ARTICLE

Lack of evidence of *Brucella ovis* infection in rams in Quebec

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Abstract — A study was conducted to estimate the seroprevalence of *Brucella ovis* infection in rams in the Estrie and Bas-Saint-Laurent regions (Quebec). Rams sera (n = 258) were serologically evaluated from 224 rams in 30 commercial flocks and from 34 rams at 2 slaughterhouses by using an enzyme linked immunosorbent assay. Epididymides and testes were examined by palpation on farms and microscopically for culled rams. No ram was seropositive to *Brucella ovis* or had lesions suggestive of brucellosis from the farm or slaughterhouse surveys.

Résumé — Absence d'évidence de l'infection par *Brucella ovis* chez des béliers du Québec. Une étude a été réalisée afin de déterminer la séroprévalence de l'infection par *Brucella ovis* chez des béliers des régions de l'Estrie et du Bas-Saint-Laurent (Québec). Les sérums de 224 béliers répartis dans 30 troupeaux commerciaux et de 34 béliers de réforme échantillonnés dans deux abattoirs ont été soumis pour évaluation sérologique par un test ELISA. Les épididymes et les testicules des béliers ont été examinés par palpation en ferme et par examen histologique pour ceux prélevés à l'abattoir. Aucun bélier n'était séropositif à *Brucella ovis* ou ne présentait de lésions suggestives de cette infection autant dans les élevages que dans les abattoirs.

(Traduit par les auteurs)

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Introduction

Brucella ovis is a specific cause of epididymitis in rams (1). Although it has mainly been associated with reduced fertility in rams, infection in ewes can result in failure to conceive, embryonic resorption, abortion, stillbirth, and weak newborn lambs (1). Pathological changes caused by *B. ovis* are generally confined to the epididymides and accessory sex glands (1–3). Diagnosis of infection in flocks is based on scrotal palpation, semen culture, serological testing, or all 3 (1,3–5).

In Canada, by using serologic testing and semen culture, it was estimated that 8.6% of flocks in Alberta were infected (4). Infection was also diagnosed in rams in a commercial sheep flock in southern Ontario in 1984, resulting in a test and cull policy for *B. ovis* being implemented in this flock (2). In the Bas-St-Laurent region of Quebec, the disease was diagnosed in 1986 in rams imported from New Zealand; infected rams from this flock were culled (6). To our knowledge, no study on the prevalence of *B. ovis* infection has been conducted in eastern Canada.

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This study was performed to estimate the seroprevalence of *B. ovis* infection in mature rams in commercial sheep flocks and at slaughterhouses in the Bas-St-Laurent and Estrie regions of Quebec. Ram serostatus was evaluated by using an enzyme linked immunosorbent assay (ELISA) selected on the basis of its commercial availability and validation in North America. In addition, testes and epididymides of selected rams in flocks were palpated to detect any induration, and testes and epididymides of culled rams were examined microscopically.

Materials and methods

Flock survey

This survey was part of a broader research project conducted in 2 regions of Quebec. Flocks with at least 60 ewes assumed to be pregnant in November 1999 were eligible to participate. Volunteer producers whose flock satisfied this criterion were enrolled until 10 flocks in the Estrie and 20 in the Bas-St-Laurent region had been obtained. Selected flocks ranged in size from 95 to 1707 (mean 408) reproductive ewes. The serological status of the flocks for *B. ovis* was unknown and no vaccine against this bacterium had ever been used in those flocks.

All mature rams to a maximum of 10 per flock were selected. In large-sized flocks, a method of random sampling, stratified for breed and age was used. The testes and epididymides of each ram were palpated, and a blood sample was collected by jugular venipuncture. Producers were asked if the testes of rams were routinely palpated before the mating season, on introduction of a new ram to the flock, or both, for the period from January 1999 to January 2000.

Slaughterhouse survey

From January to November 2000, inclusively, culled rams, to a maximum of 5 per slaughterhouse, were selected once a week for 44 wk in the Estrie region and for 30 wk in the Bas-St-Laurent region. If needed, a systematic random sampling method was used for selection. In the Bas-St-Laurent, all the rams selected were from farms of the region; in Estrie, this information was not available. A blood sample was taken from the selected rams during the exsanguination procedure. Age of rams was estimated by examining the incisor teeth. The testes and epididymides from all selected rams were removed, kept on ice, and transported to the local diagnostic laboratory.

Within 24 h of collection at the slaughterhouse, the testes and epididymides were fixed in 10% neutral-buffered formalin. Complete processing (embedding in paraffin, cutting at 5 μm, and staining with hematoxylin, phloxine, eosin, and saffron) of a transverse section of the tail of both epididymides and testes followed. Microscopic examination was performed by the same pathologist (C.G.) without any knowledge of the serologic results. Epididymes or testes with any detected lesions were stained by the immunoperoxidase technique, using *B. ovis* or *B. abortus* RB 51 polyclonal antisera diluted 1: 500. Positive and negative controls were also included (Dr. Steven C. Olsen, National Animal Disease Center, Ames, Iowa).

Serologic testing

All blood samples were kept on ice and then centrifuged within 24 h of collection. Serum was removed and kept frozen at -70°C. All selected rams were tested for antibodies to *B. ovis* by using an ELISA based on an autoclave-extracted soluble antigen (7). Serologic testing was performed at the California Animal Health and Food Safety Laboratory in Davis, California, USA. The test has a sensitivity and specificity of 98% and 92%, respectively (Sharon Hietala, personal communication, 2003). The test validation was based on sheep samples from California, Texas, Montana, and Colorado. The quality controls for the assay included a negative, a weakly positive, and a strongly positive sample obtained from the National Veterinary Services Laboratory in Ames, Iowa, USA. Those samples were tested on every plate.

Results

A total of 258 rams were sampled: 224 from within flocks and 34 (21 in the Bas-St-Laurent and 13 in Estrie) from slaughterhouses. The low number of rams selected from the slaughterhouses was due to the absence of rams among culled sheep for many of the selection weeks. The age distribution of all selected rams was as follow: 1-year-old, 36% (n = 93); 2- or 3-years-old, 34.5% (n = 89); and ≥ 4-years-old, 29.5% (n = 76).

Rams selected within flocks were of many different breeds, mostly Polypay (n = 45), Suffolk (n = 43), Dorset (n = 36), Hampshire (n = 28), Romanov (n = 24), and Arcott Canadian (n = 14). Testes were routinely palpated on newly introduced rams in 25/30 of the flocks studied. This examination was performed either by producers themselves (n = 21) or by a veterinarian (n = 4). Testes were also palpated before mating season in 18/30 flocks by the producers and in 1 flock by a veterinarian.

Antibody against *B. ovis* was not detected in the serum from any ram, although 3 rams selected from within flocks had serological reactions that were outside the normal range for negative animals but not sufficiently to be classified as positive. A 2nd blood sample was taken from these rams in May 2000, approximately 6 mo following the 1st sample, and found to be seronegative. No induration was found during on-farm palpation of testis and epididymides and no lesion was found in the testes or epididymides of the 34 culled rams, with the exception of 1 ram with a spermatic granuloma limited to 1 testis. The immunoperoxidase test was done on the granuloma and found to be negative for *B. ovis*.

Discussion

According to a census performed in the first 6 mo of the year 2000, 42% of the total sheep population in Quebec was located in the Estrie and Bas-St-Laurent regions (8). Considering the sensitivity (98%) of the ELISA used and the absence of lesion suggestive of brucellosis, *B. ovis* infection was likely absent from rams selected for our study.

In a total random selection of the 258 seronegative rams, the maximal possible seroprevalence would be of 1% at a confidence level of 95%, if 1900 is used as a calculated estimate of the total number of adult rams within these regions (8,9, WinEpiscope 2.0 [CLIVE; Royal Dick School of Veterinary Studies, Edinburgh, Scotland]). However, since a convenience sample of flocks was used, the seroprevalence estimate might be biased, since producers were enrolled on a voluntary basis, which might be influenced by the disease history of their flock. Furthermore, husbandry practices in many flocks included the palpation of testes of new rams before their introduction and, often, also before mating season. Since these husbandry practices should reduce the risk of a flock being infected, they may have led to the seroprevalence being underestimated if they were different from the practices of other flocks within the same regions. To our knowledge, the flocks selected were not different from others in Quebec; breeds of sheep, number of years in production, flock size, lamb preweaning mortality percentage, and lambing rate were various and representative of ovine production in Quebec. Furthermore, these same flocks were selected initially to conduct a within-farm study on the seroprevalence of antibodies to mædi-visna virus; all producers but one were unaware of their flock's serostatus, and estimated flock seroprevalence showed a large range. Also, none of the selected flocks were closed flocks, suggesting that if infection with *B. ovis* was present in the areas, it was not widely distributed. In order to palliate the weakness of not using a true random sample of flocks, a random sample of culled rams selected at slaughterhouses over an 11-month period was also used. Since rams infected by *B. ovis* would be more likely to be culled than others, *B. ovis* infection would probably have been detected if it were present in those areas. In Estrie, it was not possible to confirm that culled rams were from that region.

Considering that the specificity of the ELISA used is not 100%, some false-positive results could be expected

in the absence of the infection. Since the ELISA used was validated in the western United States, it is possible that different pathogens, antigens, or both, may have stimulated a false positive response; this includes the use of footrot vaccine (Dr. Sharon Hietala, personal communication). This vaccine was not used within sampled flocks, according to producers.

Brucella ovis infection in sheep flocks has been associated with a reduction in the lambing percentage up to 30% in newly exposed flocks, and 15% to 20% in flocks in which it is endemic (1). Thus, Quebec sheep producers should be careful to maintain the absence, or the very low level, of infection indicated by this study. Clinical examination of rams to determine the presence of epididymitis caused by *B. ovis* is of limited value for the diagnosis of infection, because of the existence of infected rams without clinical signs (5,10). Semen culture can also be used to detect infected rams, but this method is not practical for use in control programs, and infected rams may show intermittent or no excretion of the bacteria (1,5). Thus, producers should buy replacement ewes and, more specifically, rams only from flocks known to be free of this infection, or they should introduce newly purchased rams into their flocks only if they are shown to be serologically negative after a quarantine of 60 d. CVJ

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