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ACTINOBACILLUS SEMINIS INFECTION IN SHEEP IN THE REPUBLIC OF SOUTH AFRICA. II. INCIDENCE AND GEOGRAPHICAL DISTRIBUTION

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ABSTRACT

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To obtain information on the incidence and distribution of *Actinobacillus seminis* infection in the Republic of South Africa, a clinical and serological survey was carried out on 409 farms situated in 29 districts. All rams submitted for certification to the Regional Laboratory from 1/1/69 to 31/1/74 were included in a separate investigation. These particular rams represented different breeds and originated from farms in over 48 districts. Examinations were also carried out on all rams on 11 stud farms in the Middelburg and adjacent districts with a high incidence of epididymitis, despite regular immunization with Elberg Rev. 1 vaccine.

These investigations confirmed that genital infection of rams still presents a major problem in the main sheep breeds and the main sheep farming areas of South Africa. A high incidence of infection with *A. seminis*, an organism which appears to be the most important one associated with genital infection in this country, was also established. Genital infection due to *A. seminis* is geographically also very widespread.

Résumé

INFECTION À ACTINOBACILLUS SEMINIS *CHEZ LE MOUTON EN RÉPUBLIQUE SUD-AFRICAINE. II. FREQUENCE ET DISTRIBUTION GÉOGRAPHIQUE*

Afin d'obtenir des informations sur le frequence et la distribution de l'infection à Actinobacillus seminis *en République sud-africaine, on a exécuté une enquête clinique et sérologique dans* 409 *fermes situées dans* 29 *districts. Tous les béliers présentés au Laboratoire Régional pour certification entre le* 1/1/1969 *et le* 31/1/1974 *ont fait l'objet d'une enquête séparée. Ces derniers animaux appartenaient à des races différentes et provenaient de fermes réparties sur plus de* 48 *districts. On a également examiné tous les béliers de* 11 *élevages dans les districts de Middelburg et adjacents, où il y avait une forte incidence d'épididymite malgré une immunisation régulière avec le vaccin Elberg Rev.* 1.

Ces enquêtes ont confirmé le fait que l'infection génitale des béliers représente encore un problème majeur pour les principales races de mountons et dans les principales régions d'élevage ovine en Afrique du Sud. On a également démontré une frequence élevée d'infection à A. seminis; *dans ce pays, cet organisme semble être le plus important de ceux qui sont associés avec l'infection génitale. L'infection génitale due à* A. seminis *possède également une très vaste répartition géographique.*

INTRODUCTION

Prior to the isolation of *Actinobacillus seminis* (Worthington & Bosman, 1968; Van Tonder & Bolton, 1968), *Brucella ovis* (Van Rensburg, Van Heerden, Le Roux & Snyders, 1958) was regarded as the only primary aetiological agent of importance in ovine epididymitis in South Africa. It was therefore considered necessary to establish the relative importance of this organism in genital infection of rams in this survey.

In a preliminary study Van Tonder (1979) found that genital infection in sheep still presented a problem, even in the complete absence of *B. ovis* infection, and that *A. seminis* and *Actinobacillus*-like organisms were the most common and the most consistent bacteria isolated from these cases. Further investigations to determine the incidence and distribution of *A. seminis* infection were thought necessary to establish the importance of *A. seminis* infection in rams in this the main sheep farming area in South Africa.

A survey conducted in Queensland (Simmons, Baynes & Ludford, 1966) after the first description of *A. seminis* in Australia had been published by Baynes & Simmons (1960), suggested that this infection was a problem in one flock only. Other reports on genital infection by this organism also in Australia (Galloway, 1966; Watt, 1966, 1970) again described only individual cases from relatively few farms. Recently, the isolation of an unidentified Gram-negative pleomorph resembling *A. seminis* from cases of traumatic epididymitis in Dorset rams in South Australia (Pulsford, Eastick, Clapp & Roberts, 1967) and the isolation of *A. seminis* from cases of polyarthritis and posthitis in 4 flocks in Western Australia (Watt, Bamford & Nairn, 1970) suggest a higher incidence and a wider distribution than earlier reports indicated.

Apart from the isolation of *A. seminis* from a single ram in America (Livingston & Hardy, 1964) and *Actinobacillus*-like and Gram-negative pleomorphs from a number of rams in New Zealand (Ekdahl, Money & Martin, 1968), no further information on the incidence and distribution of *A. seminis* infection in rams in those countries has been documented.

The confirmation of *A. seminis* infection in a single ram from the Orange Free State (Worthington & Bosman, 1968) and in 21 rams from 7 farms in the Middelburg area of the Cape Province (Van Tonder & Bolton, 1968), as well as information from the previous study (Van Tonder, 1979), indicates a wide distribution and high incidence of the disease in South Africa.

A. seminis was also isolated from foetal and uterine samples taken from aborting Dorper ewes and Boer goat does (Van Tonder, 1973), infected bovine semen (Van Tonder & Bolton, 1970), and, more recently, from uterine discharges of cows delivering still-born calves or having late abortions (unpublished data). These findings further support the conclusion that the incidence of *A. seminis* infection in sheep as well as in other species is probably widespread, and this survey was undertaken to establish the assumption.

MATERIALS AND METHODS

Animals

General survey.—In all, 409 sheep farms in over 29 districts of the Cape and Orange Free State were involved in a survey conducted during 1968. The

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animals included all stud and commercial flock rams from 2-tooth to adults, representing the Merino, Dorper and Karakul breeds.

Examined at the laboratory.—Rams submitted for certification to the Regional Laboratory, Middelburg, Cape Province from 1/1/69 to 31/1/74 were included in a separate investigation. These animals represented different breeds and originated from a large number of farms distributed over 48 districts of this province.

Specific stud farms.—Particular attention was also paid to 11 stud farms in the Middelburg and adjacent districts where a high incidence of epididymitis was found to exist, despite regular immunization with Rev. 1 vaccine.

All the animals on these farms were examined and samples were collected from them, either on the farm of origin or when they were presented for certification at the Regional Laboratory, Middelburg.

Examination of the genitalia

Examination of the genitalia was conducted on all the rams by visual inspection and palpation.

Biological specimens

Where required, semen was collected and examined according to the methods described in a previous article (Van Tonder, 1979).

In some investigations, blood was collected in boracic acid from a number of rams showing genital lesions as well as from rams without lesions.

Serology

Serum specimens were subjected to the complement fixation (CF) test to determine the incidence and distribution of antibodies to *A. seminis* infection. The presence of antibodies against *B. ovis* infection was determined in the same test.

Initially, the method for conducting the CF test described by Worthington & Mulders (1969) was adopted, using antigens prepared from the original strain (Van Tonder & Bolton, 1968) of *A. seminis* only, but subsequently a modified test procedure, to be described elsewhere, was employed, while antigens prepared from 6 different strains of *A. seminis* were also included.

In all instances titres are expressed as the reciprocal of the final dilution of serum after all the other reagents had been added. As suggested by Simmons *et al.* (1966), who conducted a similar survey, antibody titres of $^1/_{20}$ and higher were regarded as positive evidence of exposure to infection.

RESULTS

Incidence of the disease

Rams tested in a general survey.—The results of the clinical and serological survey are given in Table 1.

From this table it can be seen that, of the large number of rams examined, 2,5% showed genital lesions, and that 27,1 and 15,5% of these rams also showed antibody titres of 20 and higher against the original strain of *A. seminis*, and *B. ovis*, respectively. Similarly 15,8 and 6,6% of rams without lesions showed antibody titres of $^1/_{20}$ and higher when tested with the respective antigens.

An analysis of the results for the 3 different breeds is presented in Table 2.

TABLE 1 Incidence of clinical lesions and positive serological reactions of all rams tested in the general survey

No. of rams examined	Testicular lesions	Complement fixation test				
		Sera tested	*A. seminis*		*B. ovis*	
			Positive	%	Positive	%
34 501	Present, 845 (2,5%)	731	198	27,1	113	15,5
	Absent, 33 656	8 127	1 282	15,8	536	6,6

TABLE 2 The incidence of lesions and complement fixing antibodies against *A. seminis* and *B. ovis* in the 3 different breeds

Breed	No. of rams examined	Testicular lesions	Complement fixation test				
			Sera tested	*A. seminis*		*B. ovis*	
				Positive	%	Positive	%
Merino	30 250	Present, 641 (2,1%)	562	128	22,8	70	12,5
		Absent, 29 609	7 176	998	13,9	144	6,2
Dorper	2 814	Present, 161 (5,7%)	143	60	42,0	37	25,9
		Absent, 2 653	682	171	25,1	60	8,8
Karakul	1 437	Present, 43 (3,0%)	26	10	38,5	6	23,1
		Absent, 1 394	269	113	42,0	32	11,9

TABLE 3 A comparison between the incidence of testicular lesions in stud and flock rams

No. of rams	Breed					
	Merino		Dorper		Karakul	
	Stud rams	Flock rams	Stud rams	Flock rams	Stud rams	Flock rams
Examined	21 264	8 986	1 991	823	877	560
Lesions present	332 (1,6%)	309 (3,4%)	108 (5,4%)	53 (6,4%)	14 (1,6%)	29 (5,2%)

TABLE 4 Number of farms where clinical lesions and complement fixing antibodies were encountered during the survey

Breed	No. of farms	Clinical lesions		Complement fixing antibodies			
		No. of farms infected	% of farms infected	*A. seminis*		*B. ovis*	
				No. of farms infected	% of farms infected	No. of farms infected	% of farms infected
Merino	328	202	61,6	246	75,0	170	51,8
Dorper	56	34	60,7	42	75,0	25	44,6
Karakul	25	10	40,0	18	72,0	14	56,0
Total	409	246	60,1	306	74,8	209	51,1

TABLE 5 The incidence of exposure to dual infection by *A. seminis* and *B. ovis* as determined in the survey

Presence of complement fixing antibody									
Rams with clinical lesions					Rams without lesions				
No. of sera tested	Positive *A. seminis*	Positive *B. ovis*	Positive *A. seminis* & *B. ovis*	Negative	No. of sera tested	Positive *A. seminis*	Positive *B. ovis*	Positive *A. seminis* & *B. ovis*	Negative
731	168 (23,0%)	83 (11,4%)	30 (4,1%)	450	8 127	1 095 (13,5%)	349 (4,3%)	187 (2,3%)	6 496

The incidence of clinical lesions was found to be highest in the Dorper breed (5,7%), followed by the Karakul (3,0%) and the Merino breeds (2,1%). The extent of exposure to *A. seminis* and *B. ovis*, as indicated by the presence of antibody in clinically affected rams, showed the same pattern in all 3 breeds, while in the unaffected rams the positions of the Karakul and Dorper breeds were reversed.

The incidence of *A. seminis* infection in flock rams as opposed to stud rams is given in Table 3.

The results presented in Table 3 show that the incidence of testicular lesions in all 3 breeds was higher in the flock than in the stud rams.

An analysis of the number of farms where evidence of exposure to *A. seminis* and *B. ovis* infection was found is given in Table 4.

From these results it is apparent that clinical lesions in rams were found on 60,1% of all the farms surveyed. The complement fixation test on the other hand indicated that exposure to *A. seminis* infection occurred on 74,8% and to *B. ovis* infection on 51,1% of these farms.

The results obtained in the survey were also analysed to establish to what extent antibodies against both these bacteria occurred in the same animal. The results of this analysis are summarized in Table 5.

These results clearly indicate that exposure to *A. seminis* and *B. ovis* infection as shown by the presence of antibodies to both these organisms in the same animal did in fact occur.

Rams tested at the laboratory.—Results of the examination of rams at the Regional Laboratory are summarized in Table 6.

As in a previous study (Van Tonder, 1979), rams were classified into clinical, subclinical and negative or unaffected categories by clinical and semen smear examination. From these results it can be seen that, of the rams examined during the specified period, 22,7% had clinical lesions and a further 25,3% showed the presence of neutrophils in their semen. During a bacteriological examination of semen specimens from a large number of rams within both these groups, *A. seminis* was isolated from 61,8%

of the clinical and 59,3% of the subclinical cases, respectively. *A. seminis* was also isolated from 13,8% of those cases classified as clinically and subclinically negative, that is, where no neutrophils could be found on semen smear examination. The incidence of antibodies to *A. seminis* compared with that of *B. ovis* was in all instances much higher and there was also a significant increase when antigens of 6 different strains were employed.

Rams tested on specific stud farms.—The incidence of genital infection on 11 stud farms is presented in Table 7.

The data given in Table 7 clearly indicate that genital infection in both the clinical and subclinical forms constituted a serious problem on these farms. A bacteriological examination of the semen specimens of all these rams and the isolation of *A. seminis* from the vast majority of them were a further indication of the importance of this infection.

Distribution of A. seminis *infection*

An analysis of the origin of clinically affected animals as well as the donors of positive samples showed that the disease was very widely distributed over the area surveyed. The fact that affected animals were encountered in every district from which specimens were collected was a clear indication of this.

TABLE 6 Summary of the results of the examination of rams submitted to the laboratory

Item	Clinically positive				Subclinically positive(1)				Clinically & subclinically negative			
	526 (22,7%)				587 (25,3%)				1 207			
	No. tested	Positive *A. seminis*	Positive *B. ovis*	Negative(2)	No. tested	Positive *A. seminis*	Positive *B. ovis*	Negative(2)	No. tested	Positive *A. seminis*	Positive *B. ovis*	Negative(2)
Bacteriological examination.	275	170 (61,8%)	17 (6,2%)	90 (32,7%)	270	160 (59,3%)	5 (1,9%)	105 (38,9%)	513	71 (13,8%)	0 (0,0%)	442 (86,2%)
Routine CF test	190	70 (36,8%)	53 (27,9%)	81 (42,6%)	80	24 (30,0%)	7 (8,8%)	52 (65,0%)	128	28 (21,9%)	2 (1,6%)	108 (84,4%)
Modified CF test.....	263	166 (63,1%)	65 (24,7%)	61 (23,2%)	119	58 (48,7%)	9 (7,6%)	56 (29,5%)	502	136 (27,1%)	8 (1,6%)	358 (71,3%)

(1) Classified on semen smear examination
(2) Cultures showing no bacterial growth after 48 h or overgrown by contaminants were regarded as negative

TABLE 7 Incidence of clinical and subclinical genital infection on 11 stud farms

Farm	Breed	Year of examination	No. of rams examined	Result of examination			
				Clinically positive		Subclinically positive(1)	Clinically and subclinically negative
				No. of rams	% rams positive		
1.....	Merino.....	1969....	67	1	1,5	35	31
1.....	Merino.....	1970....	85	5	5,9	29	51
1.....	Merino.....	1971....	151	3	2,0	26	122
1.....	Merino.....	1972....	96	2	2,1	29	65
2.....	Merino.....	1969....	42	3	7,1	5	34
2.....	Merino.....	1971....	30	3	10,0	8	19
3.....	Merino.....	1969....	93	0	0,0	32	61
3.....	Merino.....	1969....	70	14	20,0	27	29
3.....	Merino.....	1973....	248	22	8,9	ND	ND
4.....	Merino.....	1969....	33	0	0,0	20	13
5.....	Merino.....	1972....	192	20	10,4	ND	ND
5.....	Merino.....	1973....	213	15	7,0	ND	ND
6.....	Dorper.....	1969....	72	6	8,3	24	42
7.....	Dorper.....	1969....	41	0	0,0	26	15
7.....	Dorper.....	1970....	42	2	4,8	8	32
7.....	Dorper.....	1971....	34	2	5,9	9	23
7.....	Dorper.....	1972....	50	0	0,0	10	40
8.....	Dorper.....	1969....	210	31	14,8	ND	ND
9.....	Dorper.....	1971....	46	3	6,5	16	27
9.....	Dorper.....	1972....	50	0	0,0	20	30
10.....	Dorper.....	1972....	71	8	11,3	39	24
11.....	Dorper.....	1970....	71	6	8,5	ND	ND
11.....	Dorper.....	1971....	166	14	8,4	ND	ND
11.....	Dorper.....	1972....	109	6	6,5	ND	ND
11.....	Dorper.....	1973....	233	8	3,4	ND	ND

(1) Classified on semen smear examination
ND—Not done

FIG. 1 Distribution of *A. seminis* infection

Discussion

From the 34 501 rams examined during the survey, 2,5% had testicular lesions. Although this figure appears to be low, those rams that were to be eliminated as breeding animals represented an economic loss of R139 000, even at a time when extremely low prices prevailed.

Subsequent to the confirmation of *B. ovis* as a cause of epididymitis of rams in South Africa (Van Rensburg *et al.* 1958) and the introduction of Rev. 1 vaccination (Van Drimmelen, 1960), the elimination of clinically affected animals as part of the control programme became a standard procedure. The persons concerned were alerted to the condition with the result that, on the average, stud farm clinical palpation and elimination of rams with genital abnormalities were carried out at regular intervals throughout the year. Since only stud farms were included in the survey, the percentage of rams with testicular lesions encountered cannot be regarded as a true reflection of the prevalence of the disease. On most farms the clinical cases identified during these investigations represented only those rams which had not previously been detected or which had developed lesions after the prior routine inspection, which had been conducted after intervals varying from a few days to a few months prior to this survey. Further confirmation for this claim is provided by the higher incidence of lesions in flock rams that were less frequently examined or on the 11 stud farms where all rams were available for examination.

The difference in the incidence of genital lesions between the Merino and the other 2 breeds can in part be explained by the fact that Merino rams and notably stud and sale rams are more frequently handled and examined.

In view of the specific antibody response to *A. seminis*, as demonstrated in the initial experiments (Baynes & Simmons, 1960; Livingstone & Hardy, 1964; Worthington & Bosman, 1968; Van Tonder & Bolton, 1968) and the application of the complement fixation (CF) test in their survey (Simmons *et al.*, 1966), this test was used to determine the incidence and distribution of antibodies and therefore as an indicator of exposure of rams to infection by this organism. As suggested by Simmons *et al.* (1966), titres of $^{1}/_{20}$ and higher were regarded as positive evidence of exposure. The results obtained in the serological survey indicated a more widespread and higher exposure to *A. seminis* infection than that to *B. ovis*, although antigens prepared from the original strain only were used. Furthermore, an analysis of

these results showed that exposure to both *A. seminis* and *B. ovis*, as indicated by the presence of antibody to both antigens, did in fact occur in a relatively small percentage of rams.

In the examination of rams submitted to the Regional Laboratory, clinically affected rams were identified by palpation of the genitalia, while the semen smear examination was used to determine the presence of neutrophils in the semen. Here again it was found that, although some rams were free of clinical signs of infection, they nevertheless excreted large numbers of neutrophils in their semen. As in the previous investigation, these animals were classified as subclinical cases.

A high incidence of clinical and subclinical infection was found in those rams examined for the purpose of certification. In both groups, *A. seminis* was isolated from the majority of semen samples collected, while *B. ovis* was isolated in a relatively small percentage of clinically or subclinically affected rams. The isolation of *A. seminis* from the semen of 13,8% of rams without lesions or neutrophils in the semen was ascribed to a possible latent or inactive stage of the infection.

In both the clinically and subclinically affected rams the presence of antibodies again indicated a higher rate of exposure to *A. seminis* when compared with *B. ovis* infection. This difference became more pronounced when the incidence of antibodies to *A. seminis* almost doubled when antigens prepared from 6 different strains of *A. seminis* were included in the test.

The high incidence of clinical and subclinical genital infection on 11 stud farms where *B. ovis* infection was completely eliminated by Rev. 1 vaccination provides additional evidence of the importance of this disease. The isolation of *A. seminis* from the semen of most of these rams strongly indicated the importance of the role played by this organism.

The fact that the presence of *A. seminis* infection was established by various tests on the majority of farms distributed over 29 districts in the clinical and serological survey or in rams submitted to the laboratory from farms from 48 districts in the Cape and Orange Free State is proof of the widespread distribution of this infection. The results also indicate that this infection has a high incidence in Merino, Dorper and Karakul breeds in this country.

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