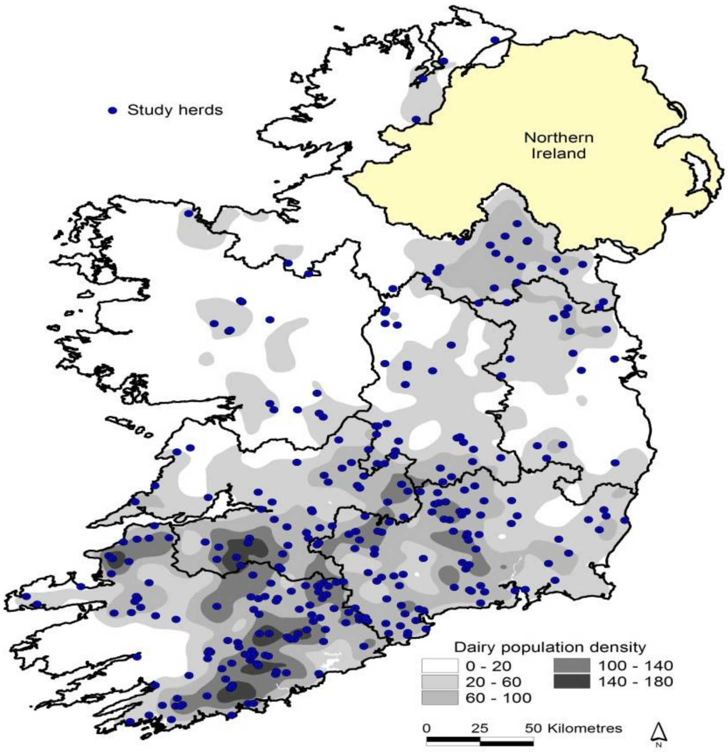

Figure. 1. Location of study herds in seven geographical regions and the kernel density of the population of dairy cows in the Republic of Ireland during 2008 (10 km search radius).

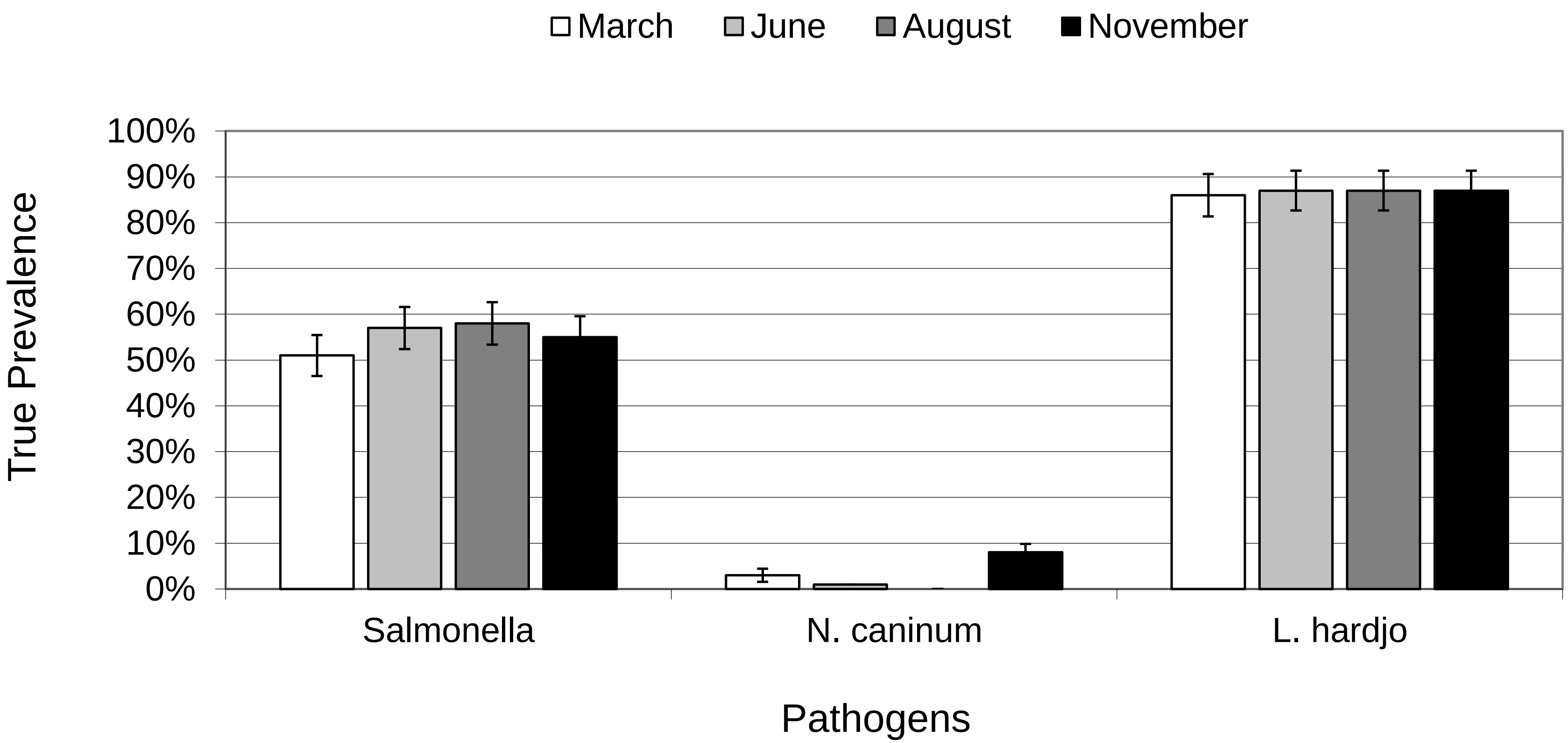

Figure. 2. True prevalence of *Salmonella*, *N. caninum*, and *L. hardjo* at each sampling time point.

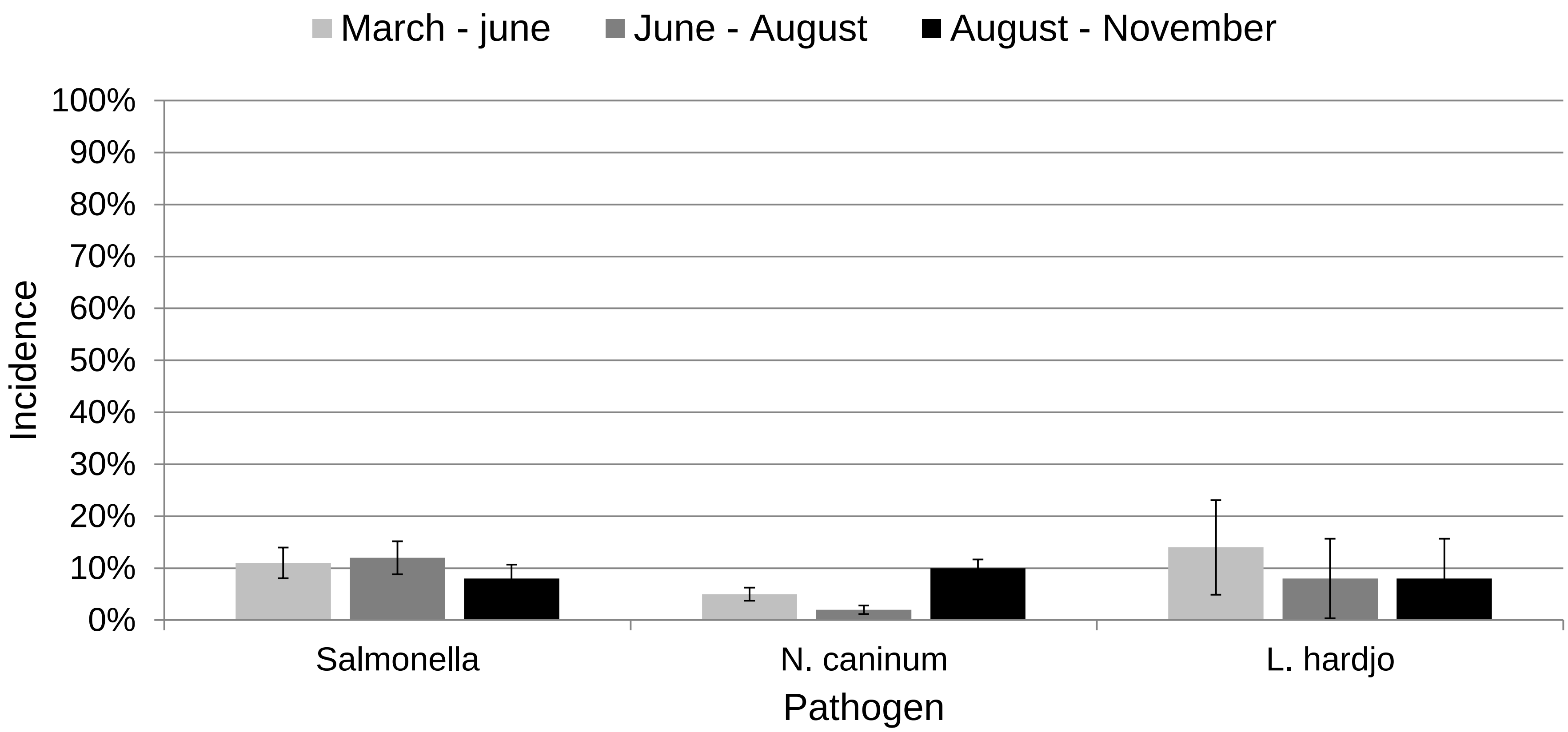

Figure. 3. Incidence of *Salmonella*, *N. caninum*, and *L. hardjo* between March and June, June and August and August and November.