ABSTRACT
A survey of 930 ovine sera and kidneys from 33 sheep was conducted to assess the rate of leptospiral infection in sheep slaughtered in Alberta. Sera were tested for the presence of agglutinins to indigenous serovars of *Leptospira interrogans*. Kidneys with gross lesions were examined for the presence of leptospires by means of an indirect fluorescent antibody test (FAT) and by culture. Antibodies to serovars *pomona* and *hardjo* were present at rates of 1.0% and 0.4%, respectively, in sheep from Saskatchewan, Alberta and British Columbia. Sera from 120 feedlot lambs shipped from Oregon reacted to serovars *pomona*, *hardjo* and *grippotyphosa* at rates of 1.7%, 61.7% and 59.1%, respectively. Fluorescent antibody test detected serovars (presumptively) *hardjo* in 52% of Oregon feedlot lambs and *grippotyphosa* in 32% of the same group, a finding supported by the isolation of both of these serovars from a pool of two fluorescent antibody test-positive kidneys. The *grippotyphosa* strain was highly virulent for hamsters, producing intense icterus and death. Leptospires, presumptively serovar *grippotyphosa* were demonstrated by fluorescent antibody test in one Alberta lamb kidney. The possibility of spreading leptospirosis by movement of breeding stock through public facilities and by assembling lambs in feedlots is discussed.

Key words: Leptospirosis, sheep, Saskatchewan, Alberta, British Columbia, Oregon, *hardjo*, *grippotyphosa*, *pomona*.

RéSUMÉ
Leptospirose chez les moutons de l'Ouest canadien
Cette étude visait à déterminer la fréquence de la leptospirose, chez les moutons abattus en Alberta. L'auteur rechercha à cette fin la présence d'agglutinines à l'endroit de sérotypes indigènes de *Leptospira interrogans*, dans 930 échantillons de sérum; elle rechercha aussi des lésions macroscopiques dans les reins de 33 moutons. Elle tenta ensuite de démontrer des leptospires dans ceux qui présentaient de telles lésions, au moyen de l'épreuve de l'immunofluorescence indirecte et par la culture. Les moutons de la Saskatchewan, de l'Alberta et de la Colombie-Britannique possédaient des anticorps sériques contre les sérotypes *pomona* et *hardjo*, dans les proportions respectives de 1% et 0.4%. Le sérum de 120 agneaux en provenance de parcs d'engraissement de l'Oregon réagit de façon positive aux sérotypes *pomona*, *hardjo* et *grippotyphosa*, dans les proportions respectives de 1.7%, 61.7% et 59.1%. L'épreuve de l'immunofluorescence indirecte permet de détecter des anticorps, présumément contre le sérotype *hardjo*, chez 52% des agneaux de l'Oregon, ainsi que contre le sérotype *grippotyphosa*, chez 32% de ces agneaux, constatation confirmée par l'isolement des deux sérotypes précités à partir d'un échantillon prélevé aux deux reins qui avaient réagi de façon positive à l'épreuve d'immunofluorescence indirecte. La souche du sérotype *grippotyphosa* s'avéra très virulente pour les hamsters chez lesquels elle entraîna un ictere marqué et la mort. L'immunofluorescence indirecte permet de démontrer des leptospires, présumément du sérotype *grippotyphosa*, dans les reins d'un agneau de l'Alberta. L'auteur commente la possibilité de disséminer la leptospirose par le transport de moutons reproducteurs dans des camions ou des wagons et par le rassemblement d'agneaux dans des parcs d'engraissement.

Mots clés: leptospirose, moutons, Saskatchewan, Alberta, Colombie-Britannique, Oregon, *Leptospira interrogans* var. *hardjo*, *pomona*, *grippotyphosa*.
Materials and Methods

Specimens

Blood samples were collected at slaughter from culled rams and ewes from Saskatchewan, Alberta and British Columbia, from Oregon feedlot lambs, and during routine monitoring of a university flock. Kidneys with grossly visible lesions at slaughter were collected for study after official veterinary inspection. Initially, a series of 17 kidneys, including three pairs, were chilled and shipped to arrive within 30 hours at the laboratory. There, sections were fixed in formalin for histology, impression smears were prepared for the fluorescent antibody test (FAT) and specimens were taken for culture. Finally, 19 single kidneys were processed variously for histology and FAT and 12 were cultured at the abattoir. The choice of diagnostic procedures depended on the condition of the specimens and the nature of the lesions.

Fluorescent Antibody Test

The procedure adopted for FAT was based on the method described by Ellis (15). Impression smears were prepared by pressing freshly cut surfaces of representative renal lesions onto microscope slides coated with 0.1% aqueous gelatin. Each slide received two impressions, and slides were prepared in triplicate for FAT with three serovars. Smears were dried in air, fixed for ten minutes in cold acetone, and stored at -20°C.

The FAT was performed by the indirect method. Slides were flooded with rabbit antisera to pomona, hardjo or grippotyphosa (titer 1/10,000), diluted 1/200. After a reaction period of 30 minutes at room temperature in a moist chamber, the slides were twice washed in phosphate buffer at pH 4.0 for 20 min. Commercial antisera to rabbit globulin conjugated with fluorescein isothiocyanate (BBL, Division of Becton, Dickinson, Mississauga, Ontario) was then applied in a dilution of 1/25 to cover the smears. Slides were protected from light during the rest of the processing. Incubation and washing, as described above, were followed by counterstaining with eriochrome black for ten seconds at room temperature, followed by washing. Smears were mounted in one drop of glycerol buffered at pH 8.0 under a cover slip.

The FAT was read under a Leitz (Wild Leitz Canada Ltd., Calgary, Alberta) incident blue/violet fluorescence lighting system. The light source was a high pressure mercury vapor lamp, HBO 50W. Three wave length options were provided in a Ploem Opak filter system, all including blue excitation (band pass filter BP 450-490 or -500), dichroic mirror reflection short pass filter RKP 510, but a choice of suppression filters LP 515, BP 525/20 µ, and BP 515-560. Magnifications of 625X and 1250X were obtained with oil immersion objectives NPL Fluoart 50X, N.A. 1.00 and NPL 100X, N.A. 1.32, respectively, a tube factor of 1.25 and Periplan GW 10X coated oculars. Smears containing fluorescing structures resembling leptospires were considered to be positive. Specificity of staining was controlled by the performance of FAT on reference cultures and on tissues infected with typed isolates in parallel with field specimens.

Histology

Representative lesions were trimmed, mounted, sectioned and stained with hematoxylin and eosin for microscopic examination.

Culture

Selected blocks of kidney tissue were surface-sterilized by immersion in methanol followed by ignition. Small pieces were then excised from beneath the burnt surface aseptically, and macerated by extrusion through a syringe into 1% bovine serum albumin in phosphate buffer (TM) (16). The 10% suspension which was thus prepared was held at room temperature for 20 to 30 min, shaken briefly without foaming and diluted to 1/100 and 1/1000 in TM. One mL aliquots were transferred from these two dilutions to two albumin-tween-80 media, namely Ellinghausen-McCullough semi-solid (EM) (17) and SPL 5X Leptospira Medium (Scientific Protein Laboratories, Waunakee, Wisconsin), with 0.15% Difco flake agar added. The selective inhibitor 5-fluorouracil (5FU) was added to both media at a final concentration of 200 μg per mL to suppress contamination (18). Cultures were incubated at 29°C and examined weekly for growth.

Positive cultures were transferred to fresh media with and without 5FU and inoculated into hamsters to obtain pure cultures. When results of FAT indicated the likelihood of mixed infection, cross-protection of hamsters was used to separate the leptospiral populations, as follows. Two hamsters were inoculated with 0.2 mL of hardjo antiserum, titer 1/10,000, intraperitoneally, followed one hour later by 0.5 mL of culture. Two more hamsters were similarly protected against grippotyphosa and exposed to the culture. Two unprotected hamsters also were exposed to the culture. Hamsters were monitored for signs of disease and when moribund they were bled and killed. Gross pathology was noted.

Blood, liver, kidney and brain were cultured as described above, except that surface sterilization was omitted and one to two drops of blood were inoculated directly on semi-solid media. Hamster sera were tested for antibodies to hardjo, grippotyphosa and pomona and impression smears of tissues were examined by FAT.

Isolates were adapted to liquid medium and tested for agglutination by antisera to reference antigens pomona, hardjo, grippotyphosa, canicola, icterohaemorrhagiae, australis, autumnalis, ballum and tarassovi. Cultures have been submitted for serotyping and DNA analysis to the National Animal Disease Center, Ames, Iowa.

Serology

Ovine sera were inactivated by heating at 56°C for 30 min prior to testing, to prevent blocking of agglutination as described by Malkin (19). Agglutinins were detected by the microscopic agglutination test (MAT) conducted in a microtiter system using doubling dilutions from 1/50. The criterion for a reaction was at least 50% agglutination at 1/100 final serum dilution. Hamster sera were tested similarly to ovine sera but without prior inactivation.

Antigens used for the MAT on sera from Saskatchewan, Alberta and British Columbia sheep were limited to serovars pomona and hardjo, because evidence for the presence of other serovars in domestic animals in west-
ern Canada had not been found despite extensive serological monitoring. The sera from Oregon lambs were tested against six serovars, *pomona*, *hardjo*, *grippotyphosa*, *canicola*, *icterohaemorrhagiae* and *bratislava*.

Antigens were propagated in SPL 5X Leptospira Medium and were used at four to six days of age when the density reached at least 25 Nephelometer units, measured on a Coleman Nephelometer. Sensitivity of antigens was controlled by testing reference sera of known potency.

**RESULTS**

Sera of 810 rams and ewes from breeding flocks in Saskatchewan, Alberta and British Columbia reacted against *pomona* antigen at the rate of 1.0% and against *hardjo* at the rate of 0.4%. Sera from 120 Oregon feedlot lambs contained agglutinins to *pomona*, *hardjo* and *grippotyphosa* at the rate of 1.7, 61.7 and 59.1%, respectively (Table I). Reactions at 1/50 dilution against *icterohaemorrhagiae*, *canicola* and *bratislava* occurred in two, three and two Oregon lamb sera, respectively.

Gross lesions were usually extensive and obvious on the cortex of decapsulated kidneys. Multiple cysts, pale spots 1-5 mm in diameter, dark areas of congestion, patchy induration, and swollen, pale, knobby-textured kidneys were seen. Histological evidence of lesions compatible with a diagnosis of leptospirosis were found throughout kidney tissues from 26 Oregon and four Wyoming lambs. Lymphoid nodules were found in the medulla of one pair of kidneys from an Alberta lamb for which there was also a positive FAT for *grippotyphosa*.

The FAT results on 25 Oregon lamb kidneys paralleled the serological results given above. Leptospires were detected by *hardjo* antiserum in 52% of kidneys and by *grippotyphosa* in 32%. One of four Wyoming lamb kidneys was positive for *grippotyphosa*. Control tests confirmed the specificity of the FA test system. No *pomona* was found in kidneys by FAT.

Positive cultures were obtained from only one pool of two Oregon lamb kidneys which had been collected and processed at the abattoir. Both presumptive *grippotyphosa* and presumptive *hardjo* were isolated from these cultures, after passage through specifically protected hamsters. This further confirmed the specificity of the FAT, by which one kidney in the pool was positive for both *grippotyphosa* and *hardjo* and the other for *hardjo* alone. The presumptive *grippotyphosa* culture killed hamsters in five to seven days, producing severe icterus and intense nephrosis and glomerulitis. The presumptive *hardjo* culture produced severe but nonfatal interstitial nephritis. One-way agglutination tests with reference sera showed the isolates to be presumptively serovars *hardjo* and *grippotyphosa*. Serotyping and DNA analysis of the strains are not complete.

The results of FAT, gross and microscopic pathology, and culture are summarized in Table II.

**DISCUSSION**

The presence of leptospiral infection at a high rate in Oregon feedlot lambs was confirmed by the demonstration of antibodies in sera and leptospires in kidneys. Screening of the survey sera from imported lambs against a broad spectrum of antigens revealed the presence of *grippotyphosa* agglutinins. Consequently, this serovar was included in the FAT antisera, which led in turn to the confirmation of serological results and to the correct choice of serovars in the hamster cross-protection procedure for isolating two mixed leptospiral populations.

Ram and ewe sera, which were used to represent Saskatchewan, Alberta and British Columbia sheep, provided little evidence of the presence of leptospirosis due to either *pomona* or *hardjo* which are indigenous to cattle in western Canada. Had *grippotyphosa* infection been suspected in Canadian sheep before 1984, this serovar would have been added to the MAT antigens.

Few kidneys from Canadian slaughter lambs, rams and ewes were included in the study because the incidence of gross lesions was low. This suggests that the conclusion drawn from the ram and ewe serology was correct. The situation could change, considering the presence of agglutinins for *pomona* and *hardjo*, albeit at a low level, the suspected *grippotyphosa*.

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**TABLE I**

<table>
<thead>
<tr>
<th>Date</th>
<th>Serum Source</th>
<th>Donor Status</th>
<th>Number Tested</th>
<th>Serovar <em>pomona</em></th>
<th>Serovar <em>hardjo</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan/82</td>
<td>AB</td>
<td>Cull rams and ewes</td>
<td>57</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mar/82</td>
<td>AB</td>
<td>&quot;</td>
<td>11</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Mar/82</td>
<td>SK</td>
<td>&quot;</td>
<td>24</td>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>May/82</td>
<td>AB</td>
<td>&quot;</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>July/82</td>
<td>AB</td>
<td>&quot;</td>
<td>145</td>
<td>0</td>
<td>1; 0.7%</td>
</tr>
<tr>
<td>Aug/82</td>
<td>AB</td>
<td>&quot;</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sept/82</td>
<td>AB</td>
<td>University flock</td>
<td>52</td>
<td>0</td>
<td>1; 1.9%</td>
</tr>
<tr>
<td>Oct/82</td>
<td>AB</td>
<td>Cull rams and ewes</td>
<td>97</td>
<td>3; 3.1%</td>
<td>0</td>
</tr>
<tr>
<td>Nov/82</td>
<td>AB</td>
<td>&quot;</td>
<td>112</td>
<td>1; 0.9%</td>
<td>0</td>
</tr>
<tr>
<td>Nov/82</td>
<td>BC</td>
<td>&quot;</td>
<td>21</td>
<td>1; 4.8%</td>
<td>0</td>
</tr>
<tr>
<td>Nov/82</td>
<td>SK</td>
<td>&quot;</td>
<td>72</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Dec/82</td>
<td>AB</td>
<td>&quot;</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feb/83</td>
<td>AB</td>
<td>&quot;</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feb/83</td>
<td>AB</td>
<td>Breeding rams</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nov/83</td>
<td>AB</td>
<td>Cull rams and ewes</td>
<td>78</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nov/83</td>
<td>SK</td>
<td>&quot;</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dec/83</td>
<td>AB</td>
<td>&quot;</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Apr/84</td>
<td>OR</td>
<td>Feedlot lambs</td>
<td>120</td>
<td>2; 1.7%</td>
<td>74; 61.7%</td>
</tr>
</tbody>
</table>

*Agglutinins were measured by the microscopic agglutination test (MAT).*

*Exceptions were one group of 52 sera collected for disease monitoring in a university flock, and one group of five sera collected from breeding rams.*

*Reactor sera agglutinated at least 50% of leptospires in the test antigen, at a final serum dilution of 1/100 in the test.*

*Serovar from Oregon lambs were tested also with *Leptospira* serovar *grippotyphosa* antigen. The reactor rate was 71/120, 59.1%.*
TABLE II
EVIDENCE OF LEPTOSPIRAL INFECTION IN OVINE KIDNEYS AT SLAUGHTER IN ALBERTA (1984)

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of kidneys</th>
<th>FAT*</th>
<th>Gross pathology*</th>
<th>Histology</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacombe, Alberta</td>
<td>1 pair</td>
<td>Negative</td>
<td>Cysts 1-3mm</td>
<td>Congenital cysts</td>
<td>ND^ — unfit</td>
</tr>
<tr>
<td>Patricia, Alberta</td>
<td>1 pair</td>
<td>Negative</td>
<td>Pale stippling</td>
<td>ND — unfit</td>
<td>ND — unfit</td>
</tr>
<tr>
<td>Sexsmith, Alberta</td>
<td>1 pair</td>
<td>Positive —</td>
<td>Darkly congested; pale foci 1-2mm</td>
<td>Foci of intense interstitial mononuclear cells; fibrosis</td>
<td>Negative: heavily contaminated</td>
</tr>
<tr>
<td>Oregon</td>
<td>11</td>
<td>Positive —</td>
<td>Pale foci 1-3mm</td>
<td>Congested areas</td>
<td>Negative</td>
</tr>
<tr>
<td>Oregon</td>
<td>13</td>
<td>Positive —</td>
<td>Pale foci 1-5mm</td>
<td>Swollen, knobby texture on 3</td>
<td>Positive —</td>
</tr>
<tr>
<td>Oregon</td>
<td>2</td>
<td>Positive —</td>
<td>Pale foci 1-5mm</td>
<td>Intertstitial nephritis</td>
<td>Positive —</td>
</tr>
<tr>
<td>Wyoming</td>
<td>4</td>
<td>Positive —</td>
<td>Pale foci 1-5mm</td>
<td>Focal and diffuse interstitial nephritis</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Fluorescent antibody test (FAT) was done to detect leptospiral serovars pomona, hardjo and grippotyphosa. Negative refers to all three serovars.

*Observations of whole kidney after decapsulation.

^ND = Not done.

infection in one Alberta lamb, and the extensive movement of sheep between test stations, sales barns, breeding premises and feedlots.

The value of indirect fluorescent antibody staining as a survey and diagnostic tool was proven in this study. Death of leptospires and overwhelming contamination during shipment of kidneys to the laboratory precluded isolation of leptospires except by travelling to the abattoir and setting up a field laboratory for immediate processing of kidneys. The FAT provided a quick, reliable, serovar-specific method of demonstrating leptospires independent of their survival and of contamination. An optical system and lighting of high quality, such as the one described here, is crucial to the success of this technique.

Serotyping of the isolates by the World Health Organization standard method requires the growth of dense liquid cultures. The isolates have not yet been sufficiently adapted to liquid medium to permit their serotyping at Lethbridge or Ames.

The need for inactivation of ovine sera for the MAT to ensure consistent freedom of agglutinins to react (19) must be emphasized. Prior to the study reported here, a mixed culture of pomona and hardjo had been isolated from urine of a ewe lamb whose sera, in common with seven other sheep in the group, produced erratic test results and an atypical form of agglutination before inactivation.

The presence or potential presence of leptospirosis in Canadian sheep creates the following possibilities: lamb losses due to congenital infection, starvation of lambs of acutely infected ewes and fatal infection in feedlots; the development of foci from which other domestic species can become infected; and human infection in producers, truckers and abattoir workers.

Blackmore (20) considers sheep not to be natural maintenance hosts for pomona or hardjo. Therefore, sheep are likely to have infections of relatively short duration producing severe pathological effects. This view is consistent with observations in the reports of outbreaks cited above. Ellis (10) implicates the acute agalactic phase of hardjo infection in ewes with starvation in newborn lambs. The fatal effect of pomona on ovine fetuses was shown by the experimental studies of Smith (11).

Van der Hoeden (7) over 30 years ago described graphically the severe icterus and high mortality produced by grippotyphosa in goats herded with sheep in Israel. Although the sheep became infected, they remained clinically normal. The grippotyphosa strain isolated in the current study produced a rapidly fatal, icteric infection in hamsters, while the lambs from which it originated showed no icterus or debility.

Leptospirosis has been recognized for many decades as a serious zoonosis. The intimate contact between producers and sheep at lambing time, and the exposure of handlers to urine during transportation of sheep, provide opportunities for transmission of infection to man.

The prevalence of leptospirosis appears to be low in western Canadian sheep. The following measures should be considered for preventing an increase in this prevalence:

1. monitoring breeding stock by blood testing representative numbers of sheep at opportune times such as their movement to test stations or to sales;
2. monitoring commercial flocks through spot-checking of groups of sera and kidneys from cull ewes and slaughter lambs;
3. blood-testing samples of sheep going onto community pastures;
4. encouraging laboratory submission of lambs which are born dead.
or which die in the first week of life, for leptospirosis diagnosis;
5. testing imported sheep for a wide range of serovars and recognizing the significance of low titers;
6. treating and vaccinating only where the need is clearly established;
7. ensuring that cattle liners transporting imported feedlot lambs over Canadian highways pass border points without delay and be bedded to contain urine, thus minimizing the hazard of disseminating infection.

ACKNOWLEDGMENTS
The author thanks Dr. W.D.G. Yates, Pathologist and Director, Animal Pathology Laboratory, Saskatoon, Saskatchewan for his expert assistance by performing the histological examination of the tissues, and Mrs. Ina Harding for her meticulous care in conducting the serological, cultural and fluorescent antibody tests. The author also thanks Dr. G. Godkin, Inspector, Meat Hygiene Division for collecting ovine specimens. The continuing interest of Dr. A. Thiermann, National Animal Disease Center, Ames, Iowa in typing the isolates, is gratefully acknowledged.

REFERENCES

ABSTRACT

Case records of adult dairy cattle with abomasal ulcers admitted to the University of Pennsylvania from 1968 to 1980 were evaluated. Of 6385 adult dairy cattle admitted during that period, 69 had abomasal ulcers of clinical importance. Of 43 ulcers that had perforated, 17 were associated with local peritonitis and 22 were associated with diffuse peritonitis; 4 had perforated so recently that peritonitis had not had time to develop. Perforated ulcers were found in 40 cows that had recently calved (32 cows with ulcers that had perforated within 4 weeks after calving) and often had concurrent disease(s) (39 cows with at least one other disease). Ten cows with local peritonitis survived, whereas only two of the cows with diffuse peritonitis survived. Cows with abomasal ulcers resulting in diffuse peritonitis had an acute onset and rapid progression of signs attributed to septic shock. Cows with abomasal ulcers resulting in local peritonitis had variable clinical signs which were often so vague that diagnosis could not be made before exploratory surgery.