QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

DIVISION OF ANIMAL INDUSTRY BULLETIN No. 165

THE SEROLOGICAL AND MICROSCOPIC MONITORING OF A NATURAL OUTBREAK OF EPERYTHROZOON INFECTION IN SHEEP

by K. N. DADDOW, B.Sc., D.M.T., A.A.I.M.T., and L. B. DUNLOP, W.D.A.

SUMMARY

The course of a natural infection of *Eperythrozoon ovis* in 50 ewes and their lambs was monitored by a Complement Fixation Test (CFT) and examination of stained thin blood smears. Evidence of infection was detected in 39 of the 50 lambs. Thirty-eight lambs had a serological reaction to *E. ovis* in the CFT, 17 of these and one other lamb had *E. ovis* organisms in blood smears. Of the 50 ewes, 17 had serological reactions in the CFT; three of these and five others had organisms in blood smears.

The ewes appeared to be the source of infection for their lambs and the infection may have been spread by mosquitoes or sandflies that were plentiful at the time.

I. INTRODUCTION

Until recently *Eperythrozoon ovis* infection in sheep could be detected only microscopically or by the Coombs test Sheriff (1967) and Sheriff and Geering (1969). Daddow (1977) described a Complement Fixation Test (CFT) using antigen derived from sonically disrupted organisms. The CFT and microscopic examination of stained thin blood smears were used to monitor the course of a natural outbreak of *E. ovis* infection. The opportunity was taken to compare the two methods for their efficiency in detecting infection. A study of the correlation of infection of ewes and their lambs was made, and the presence of insects and external parasites was noted for their possible role in the mode of transmission.

II. MATERIALS AND METHODS

Fifty ewes, each with approximately month old lambs at foot, were selected from a commercial flock of about 2000 ewes. The ewes and lambs were tagged. The ewe numbers were initially 1 to 50. Six of these were later retagged with numbers 693, 694, 698, 699, 700, NT2. The lamb numbers were initially 51 to 100. One was later retagged 500. Ewe and lamb pairs were noted when observed. The ewes and lambs were bled for serum and uncoagulated blood in EDTA and thin smears of jugular blood were made except in the initial batch when the lambs' samples were collected from freshly docked tail butts, and in the final batch when only the 35 lambs that still had tags were sampled. There were five batches of samples over an 8-month period. The thin blood smears were fixed in methanol and stained with Giemsa's stain and examined at the magnification of 1 125 using an oil objective. When sera could not be tested soon after receipt at the laboratory, they were stored at -12°C until tested.

The EDTA samples from the initial batch were pooled and aliquots of 40 ml were inoculated intravenously into each of two susceptible splenectomized lambs which were housed in an isolation unit. Two months later, the lambs were inoculated intravenously with infected blood to determine whether the lambs were still susceptible to infection.

At each sampling, the flock was examined for ectoparasites and the prevalence of biting insects was noted.

III. RESULTS

The serological and smear examination results are shown in table 1.

TABLE 1

Batch		Test	P or S	Results and Ewe or Lamb No. and Subscript	
Date	Number			Ewes	Lambs
26 Nov 74	1	CFT	P	1; 26; 32; 42	
			S	10; 18; 26; 40	All lambs negative
		Smear	P	32; 46	
16 Jan 75	2	CFT	P	1_1 4; 32_1; 36; 38; 694; 700	62; 80; 93; 94; 95
			S	10_1; 33; 42_1; 45	63
		Smear	P	9; 36	55; 62; 77; 80; 93; 94; 95
24 Feb 75	3	CFT	P	36_2; $42_{1,2}$; 45_2; 693	56; 57; 59; 64; 72; 73; 74; 75; 77_2; 78; 80_2; 82; 84; 85; 99
			S		55_2; 94_2
		Smear	P	18; 48	53; 56; 57; 64; 69; 71; 78; 82; 93_2; 99; 500
15 Apr 75	4	CFT	P	35; $36_{2,3}$; $42_{1,2,3}$; 693_3; 694_2	53_3; 54; 57_3; 58; 59_3; 64_3; 66; 67; 69_3; 74_3; 78_3; 79; $80_{2,3}$; 81; 85_3; 90; 99_3
			S		71_3
		Smear	P	694_2	61; 71_3; $93_{2,3}$
25 Jul 75	5	CFT	P		52; 54_4; 58_4; 66_4; 70; $71_{3,4}$; $77_{2,3}$; $78_{3,4}$; 79_4; 83; 90_4; 91
			S	Not sampled	$53_{3,4}$; 56_3; $59_{3,4}$; $69_{3,4}$; 74_3; $80_{2,3,4}$; 81_4; $85_{3,4}$; 88; 94_2; 97; $99_{3,4}$; 500_3
		Smear	P		$71_{3,4}$

LEGEND TO TABLE 1

Ewe No. 1 to 50, 593, 694, 698, 699, 700, NT2

Lamb No. 51 to 100, 500

CFT = Complement Fixation Test

Smear = smear examination

P = positive

S = suspect

Subscript refers to the earlier batch number or numbers in which the ewe or lamb sample was CFT positive or suspect or smear examination was positive.

Of the 50 lambs originally tagged, 39 became infected with *E. ovis* during the 8-month period as indicated by the CFT or smear examination, or both. Twenty-one of the lambs were identified serologically only, one was identified by smear examination only, and 17 were identified by both the CFT and smear examination.

Reference to table 1 will show that, in the first batch, eight ewes only were shown to be infected. In the second batch, 13 ewes together with eight lambs were shown to be infected. In the third and fourth batches, there were respectively six and six positive ewes and respectively 17 and eight more lambs were shown to be infected. In the final batch when only the lambs were sampled, a further six lambs were shown to be infected.

Three out of eight, 10 out of 16, and seven out of eight lambs had CFT titres of at least 6, 5 and 4 months' duration respectively.

Over the 8-month period 20 of the 50 ewes were shown to be infected by *E. ovis*. Twelve were detected by the CFT only, three by smear examination only and five by both the CFT and smear examination.

Of the 10 positive ewes which were paired with lambs, nine had positive lambs while 10 of the 17 ewes, which had no indication of infection and which were paired with lambs, had positive lambs.

Neither of the lambs inoculated intravenously with aliquots of pooled EDTA blood from the first lamb batch reacted to indicate infection in the sampled lambs. On challenge 2 months later, the inoculated lambs were found to be susceptible to the challenge inoculum.

No ectoparasites were noted in the flock but sandflies and mosquitoes were present over the observation period particularly during the period between the second and third batches.

IV. DISCUSSION

No infection was detected in the lambs in the initial batch, even though some lambs were at least 1 month old and even though there was a source of infection in the ewes and potential vectors were present. This may indicate a fairly long incubation period in the natural disease.

As can be seen from the subscripts to the sheep numbers in table 1, most sheep undergo relapses as evidenced in the cases of ewe 694 and lambs 53, 74, 77, 90 and 500 in particular. It is also likely that all sheep that become infected remain carriers for a long period. Laws and Daddow (1976) recorded a carrier state of at least 5 years.

It has been clearly shown that the CFT is a far more efficient means than smear examination for detecting evidence of *E. ovis* infection. This applies particularly to new infections as in the figures for the 39 lambs. Of these, 38 could have been identified by the CFT alone, while only 18 would have been identified by smear examination alone.

In the detection of evidence of infection in the ewes, the CFT also proved more efficient than smear examination. If the CFT only were used, 17 of the 20 ewes could have been identified while only eight would have been identified if the smear examination was used.

There was no correlation between infected and non infected ewes and their lambs as could be expected when the social behaviour of sheep and the strong possibility of all the ewes being carriers is considered.

The presence of sandflies and mosquitoes during the observation period, and particularly during the period between the second and third batches, may be of importance in the transmission of the disease, but this remains to be investigated.

V. ACKNOWLEDGEMENTS

The authors thank Mr. W. T. K. Hall, Director of Pathology, and Mr. R. J. Rogers, Chief Pathologist, Animal Research Institute for their assistance in preparing this paper, and Mr. A. T. Bell, Director of Sheep and Wool Branch for his support of this interbranch project. They also thank Mr. A. M. Lethbridge, 'Hythe', Charleville, for the use of his stock and facilities, Mr. D. R. Niven and Mr. E. Wood and the Department of Primary Industries staff at Charleville for the technical assistance.

REFERENCES

DADDOW, K. N. (1977)—A Complement Fixation Test for the Detection of Eperythrozoon Infection in Sheep. *Aust. vet. J.* 53 (3): 139-143.

LAWS, L. and DADDOW, K. N. (1976)—The Occurrance of *Eperythrozoon ovis* in Queensland. *Aust. vet. J.* 52 (5): 243.

SHERIFF, D. (1967)—*Eperythrozoon ovis* Infection and the Antiglobulin Test. *Nature, Lond.*, 215:102.

SHERIFF, D. and GEERING, M. C. (1969)—The Antiglobulin (Coombs) Test in *Eperythrozoon ovis* Infection in Sheep. *Aust. vet. J.* 45: 505-507.

(Received for publication 11 March 1976)

Mr. K. N. Daddow is an officer in the Pathology Branch, Animal Research Institute, Yeerongpilly; and Mr. L. B. Dunlop is an officer in the Sheep and Wool Branch, Charleville.

S. R. HAMPSON, Government Printer, Brisbane