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SEROPREVALENCE AND RISK FACTORS FOR DICTYOCAULUS VIVIPARUS INFECTION IN 1ST-LACTATION DAIRY COWS: A SURVEY OF QUÉBEC HERDS

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Une étude a été entreprise au Québec pour évaluer la prévalence de troupeaux laitiers ayant des vaches en 1ère lactation infectées à Dictyocaulus viviparus (D.v.) et pour déterminer les facteurs de risque pouvant être associés à l'infection. Des prélèvements sanguins et fécaux ont été récoltés chez 5 vaches de première lactation dans 177 troupeaux en Août 1995. Des données sur l'élevage et les pâturages ont été compilées. Un seuil de troupeau pour la détection de D.v. a été choisi pour un test ELISA. Le meilleur seuil (3 des 5 vaches au-dessus d'une densité optique pré-déterminée) a permis d'obtenir une spécificité de 90%, une sensibilité de 67% et des valeurs prédictives positives et négatives de 80% et 81%.

La prévalence provinciale était de 31% (int. conf.:16%-46%) et une variation existait entre les régions (G= 14.1, p≤0.05). L'infection chez les vaches de 1ère lactation d'un troupeau était plus fortement associée à la mise au pâturage des vaches (RR:2.72 (1.17-6.33)) qu'à la mise au pâturage des génisses (risque relatif:2.21 (0.87-5.58)). Les autres facteurs de risque associés d'après la régression logistique étaient: le fauchage du pâturage des génisses (OR: 2.1, p=0.09), plus de 70 vaches dans le troupeau (OR:7.2, p=0.03), densité de population élevée (OR:0.34, p=0.11), changements de pâturage (OR:0.2, p=0.02), ouverture de nouveaux pâturages en gardant accès au premier (OR: 2.4, p=0.13) et la vermifugation (OR:0.49, p=0.14).

La prévalence de troupeaux où l'infection à D.v. existe chez des vaches en 1ère lactation est donc assez élevée au Québec et il semble qu'au moins certaines vaches se contaminent au pâturage après le vêlage. La connaissance des méthodes de gestion des pâturages en place peut aider les vétérinaires dans leurs décisions concernant le diagnostic et la prévention du ver du poumon.

INTRODUCTION

Lungworm disease has been well described in young calves and yearlings, with a rare occurrence of re-infection in adults. The condition has decreased in incidence among North American dairy herds with the widespread use of endectocides in replacement stock. Anecdotal reports give the impression that incidence has increased in adult lactating cattle in the last decade in Eastern Canada. Prevalence information for this part of the world is incomplete and dated (Gupta and Gibbs, 1969). Risk factors have been studied in Europe (Schnieder et al, 1993), but young animals were usually the unit of interest and it is uncertain that results can be extrapolated to Canadian climate and pasture conditions. Therefore, bovine practitioners lack scientific information to help with decisions regarding diagnosis and prevention of *Dictyocaulus viviparus* (*D.v.*) infection.

The reference diagnostic test for lungworm disease, Baermann coprology, has relatively low sensitivity, particularly in adult animals where larvae are often absent in feces (Jørgensen, 1981). A variety of immunological tests have been used in Europe for screening or diagnostic purposes (Boon et al, 1982). Recently, a very promising ELISA test using purified *D.v.* antigen has been developed at the University of Missouri in Columbia (Bates et al, 1997). The aim of this assay is to limit cross-reactivity with other nematode antigens, but still recognize heterogeneous antibody responses to *D.v.*.

A randomized cross-sectional survey was conducted to establish the prevalence of herds with *Dictyocaulus viviparus* (D.v.) infected first-lactation cows in the province of Québec using an ELISA test and to evaluate risk factors associated with herd infection.

MATERIALS AND METHODS

The province of Québec was divided into 7 regions (strata) within which 28 veterinary clinics were randomly selected (clusters). In each clinic's clientele, a random sample of 8 dairy farms was chosen. In each farm, the 5 most recently calved 1st-lactation cows were chosen to detect infection. Whole blood and feces, collected by participating veterinarians, were sent on ice to Saint-Hyacinthe where they were processed and frozen (serum) or examined (feces) within 48 hours of sampling.

A pooled fecal sample was submitted to a Baermann test for each herd with results reported as positive or negative. Gastro-intestinal nematode (gin) eggs were detected and counted (eggs per 5 grams) using the Wisconsin technique. Individual serum samples were submitted to an ELISA test in Columbia, Missouri as triplicates with results reported as positive or negative at a pre-determined cutoff (2 sd above the mean optical density of negative controls on plate). A questionnaire on replacement and pasture management practices was sent by mail to all farms and herd owners answered each question by telephone, with a copy in hand. After validation by identification of protocol deviations by participating veterinarians, 31 farms were excluded from the analyses. Frequencies were compiled for all categorical variables. Stocking rate (number of

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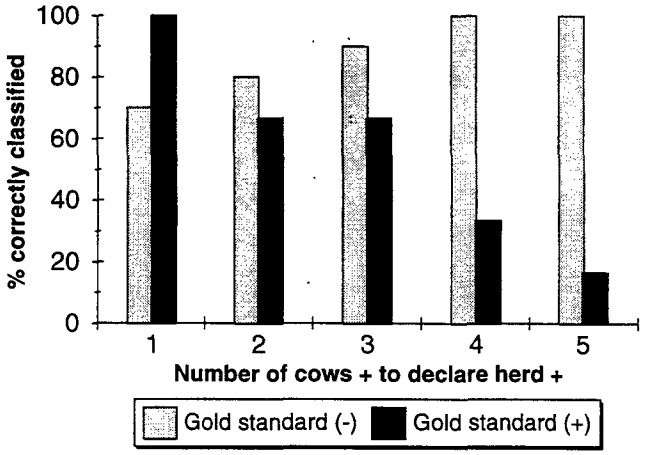
animals/ha) was submitted to a logarithmic transformation and subsequently transformed into categories (high stocking rate or not) since frequency distributions revealed a clearly bimodal nature. The categorization cutoff for this variable (10 animals/ha) was obtained by cluster analysis (centroid method).
From the original random sample, all six farms where *D.v.* was identified by Baermann coprology and ten farms where animals had never been outside and had no gin eggs were chosen respectively as positive and negative gold standards in order to chose a herd ELISA cutoff.
Regional prevalences with 95 % confidence intervals were calculated. A provincial prevalence, weighted for the actual number of herds in each region, was calculated. Independence between infection of herds and region was tested using the G-test with Williams' correction. *A posteriori* comparisons between regions were also done using the G-test, controlling for experimentwise α error.
Risk ratios for infection were calculated on the whole sample (177 herds) for two risk factors: exposure of cows to pasture and exposure of heifers to pasture. In order to test the association of all risk factors with infection, three multiple logistic regression models were created to represent the three following situations: 1) Farms where heifers and cows are pastured; 2) All farms where heifers are pastured; 3) Farms where lactating cows are pastured. Final models were built in a stepwise fashion with variables chosen at an $\alpha = 0.15$ level. Dichotomous and ordinal variables were entered one at a time whereas nominal variables were entered or removed as blocks of dummy variables.

RESULTS AND DISCUSSION

The best herd cutoff to evaluate prevalence as determined by cross-tabulation of the 16 available gold standard herds was: 3 positive cows out of the 5 sampled at the individual ELISA (Figure 1). This cutoff yielded the following test characteristics: sensitivity: 67%, specificity: 90%, predictive value of positive and negative results: 80% and 81% respectively. Likelihood ratio for infection given a positive or a negative result were of 4.02 and 0.22 respectively.
These values do not qualify the ELISA test itself but the herd-level use that was made of it with adult animals. A better cutoff could probably have been chosen if more gold standard herds had been available and if more animals had been sampled per herd. The Baermann's 100% specificity made it possible to chose true positive gold standards but its low sensitivity explains the low number of them detected in the sample.

Figure 1
Percentage of correct classification for negative and positive gold standard herds at various cutoffs.



The weighted provincial prevalence was of 31% (C.I.:16%-46%) and between-region variation was significant ($G=14.1$, $p \leq 0.05$) with only one pair of regions demonstrating a significant difference in prevalences. The difference between coprological and serological prevalence (3.3 % vs. 31%) is not surprising and has been observed in other studies (Schnieder et al, 1993).
Presence of D.v. in a herd's first-lactation cows demonstrated a stronger association with grazing of cows (RR:2.72 (1.17-6.33)) than with grazing of pre-calving heifers (RR:2.21 (0.87-5.58)). This suggests that a significant proportion of 1st-lactation cow infections are not due to resurgence of inhibited forms but to self-contamination at pasture after calving. Odds Ratios and p-values for the Wald Chi-square statistic for important risk factors of all 3 final logistic regression are reported in Table I. Risk factors associated to an increased risk of infection were: number of cows greater than 70 in herd, exposure of lactating cows to pasture, exposure of heifers to pasture, mechanical mowing of heifer's pasture during the grazing season and opening of new pasture parcels while keeping access to the original pasture. Risk factors associated to a decreased risk of infection were: complete paddock changes during grazing season, very high stocking rate and anthelmintic treatment of heifers. Intensive pasture rotation and level of supplementary feeding for heifers were not associated with an increased or decreased risk of infection.

Table I
Risk factors for infection of first-lactation cows with *D. viviparus* in dairy herds

	Farms where heifers and cows are pastured (n=122)		Heifers are pastured (whether or not cows are) (n=151)		Lactating cows pastured (whether or not heifers are) (n=139)	
	Odds Ratio	p-value	Odds Ratio	p-value	Odds Ratio	p-value
> 70 cows in herd	7.2	0.03	4.1	0.07	3.5	0.09
Heifer's pasture mowed	2.1	0.09	2.1	0.06	---	---
Heifers = complete paddock changes	0.2	0.02	0.2	0.05	---	---
Heifers = continuous access to same paddock	2.1	0.13	2.1	0.08	---	---
Heifers grazed	---	---	---	---	4.1	0.08
Heifers treated (anthelm.)	0.5	0.14	NS[1]	NS	NS	NS
Cows grazed	---	---	4.7	0.02	---	---
High stocking rate	0.3	0.11	---	---	0.3	0.05

[1]NS= Not statistically significant at $\alpha = 0.15$ (level for inclusion in the logistic model)

Pasture mowing before turnout has previously been associated to a risk decrease (Schnieder et al, 1993b). The increased risk associated to pasture mowing during summer observed in this study may be explained by the fact that *D. v.* infective larvae are less mobile than other nematode L3 forms and have a limited autonomous dispersion capacity, possibly improved by a practice such as mowing. The number of grazing animals in a herd has also been correlated with infection before (Boon et al, 1986) the suggested explanation being that the risk of having one « high-excreting » animal is higher when more animals are present.

CONCLUSIONS

These results indicate that the prevalence of herds with *D .v.* infection in 1st-lactation cows is relatively high in Québec and that at least some lactating cows seem to contaminate themselves at pasture after calving. Results also suggest that knowledge of pasture management techniques on a farm can help veterinarians in their decisions regarding lungworm diagnosis and prevention.

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