Ovine Brucellosis in Alberta

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ABSTRACT

Two parallel surveys of rams from Alberta sheep flocks were conducted to determine the presence of infection with *Brucella ovis*. In a retrospective study over a period of 24 months, using complement fixation test, 12 flocks out of 142 tested were considered infected. In another 17-month survey of slaughter rams by serology and culture methods 11 flocks out of 124 were found to be infected. The overall prevalence of ovine brucellosis was 8.6% of the flocks tested which represented 12.5% of the estimated sheep flocks in Alberta. Up to 67% of rams in infected flocks reacted to complement fixation test.

The complement fixation test was evaluated for its efficiency in the diagnosis of ovine brucellosis and compared with a limited number of an enzyme-linked immunosorbent assay (ELISA) results and clinical criteria. The complement fixation test as well as ELISA identified all culture positive rams. Both serological tests appeared satisfactory for the diagnosis of *B. ovis* epididymitis when the results could be interpreted in the light of flock history and clinical findings.

Key words: *Brucella ovis*, ram epididymitis, sheep diseases.

RÉSUMÉ

Brucellose ovine en Alberta

Cet article présente les résultats de deux relevés parallèles qui portaient sur les béliers de troupeaux de moutons de l'Alberta et qui visaient à déterminer la présence de l'infection par *Brucella ovis*. Une étude rétrospective qui s'étalait sur une période de 24 mois et dans laquelle on utilisa l'épreuve de la déviation du complément, permit de considérer 12 troupeaux comme infectés, sur un total de 142. Un autre relevé qui s'étalait sur une période de 17 mois et qui consistait à effectuer la sérologie et la bactériologie, à partir de tissus de béliers envoyés à l'abattoir, permit de diagnostiquer la brucellose dans 11 des 124 troupeaux impliqués. On détecta la maladie dans 8,6% des troupeaux éprouvés à cette fin, lesquels représentent environ 12,5% de tous les troupeaux de l'Alberta. Jusqu'à 67% des béliers des troupeaux infectés réagirent à l'épreuve de la déviation du complément. On en évalua l'efficacité pour le diagnostic de la brucellose ovine en comparant ses résultats avec un nombre limité de ceux de l'épreuve immunoenzymatique ELISA et de critères cliniques. Les deux épreuves précitées permirent d'identifier tous les béliers desquels on isola *B. ovis* et elles semblèrent satisfaisantes pour le diagnostic de l'épididymite brucellique, quand on pouvait interpréter leurs résultats à la lumière de l'histoire du troupeau et des observations cliniques.

Mots clés : *Brucella ovis*, épididymite des béliers, maladies ovines.

INTRODUCTION

Ovine brucellosis is an insidious and chronic infectious disease of sheep, caused by *Brucella ovis* and characterized by various degrees of epididymitis in rams, leading to reduced reproductive performance or infertility. Serological testing of rams from Albertan sheep flocks for the presence of antibody to *B. ovis* in 1981-1983 uncovered 23 flocks containing rams with positive reactions in the complement fixation test (CFT) (1). Ovine brucellosis was confirmed in six of these flocks by cultural isolation of *B. ovis* from testes of the affected animals. The remainder of the flocks were assumed to be infected on the basis of a large proportion of high-titer CFT reactions in the flocks and poor reproductive performance. However, this information was fragmentary.

In order to determine the prevalence of ovine brucellosis in Alberta and try to assess the efficacy of current diagnostic methods of this disease the following study, composed of two parallel surveys, was undertaken. It involved analysis of serological results of diagnostic testing over a two-year period, coupled with available clinical data and bacteriological examination of testes from rams shipped for slaughter.

MATERIALS AND METHODS

Survey I

This retrospective investigation was based on blood samples from rams submitted by veterinary practitioners to Animal Diseases Research Institute, Lethbridge (ADRI) for diagnostic or sale purposes and it covered a 24-month period from June 1983 to May 1985. Identification and description of animals, flocks, clinical observations, and other pertinent information, together with the test results for *B. ovis* antibody, were recorded.

Survey II

This involved mature rams shipped by farmers for slaughter to Lambco (a Division of Alberta Agricultural Development Corp.) in Innisfail. The

testes and epididymides of these animals were collected, placed in separate plastic containers and frozen for submission to the Edmonton Diagnostic Laboratory periodically in batches. Also blood samples were collected, the serum separated, frozen and forwarded to ADRI for serological testing which was carried out independently without any prior knowledge of the bacteriological results. This survey covered a 17-month period from September 1983 to January 1985. Sampling data were correlated as to the origin of the animals to avoid duplication between the two surveys.

Laboratory Examination of Testes

The submitted paired organs were thawed out and examined for the presence of gross abnormalities such as malformation, fibrosis, abscessation and swelling. Using aseptic methods, portions of both epididymides were cultured on blood agar plate (BAP) and McConkey's agar and incubated at 35°C for 48 h. Both epididymides were cultured also on BAP and chocolate agar and incubated for five days at 35°C in a 5% atmosphere of CO_2. Suspicious colonies were examined using a Gram's smear. Small Gram-negative coccobacilli were further identified by oxidase, catalase and urease tests. All isolates were negative for these tests, required increased CO_2 and conformed to other growth inhibition tests consistent with known characteristics of *B. ovis* (2).

Serology

A standard direct cold CFT (tube method, 18 h fixation at 4°C) was used for detection of antibody to *B. ovis*. The sera were tested in 1:5, 1:10, 1:25 and 1:50 dilutions with serum controls in 1:5 and 1:10 dilutions. Antigen was an autoclaved aqueous extract of *B. ovis* strain 3572, preserved with 0.5% phenol, prepared at ADRI, Nepean, Ontario. A positive reference serum was raised in a ram with killed *B. ovis* strain 3572 and a negative control serum originated from known uninfected rams. The test was based on three 50% hemolytic units of guinea pig complement and the reactions read against color standards which were determined spectrophotometrically at 5% intervals ranging from 0% to 100% hemolysis. Hemolysis of 50% or less in a given serum dilution was considered significant, indicating at least 50% of the complement was fixed. For interpretation, reciprocal titers of 5 and 10 were considered "suspect" while those of 25 and 50 were designated as "positive". Occasionally, samples which gave unclear titers or showed evidence of prozone reaction were tested in extended doubling dilutions of up to 1:400. The principles of CFT and its technology have been described elsewhere (3).

An enzyme-linked immunosorbent assay (ELISA) was employed, with the CFT, to test the samples in survey II. This test was similar to the method used for another antigen-antibody system (4), except that the antigen which was the same preparation as used for the CFT, and peroxidase-conjugated rabbit anti-ovine IgG, were diluted to obtain optimum activities, and 1.0% heat inactivated horse serum was substituted for 0.1% egg albumen. The tests were performed in triplicate plates in 1:50 and 1:100 dilutions of sera. An average signal/noise ratio at A492 densitometry, which was calculated by dividing the A492 value of test samples by the average A492 of reference negative sera, was obtained from the triplicate test and used for interpretation. Thus, any sample giving an average signal/noise ratio of 2.00 or greater was considered "positive" at that dilution. Samples with the ratio of less than 1.50 were termed "negative" whereas those yielding intermediate values were classed as "suspect".

TABLE I
RESULTS OF COMPLEMENT FIXATION TEST (CFT) FOR *B. OVIS* FROM ALBERTAN RAMS (SURVEY I)

Flocks		
No. tested	142	
No. positive	12	(8.4%)
No. suspect	31	(22%)
Samples		
No. tested	2,343	
No. positive	129	
No. suspect	312	
No. negative	1,873	
No. anticomplementary	29	

RESULTS

Survey I

A total of 2,343 sera of rams from 142 Albertan sheep flocks were tested for *B. ovis* antibody by the CFT during the 24-month period. This figure includes some retests of suspects, but excludes spoiled samples, samples from outside Alberta, vaccinated animals, ewes, submissions with insufficient information, and those covered by survey II. The results are given in Table I.

From the above total, 129 rams from 12 flocks gave positive reactions to CFT (8.4%). In addition, there were 312 animals in the suspect category which included rams from the positive flocks as well as those from 31 flocks containing suspect and negative animals only. Sample numbers from the positive flocks ranged from four to 31, with the smallest number yielding one positive reactor. Eight such flocks contained more than ten CFT positive rams. These flocks are listed in

TABLE II
PROPORTION OF REACTORS IN EIGHT CFT POSITIVE FLOCKS OF MORE THAN TEN RAMS WHEN FIRST TESTED (SURVEY I)

Flock No.	No. Tested	No. +	No. ±	No. -
1	12	2	8	2
2	27	10	7	10
3	29	17	6	6
4	22	6	12	4
5	15	7	4	4
6	31	7	6	18
7	30	18	9	3
8	21	5	2	14
Total	187	72 (38%)	54 (29%)	61 (33%)

+ = Positive
± = Suspect
- = Negative

TABLE III
ASSOCIATION OF PALPATED TESTICULAR LESIONS WITH CFT REACTIONS IN 164 RAMS FROM CFT POSITIVE FLOCKS (SURVEY I)

CFT	No. Tested	No. with Lesions
+	23	15 (65%)
±	45	14 (31%)
-	96	9 (9%)

+ = Positive
± = Suspect
- = Negative

TABLE V
COMPARISON OF CFT AND ELISA REACTIONS WITH CULTURAL RESULTS IN 41 RAMS FROM SIX INFECTED FLOCKS (SURVEY II)

Test Group		*B. ovis* Culture (No).		
CFT	ELISA	+	-	Total
+	+	16	4	20
+	-	0	0	0
±	+	2	3	5
±	-	0	8	8
-	+	0	0	0
-	-	0	6	6
AC	+	0	1	1
AC	-	0	1	1
Total		18	23	41

+ = Positive
± = Suspect
- = Negative
AC = Anticomplementary

Table II to illustrate the degree of flock infection when first tested for diagnosis by the CFT method.

Scrotal palpation for testicular abnormalities was carried out by several veterinarians. Table III lists the palpation findings in 164 rams originating from flocks with any number of CFT positive reactors. The occurrence of lesions is tabulated for correlation with CFT reactions in individual animals.

Survey II

During the 17-month period which coincided with survey I, a total of 319 rams from 132 flocks were tested. Of this total, 18 rams from six flocks originated from Saskatchewan and six rams from two flocks came from British Columbia. The number of rams sampled per flock ranged from one to 11, with a mode of 2. Table IV summarizes overall test results on the basis of the three criteria used: testicular or epididymidal lesions, *B. ovis* culture, and CFT reactions. *Brucella ovis* was isolated from 19 of 43 rams which originated from seven flocks. These flocks were termed infected. The samples from one of these infected flocks, represented by two Albertan rams, could not be individually identified and were therefore deleted from subsequent serological comparisons, leaving six flocks for this purpose. These six flocks include one from British Columbia with five rams tested, two of which were both culture and CFT positive, two CFT positive and one CFT suspect. The remaining out of the province animals were negative to all examinations. Table V compares the CFT results with those of ELISA in the six infected flocks in respect to cultural confirmation of *B. ovis* infection.

In addition to the six culturally confirmed infected flocks, there were five rams out of five flocks (one to five rams in each sampled) that gave positive CFT results, but were negative on culture. These were considered also infected on basis of serological results.

The rams originating from outside Alberta were included in Tables IV and V for purposes of test comparisons. For estimation of prevalence of *B. ovis* epididymitis, only the Albertan sheep flocks were considered, of which there were six flocks culture-positive and five CFT positive, totalling 11 flocks presumed infected out of 124 tested (8.9%).

DISCUSSION

The prevalence rate of infected flocks in survey I was 8.4% and that of survey II was 8.9%, yielding an overall prevalence of 8.6% of 266 Albertan flocks tested. This infection rate appears very similar in both surveys considering the differences in sample

TABLE IV
SEROLOGICAL AND TESTICULAR EXAMINATION RESULTS ON 319 RAMS FROM 132 FLOCKS (SURVEY II)

Testes		CFT (No.)				
Lesions	*B. ovis* Culture	+	±	-	AC	Total
+	+	3	1	0	0	4
-	+	13	1	0	1[a]	15
+	-	1	6	8	2	17
-	-	8	32	220	23[a]	283
Total		25	40	228	26	319

+ = Positive
± = Suspect
- = Negative
AC = Anticomplementary
[a]Identity of two samples in one flock not confirmed

selection as a larger number of animals per flock were selected in survey I than in survey II. The results also indicate that attempting to determine the prevalence of this disease on the basis of total sheep population rather than flocks or farms would be misleading. Statistics Canada data (Agriculture Statistics Division, Ottawa, Ontario), based on 1981 Agricultural Census, refer to 2,121 farms in Alberta reporting sheep one year and older on the premises. This figure is not expected to be substantially different at present although the total number of sheep of this age in January 1985 was estimated to be 92% (90,300) of the 1981 figure (98,044). Assuming the above figures valid, the 266 flocks tested would then represent 12.5% of Albertan sheep flocks. In a cursory examination of the farm locations of sample origin in both surveys, but especially in survey II, the flock numbers appear approximately proportionally distributed among the estimated sheep populations within the 15 Census Divisions of the province. This indicates a reasonable geographical coverage of the Albertan sheep population.

The true prevalence of infection is difficult to estimate in this case due to a number of uncertainties in the collected data. Some of the suspect CFT reactors in limited numbers of samples may represent infected animals, and some lightly infected flocks without major clinical problems may have escaped detection, thereby increasing the demonstrated 8.6% prevalence rate. On the other hand, flock selection in survey I was somewhat biased toward infected flocks beause of obvious breeding problems as a motive for testing; this would consequently yield an over-estimate of the disease prevalence. Any bias in survey II could not be ascertained, but marketing practices may have an influence. Prevalence rates also change with time as farmers eradicate infection from their flocks, or new flocks may become infected, but the trend presently appears to be toward the former and this would diminish the prevalence.

Although a few rams from Saskatchewan and British Columbia found their way into survey II, the prevalence of *B. ovis* infection in these provinces is not known as this study was the first such investigation conducted in Canada. In the neighboring United States, however, a high prevalence of this infection in commercial sheep flocks of Idaho has been noted (5).

The CFT is widely used for serological diagnosis of ovine brucellosis and it has been the main diagnostic tool in the eradication of this disease from Australia and New Zealand. There are many versions of this test, based on different methods of antigen preparation, time and temperature of complement fixation, tube or microtiter techniques, and serum dilution ranges. Each version has given different results in the hands of different investigators. In general, the cold fixation technique, as used in this survey, has been considered more sensitive than the warm method (6,7) although the latter had been used as a standard test in Australia (8,9). Various investigators have reported the sensitivity of CFT (freedom from false negatives) ranging from 96.3% to 100% and its specificity (freedom from false positives) as 98.4% to 99.9% (6-11). Our CFT in survey II identified all culture positive animals, but due to a small number of tests compared, its sensitivity percentage would not be valid for a statistical inference. Similarly, false positives (specificity) could not be calculated due to lack of reliable references.

Burgess and Norris (7), recommended that a ram with a CFT titer (cold fixation) of 16 or more be regarded as being infected while a titer of eight would be suggestive of infection and presumably infected if found in an infected flock. Searson (9), using an extended warm fixation method, considered a titer of eight or greater as a satisfactory indication of *B. ovis* infection in conjunction with clinical examination and flock history. The importance of considering flock history in the interpretation of serological titers is also stressed by other investigators based on extensive practical experience in major sheep raising countries (12,13). In the present investigation the "suspect" titers of five and ten are considered nonspecific reactions if they occur in small numbers in otherwise healthy flocks without accompanying positive reactions, clinical lesions or other flock history suggestive of infection or vaccination. As the data were compiled there was no practical way to ascertain the diagnoses of the reported suspect animals as there was very little feedback from the field. Animals with suspect titers in a flock containing CFT positive rams, however, are presumed infected. Usually these titers occur in a large proportion of an infected flock.

Demonstration of *B. ovis* on culture is the most specific method of diagnosis and has been used by most investigators in the form of semen culture as a reference against which serological and other diagnostic methods have been evaluated. Occasionally, this method may also fail to detect some infected animals (8). In survey II there were four CFT positive rams in the culturally confirmed infected flocks that did not yield *B. ovis* on culture. In absence of supportive evidence, these could be interpreted as either false positive CFT reactions or false negative culture results. However, since the animals originated from an infected flock which is supportive evidence, it is reasonable to accept the CFT results for a positive diagnosis, including 11 CFT suspects in this group.

Scrotal palpation of rams as a means of detecting clinical epididymitis has been practiced with apparent success before laboratory tests for *B. ovis* became generally available (14). The procedure is simple, rapid, and economical and has often been considered as a standard procedure along with currently available serological tests (12,13,15,16) although its reliability in individual rams has been refuted (8). The palpation results in survey I showed a much greater proportion of abnormalities associated with CFT positive or suspect rams than with CFT negative animals (65% and 31%, respectively, vs. 9%). In survey II, correlation of testicular lesions with positive serological or cultural results was not evident. This difference of findings between the two surveys can be explained on the basis of sample selection as discussed before. Nevertheless, palpation for lesions appears to be an additional, practical diagnostic tool. An advantage of this examination is that it also

detects clinical epididymitis caused by other microorganisms such as *Actinobacillus seminis* and *Histophilus ovis* (16) which are not readily detected by laboratory tests.

In recent years, several investigators have adapted ELISA for the detection of *B. ovis* antibody and compared its efficacy with that of CFT (11,17-19). In general, the reports seem to imply that the sensitivity and specificity of the ELISA and CFT are not significantly different, but that ELISA has some advantages, such as technical automation, standardization for quantitative antibody measurements, somewhat increased sensitivity, and capacity to give clear-cut results with anticomplementary and hemolyzed sera. In survey II, two samples could be tested by ELISA which were anticomplementary and could not be diagnosed in the CFT system. The efficacy of ELISA could not be further evaluated in the small number of samples tested.

In conclusion, the investigation on Albertan sheep flocks confirms the prevalence of *B. ovis* epididymitis to a considerable degree and indicates diagnostic problems not unlike those encountered in other parts of the world where this disease exists. For practical considerations, the following recommendations may be made: First, no diagnostic method is absolutely reliable for detecting *B. ovis* infection in all cases, but the serological tests have a relatively high degree of accuracy and practicality in routine testing.

Secondly, the test results on single rams by any method have relatively little meaning. The entire ram flock should be tested to establish whether or not the infection exists in the flock.

Thirdly, a combination of findings should be considered in arriving at a diagnosis. These are: serological test results, palpation results, flock history and, possibly, semen examination. The greater the number of positive indicators, the firmer the diagnosis for an infected flock. Conversely, all negative findings would confirm a noninfected flock.

Fourthly, a few low-titer (suspect) serological reactions in an otherwise negative flock, without unfavourable history and clinical findings could be nonspecific and may be ignored or the rams retested. Rams with suspect titers in an infected flock should be presumed infected. Eradication of the disease from an infected flock can then be accomplished by retesting and culling of infected rams.

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