Prevalence of Maedi-visna in Saskatchewan sheep

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Abstract — A study was conducted to estimate flock and individual seroprevalence of Maedi-visna in Saskatchewan and evaluate risk factors for seropositive flocks. Thirty-five percent (24/68) of flocks and 4.6% (93/2010) of individual samples were positive. Within-flock prevalence ranged from 3.3% to 96.7%. Significant flock-level predictors of flock prevalence included large flock size, purchasing > 50 sheep and respiratory problems in the previous 5 years.

Résumé — Prévalence de maedi-visna chez des moutons de la Saskatchewan. Une étude a été réalisée pour estimer la séroprévalence individuelle et dans le troupeau de maedi-visna en Saskatchewan et évaluer les facteurs de risque des troupeaux séropositifs. Trente-cinq pour cent (24/68) des échantillons des troupeaux et 4,6 % (93/2010) des échantillons individuels étaient positifs. La prévalence dans le troupeau variait de 3,3 % à 96,7 %. Les prédicteurs importants au niveau du troupeau incluaient une taille importante du troupeau, l'achat de > 50 moutons et des problèmes respiratoires au cours des cinq années antérieures.

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Maedi-visna (MV), also known as ovine progressive pleuropneumonia (OPP), is one of the major production-limiting diseases facing the sheep industry in Canada. The non-oncogenic, non-immunosuppressive virus belongs to the family *Retroviridae* and subfamily *Lentivirinae*. Animals usually become infected orally, through ingestion of colostrum or milk that contains virus, or via the respiratory tract from the inhalation of aerosolized virus (1). Most infections with MV virus are subclinical and clinical signs are most common in sheep over 4 y old (1). A slowly progressive disease, wasting and increasing respiratory distress are the main signs; coughing, bronchial exudate, depression, and fever usually only occur if there is secondary bacterial infection present (1). A noninflammatory, indurative mastitis is also a common clinical finding (1). Infected animals are likely to remain in a flock for some time, perpetuating the disease within the flock until they succumb to the disease or are culled.

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The first Canadian cases were reported in the early 1970's (2–4). Subsequent to that, MV has spread throughout Canada. A 1988 study in Ontario found 20.9% seroprevalance at the individual level and 69.9% at the flock-level (5). A national serosurvey for MV conducted during 1988 to 1989 found that the provinces of Quebec (39.9%) and Nova Scotia (26.5%) had significantly higher individual MV seroprevalence in sheep than the other provinces (6). In that study, 725 ewes selected from 15 Saskatchewan flocks had a seroprevalence of 3.1% and 7 of 15 flocks (46.7%) tested had at least 1 individual test positive. To the best of our knowledge, that was the only investigation of MV in Saskatchewan. The objective of this study was, therefore, to investigate the current prevalence of this disease in the Saskatchewan sheep population, and to evaluate factors associated with MV-positive flocks.

The research plan called for the simple random sampling of 75 flocks across Saskatchewan, with samples to be taken from 30 individual animals within each flock. The number of flocks and individuals to be sampled was decided upon primarily due to logistical and budgetary considerations. No attempt was made to differentiate between meat or dairy production. Samples were collected by private veterinarians, and the date, time, and location of sampling were arranged by the flock owner. All veterinary and laboratory costs were paid for through the study.

The sampling frame consisted of all flocks (n = 980) registered with the Saskatchewan Sheep Development Board as of December 2013. The list of registered producers was randomized using a random number generator, and producers were contacted by telephone starting at the top of the randomized list and continuing until the target number of voluntary participants was met. Initially, only flocks with 100 animals or more were targeted for inclusion; however, in order to recruit sufficient participants, this was modified to include any flock with at least

30 animals. A convenience sample of 30 animals (both rams and ewes) that were 2 y of age or older were chosen within each flock for testing. Blood was collected from each animal by jugular venipuncture using standard techniques.

A questionnaire was used to collect flock-level information including flock size and production characteristics, management practices, veterinary contact, and flock-level animal health syndromes observed in the last 5 y. Questionnaires were sent to each producer to be completed prior to the farm visit for blood collection or, in some cases, were completed during a follow-up phone call with the producer. Completed questionnaires were returned to the primary investigator and answers were entered into an Excel spreadsheet (Microsoft 2010; Microsoft Excel, Redmond, Washington).

Samples were submitted to Prairie Diagnostic Services (PDS) laboratory in Saskatoon, Saskatchewan. Serum antibody levels to MV were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (CAEV cELISA; VMRD, Pullman, Washington, USA). Antibodies in the serum sample compete with a monoclonal antibody for binding to viral antigen. The result is expressed as the percentage inhibition, and values $\geq$ 35% are considered positive. This test was validated in sheep infected with North American MV strains to have a sensitivity of 98.6% and specificity of 96.9% when used with sheep sera (7); however, recent work has shown that the VMRD ELISA has one of the poorest specificity values (80.9%) among commercially available ELISAs for MV (8). Therefore, all samples that tested positive for MV were sent to the Animal Health Laboratory (AHL), Guelph, Ontario, for verification of positive results via a different commercial ELISA (Elitest MVV/CAEV ELISA; HYPHEN BioMed, Paris, France). Samples that were "high negative" (below the cutoff value of 35.0% but above 25.0% inhibition), were also sent for verification.

To model the relationship between flock level predictor variables and flock MV sero-status, logistic regression analysis was implemented in Stata/IC 12.0 (StataCorp, College Station, Texas, USA). Analysis was done twice, once with flocks designated as MV positive if 1 or more animals in the flock tested positive for MV and once with flocks designated as MV positive if 2 or more animals tested positive. Multi-variable analysis was not undertaken because of the small sample size and because this was not the primary focus of the study, which was estimating disease prevalence and describing flock-level factors associated with MV.

Flocks were visited once between May 1, 2013 and May 31, 2014. Blood samples (n = 2041) were collected from 68 flocks across Saskatchewan. Completed surveys were returned by 65 participants. The average flock size was 200 adult animals [median 125; range: 36 to 1004 $\pm$ standard deviation (SD) 203]. Six farms were purebred operations only, 39 farms raised commercial stock only, and 19 farms raised both purebred and commercial animals; 4 farms did not specify.

Twenty-four flocks had at least 1 MV-positive test result [apparent prevalence (AP): 35.3%; 95% confidence interval (CI): 25.0% to 47.2%], while 14 flocks had 2 or more positive tests (AP: 20.6%; 95% CI: 12.7% to 31.6%). There were 93 positive samples in total (AP: 4.6%; 95% CI: 3.8% to 5.6%)

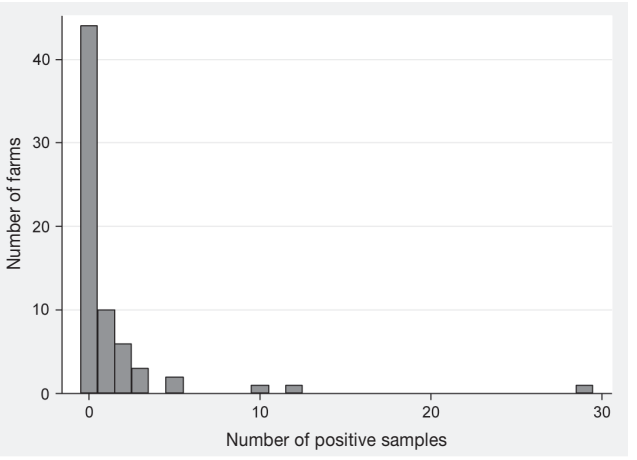

Figure 1. Within-flock distribution of Maedi-visna seropositive tests results in 68 sheep flocks in Saskatchewan.

and within-flock positive results ranged from 3.3% to 96.7% (1/30 to 29/30). The distribution of positive titers is shown in Figure 1. One hundred and two sera were sent to AHL for verification of MV test results. Of the 93 positive samples sent, 5 (5.4%) were test-negative by the Hyphen ELISA, while 4 (44.4%) of the 9 negative samples were test-positive by the Hyphen ELISA. When the Hyphen ELISA results were taken as the true results, this changed flock status for only 1 flock (from positive to negative), and then only when a cutpoint of 2 positive tests was used to define a positive flock.

Flock-level predictors of MV flock prevalence, at cutpoints of 1 or 2 positive tests per flock (based on the VMRD ELISA results), are reported in Table 1. As shown, when using a cutpoint of 1, large flock size (OR = 15.4; $P < 0.01$), was positively and significantly associated with positive MV flock status. Purchasing $>$ 50 sheep or reports of respiratory problems in the previous 5 y also tended to be associated with positive MV flock status (OR 2.9; P = 0.07 and OR 2.6; P = 0.08, respectively). At a cutpoint of 2, large flock size (OR 19.3; P = 0.01), purchasing $>$ 50 sheep (OR 7.2, $P < 0.01$) and reports of respiratory illness in the previous 5 y (OR 3.6; P = 0.04) were all positively and significantly associated with positive flock status.

The results of this study, with 4.6% and 35.3% individual and flock seroprevalence, respectively, are similar to those of the study undertaken across Canada in the late 1980's (6), which found that 3.2% of the Saskatchewan sheep sampled were test-positive and 46.7% of the Saskatchewan flocks sampled were test-positive when 1 positive test was used to define a positive flock. It is impossible to say whether a decline by 12% in flock prevalence is real or not, since considerable time has elapsed between studies, ELISA assays were not identical (indirect ELISA was used in the earlier study, *versus* competitive ELISA in the current study), and diagnostic techniques in general have evolved over time. Sampling strategies also differed, in that far fewer Saskatchewan flocks were included in the previous study and all animals in a flock were sampled (limited to 200 animals in flocks with $>$ 200 head), *versus* more flocks and fewer animals per flock samples in the current study.

Table 1. Flock level predictors of Maedi-visna seroprevalence in 68 Saskatchewan sheep flocks at cutpoints of 1 or 2 positive cELISA tests per flock, univariable logistic regression analysis

	Cutpoint 1 positive test			Cutpoint 2 positive tests		
Variable	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
> 50 sheep purchased in previous 5 years	2.9	0.91 to 9.3	0.07	7.2	1.9 to 26.3	0.00
Flock size *(≤ 70 reference category)*						
< 70	ref					
71 ≤ *125*	2.2	0.3 to 13.8	0.42	0.94	0.05 to 16.4	0.96
126 ≤ *240*	5.4	0.92 to 32.3	0.06	3.5	0.32 to 37.5	0.31
241 ≤ *1004*	15.4	2.5 to 95.0	0.00	19.3	2.0 to 183.4	0.01
Dewormed regularly	1.2	0.20 to 7.0	0.85	1.4	0.15 to 13.2	0.76
Vaccinated regularly	0.65	0.18 to 2.4	0.52	0.68	0.15 to 3.0	0.61
Cross-foster	0.84	0.30 to 2.3	0.73	1.01	0.31 to 3.3	0.99
Respiratory problems*	2.6	0.90 to 7.2	0.08	3.60	1.0 to 12.4	0.04
Unthriftiness*	1.9	0.62 to 5.5	0.27	1.5	0.42 to 5.2	0.55
Lameness*	1.1	0.37 to 3.4	0.84	0.65	0.16 to 2.7	0.56
Mammary problems*	1.4	0.51 to 4.0	0.49	1.0	0.31 to 3.3	0.99

**Problems observed in the flock within the previous 5 years.*
CI — confidence interval.

In this study, larger flock size was significantly associated with MV-positive flock status, which is consistent with findings from previous studies in Canada (8), Manitoba (9), and elsewhere (10). The positive and strong association of introduction of new animals in the last 5 years with flock level MV status observed in this study underlines the importance of this factor as a means of introduction into naive flocks, and is similar to findings of the 2008 study in Manitoba (10). It is likely that owners of larger flocks are also more likely to purchase > 50 sheep in the previous 5 y than are owners of smaller flocks; however, for reasons stated previously this was not evaluated in a multivariable analysis, nor was it examined in the Manitoba study.

Practices that may be indicative of better managed flocks, such as regular vaccination and regular deworming, may in theory be associated with a reduced risk of MV; however, there was no statistical association between the use of regular vaccination, regular deworming, or cross-fostering with flock status. A flock history of mammary problems, lameness/musculoskeletal problems or unthriftiness, syndromes often associated with MV disease, was also not found to be significantly associated with flock status. A flock history of respiratory disease, however, was found to be marginally associated ($P = 0.08$) with flock status when a cutpoint of 1 was used, and significantly associated ($P = 0.04$) when a cutpoint of 2 was used. This differs somewhat from the Manitoba study, which found that lameness/musculoskeletal syndrome was associated with flock status ($P = 0.04$) but respiratory syndrome was not ($P = 0.38$). The reason for the different findings is unknown, but it is clear that reliance on clinical signs alone is not sufficient to predict flock status for MV.

While more sensitive and specific ELISAs are available (11), there is a significant cost advantage to using the VMRD assay compared with some other commercially available assays. This, along with the fact that PDS offers the VMRD assay locally which simplified the submission process for veterinarians, made this test the logical choice for this project. Assuming a specificity of 96.9% (7), we can anticipate 3 false positives for every 100 truly MV-negative animals, and many more if a specificity of 80.9% is assumed. It is likely that some of the positive test results in this study were in fact false positives, and for this reason all positive samples were sent to Animal Health Laboratory in Guelph for verification with the more sensitive and more specific Hyphen ELISA for MV. Assuming the Hyphen test to be the accurate test, there were 5 false-positive and 4 false-negative results among samples subjected to both tests. Still, this information did not change the status of any flock, as determined by the VMRD ELISA, when a cutpoint of 1 positive test was used. When a cutpoint of 2 positive tests was used, the Hyphen test results caused a change in MV-status for just 1 flock, from positive to negative.

Test results for MV must always be interpreted with caution. One positive test in a flock of 100, for example, does not necessarily mean that MV truly exists in that animal or within the flock. The more positive tests there are, the more certain a producer can be that MV is present. Certainly, the producer with 29/30 positive tests can be very certain that MV is a serious problem in his or her flock. Because of the probability of false positive tests, we would recommend that at least 2 positive results be required to consider a flock MV-positive. Conversely, we cannot be absolutely certain either that MV is not present when all tests are negative, as false negative results can also be expected. Repeat testing is recommended as a means to ensure the continuing absence of MV from a flock.

Finally, 2/3 of flocks tested had no evidence of MV infection, indicating that the majority of Saskatchewan flocks are MV-free. To help keep the disease out of their flock, owners are encouraged to only purchase replacement stock of known MV-status and to isolate their sheep from other potentially infected sheep or goats. Operators of known infected flocks could consider eliminating the disease *via* ongoing test-and-cull, feeding colostrum/milk replacement products or heat-treated colostrum and pasteurized milk to replacement ewes, and following good biosecurity practices such as single-use needles and sterilizing other treatment equipment before use on other animals.

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