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EPIDEMIOLOGICAL OBSERVATIONS IN A CORRIEDALE FLOCK AFFECTED BY *BRUCELLA OVIS*.

Robles, C.A.[(1)] ; Uzal, F.A.[(1)] ; Olaechea, F.V.[(1)] ; Low, C.[(2)]

[(1)] National Institute for Agricultural Technology (INTA) CC: 277 - 8400 Bariloche - ARGENTINA.

[(2)] Veterinary Investigation Centre (SAC) Bush Estate, Penicuik EH26 0QE, Scotland, UK.

Correspondence address

Dr. Carlos Robles
CC: 277 - INTA
(8400) Bariloche
ARGENTINA

☎ 54 - 944 - 22731 / 29862 / 29863
Fax: 54 - 944 - 24991
e-mail: crobles@inta.gov.ar

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ABSTRACT

Brucellosis in sheep, caused by *Brucella ovis*, is primarily a chronic infectious disease of rams with epididymitis as the most characteristic lesion. Six hundred rams from an infected farm were clinically and serologically examined once a year, over a three year period. An increase from 2.1% to 6.3% in the prevalence of animals serologically positive to *B. ovis* occurred over the three years. However, the prevalence of rams with lesions in the reproductive tract, declined from 14.2% to 6.5% in the third year following one year's strict culling of clinically affected and *B. ovis* serologically positive rams. Clinical lesions found in the 179 affected rams fell into two main categories: rams with epididymitis and rams with affected lymph nodes. These results suggest that the prevalence of the disease relates, mainly to the sexual activity of the animal and not to age itself. A single cull based on the results of clinical examination and serological test results was unable to decrease the prevalence of *B. ovis* in an extensive Corriedale sheep flock.

Keywords: Brucella ovis - Disease - Epidemiology - Epididymitis - Sheep - Reproduction.

INTRODUCTION

Brucellosis in sheep, caused by *Brucella ovis*, is primarily a chronic infectious genital disease of rams with epididymitis as the most characteristic lesion (Simmons & Hall, 1953; Jebson et al., 1954; Kennedy et al., 1956; Biberstein et al., 1964; Burgess et al., 1982).

Ovine brucellosis occurs world-wide (FAO, 1992) and has a serious economic impact on sheep husbandry. This is due to poor flock fertility, increased culling of rams, occasional abortions, increased labour and restrictions on trading (Haughey et al., 1968; Moro, 1974; Afzal & Kimberling, 1986; Niilo et al., 1986; Kimberling & Schweitzer, 1989; Bulgin, 1990).

Brucella ovis was first isolated in New Zealand by McFarlane and colleagues (1952) from aborting ewes. Epididymitis caused by *B. ovis* in rams was reported simultaneously in Australia by Simmons & Hall (1953) and in New Zealand by Buddle & Boyes (1953). The organism was first reported in America by Kennedy and colleagues (1956) and in Argentina by Szyfres & Chappel (1961) from the semen of a ram with clinical epididymitis. Cedro and colleagues (1963) reported the disease in Patagonia and isolated *B. ovis* from Corriedale rams in Tierra del Fuego province. No more studies were carried out in the region until a farmer noticed a problem in his rams during the artificial insemination season. Several rams had semen of poor quality which contained neutrophil leukocytes. The demonstration of serum antibodies against *B. ovis* and the isolation of *B. ovis* from several rams confirmed the presence of the disease on the farm.

Despite the identification of contagious epididymitis due to *B. ovis* in Argentina in the early

sixties, no attempts to characterise the disease were recorded. Therefore, it was decided to initiate a study to determine the extent and characteristics of the disease in the problem flock and also to compare it with published reports in other parts of the world.

MATERIALS AND METHODS

Area of study and farm description

The study was carried out on a sheep farm located in the South of Patagonia Region, Argentina. The farm has approximately 21,000 Corriedale sheep and 1,000 Hereford cattle grazing on an area of 40,000 hectares. Sheep farming is the most common form of livestock production in the area. The system of production is very extensive and is based on the use of the natural pastures throughout the year, without any feed supplementation. Mating takes place in the autumn. The ram to ewe ratio is usually 1:25 and rams have their first mating season when 18-20 months old.

Clinical examination of the rams and sampling

All the rams were identified with ear tags at the beginning of the study and the age was estimated according to teeth eruption. During the three years of study, all the rams were clinically examined before the mating season for any lesions in the external genitalia or superficial lymph nodes. The technique used was that previously described (Van Tonder, 1977; MacLaren, 1988; Robles, 1989). Briefly, the rams were placed in an upright sitting position and size and consistency of the submandibular, parotid, prescapular, prefemoral and inguinal lymph nodes were examined. The testes and epididymis were palpated and their size, shape, consistency and location recorded. Organs found to contain lesions at clinical examination were recorded falling into one of the five following categories: Epididymis, Epididymis and Lymph nodes, Testes, Testes and Epididymis, and Lymph nodes. After the clinical examination, blood samples were taken from the jugular vein. The collected blood was kept at room temperature for 24 hours, then the serum was separated and stored at - 20°C.

Agar Gel Immunodifussion Test (AGID)

The technique used was that recommended by Centro Panamericano de Zoonosis - WHO

(CEPANZO), Argentina (1976). The test was performed on microscope slides, covered a gel composed of Noble agar in saline buffer. A puncher with 6 peripheral holes, for the control and problem sera and a central hole for the antigen was used. The heat extracted (HS) antigen, provided by CEPANZO was used at a dilution of 10 mg/ml.

Control procedures

The data from the first year's examination showed the situation on the farm without any intervention (Table 1). After the first year's clinical examination having any animals with either clinical signs or positive serology for *B. ovis* were removed from the flock and consequently all affected rams remained in the flock and were used during the second year of study. This strategy was established as being the usual system of management of the farm. After the second year's clinical examination, all the rams that were either positive for *B. ovis* serology or showed clinical lesions on their external genitalia were culled. In the third year, the results of the second year strategy was evaluated.

Management and analysis of the data

EPI INFO, Version 6.04b (USD Inc., USA, 1997) was used to store and retrospectively analyse the data and also to work out the significance of the data. Chi square distribution, chi square for trend and Odds ratio were the most common outputs used. The EPISCOPE program (Frankena & Goelema, Agricultural University, Wageningen, The Netherlands) was used to compare the degree of agreement between clinical examination of rams and AGID test through the Kappa value calculation. Confidence Interval Analysis Program, Version 1.2 (Gardner, Gardner & Winter, BMJ Publishing Group, London, 1992) was used to calculate the confidence intervals. For all the calculations a 95% confidence level was used.

RESULTS

The general results from clinical examinations of all the rams on the farm and of the *B. ovis* serology during the 3 years of the study are presented in Table 1. It is notable that the farmer increased the number of rams at service by 25 % over the three years.

The clinical data varied significantly over the three years. An increase in the prevalence of animals serologically positive to *B. ovis* occurred (Table 1). However, the prevalence of rams with lesions in their reproductive tract and changes in the related lymph nodes and the prevalence of epididymitis alone, declined in the third year after strict culling of clinically affected and AGID positive rams after the second year's clinical examination (Table 1).

The distribution of clinical lesions found in the 179 affected rams detected during the 3 years is shown in Figure 1. The rams fell into two main categories: rams with epididymitis and rams with enlarged lymph nodes. Problems in the testes were minor and were mainly related to bilateral hypoplasia.

An analysis was carried out on the distribution of the epididymal lesions from the data from 75 affected rams. There was a tendency for unilateral (66% CI: 54.8-77.1) rather than for bilateral epididymitis (34% CI: 22.9 - 45.2). The tail was the most frequently affected part of the epididymis (Table 2). Regarding the lymph nodes, lesions consisted in enlargement and increased hardness, the prefemoral and inguinal lymph nodes being the most affected.

The prevalence of epididymitis and its distribution by age for the three years is displayed in Table 3.

When the data were analysed for each year it was observed that the percentage of epididymitis was significantly different according to the age for the three years ($p> = 0.04$). However when analysed as to whether these differences increased with the age, they were statistically significant in the first ($p=0.00372$) and the second ($p=0.00150$) years, but not in the third year ($p=0.91565$). In the third year it was noticeable that 3 years old rams were the most affected (6.03%).

The results of the serology against *B. ovis* were also analysed for the three years according to the age of the rams (Table 4).

As occurred with clinical epididymitis, the prevalence of *B. ovis* positive animals increased significantly with the age during the first ($p=0.00168$) and second (0.00059) year but in the third year there was no significant trend ($p=0.06703$). Again, 3 year old rams were the most affected in the third year (14.65%).

There was only a poor correlation between rams with clinical epididymitis and positivity to the AGID test for *B. ovis* (Table 5). The Kappa value between both tests was 0.26 at the 95% confidence level.

DISCUSSION

The low serological prevalence of *B. ovis* (2.1%) in the first year, followed by the increasing serological prevalence in the second (4%) and third years (6.3%) suggests that *B. ovis* infection may have been recently introduced to the flock. Other authors have reported that high prevalences should be expected in endemic situations and when control methods are not applied (Blasco, 1990; Bulgin, 1990). The lower contact rate between the animals in our study compared with those in more intensive breeding systems may explain the small increase in prevalence observed over the three years.

The observation that the epidydimis was the most frequent reproductive organ affected and the significantly greater percentage of rams with unilateral than with bilateral epididymitis are in agreement with previous reports on contagious epididymitis due to *B. ovis* infection (Buddle, 1956; McGowan & Shultz, 1956; Kennedy et al., 1956; Moro 1974; Jansen, 1980; Blasco 1990). The finding that no lambs were either clinically affected or serologically positive to *B. ovis* is also in agreement with previous observations that *B. ovis* epididymitis is mainly a disease of adult rams with previous sexual experience (Osborne, 1955; Walker et al., 1985; Bulgin, 1990).

Both epididymitis and the serological prevalence of *B. ovis* varied with the age of the rams (Tables 3 & 4). In the first and second years both increased with the age of the rams. Since epididymitis caused by *B. ovis* is a chronic infectious disease such results are to be expected in flocks where no control measures have been applied, since a cumulative effect is produced with age (Blasco and Barberán, 1990). However, in the third year of our study, after culling of all the clinically affected and serologically positive rams during the second year, the pattern of the disease changed considerably. The prevalence of the disease then reached a peak in 3

year-old rams. This result suggests that the prevalence of the disease is in relation, mainly to the sexual activity of the animal rather than to age itself.

The presence of animals which were not serologically *B. ovis* positive in the group of rams with epididymitis could be attributed to infection with other bacteria. Organisms such as *Actinobacillus seminis* (Sponenberg et al., 1983; De Wet & Erasmus, 1984; Hajtós, et al., 1987) and *Histophilus ovis* (Claxton & Everett, 1966; Low & Graham, 1985; Hajtós et al., 1986; Robles et al., 1990) have been recognised as primary causes of epididymo-orchitis in rams. Additional studies should be carried out to determine the aetiology of ram epididymitis in Patagonia, since bacteria different from *B. ovis* could be involved and might require different control methods. The sensitivity of the test should also been taking into account, since it could varies from 84,2% to 96,4 % (Worthington et al, 1985; Marin, et al, 1989).

When strict culling was carried out after the second year's clinical examination, the prevalence of epididymitis decreased significantly, but the serological prevalence of *B. ovis* continued to increase (Table 1). This might be due to a failure of the test to detect all the infected rams (Worthington et al., 1984; Worthington et al., 1985; Marin et al., 1989). Also, it has been shown that under experimental conditions that lesions may appear 30-45 days after inoculation, while serological titres can be detected 2 to 3 weeks after challenge (Biberstein et al., 1964). Thus, the presence of recently infected rams which could not be detected by the methods employed in this study, should be considered.

A single annual culling based on the results of clinical examination and AGID test results was apparently unable to decrease the prevalence of *B. ovis*, so more sensitive diagnostic methods

are required. Under Patagonian management conditions, serological tests and culling applied both prior to and after the breeding season might afford better control of this disease. However more research is necessary to establish when and with which periodicity the clinical and serological examinations should be done to be both technically effective and economically feasible for the farmers.

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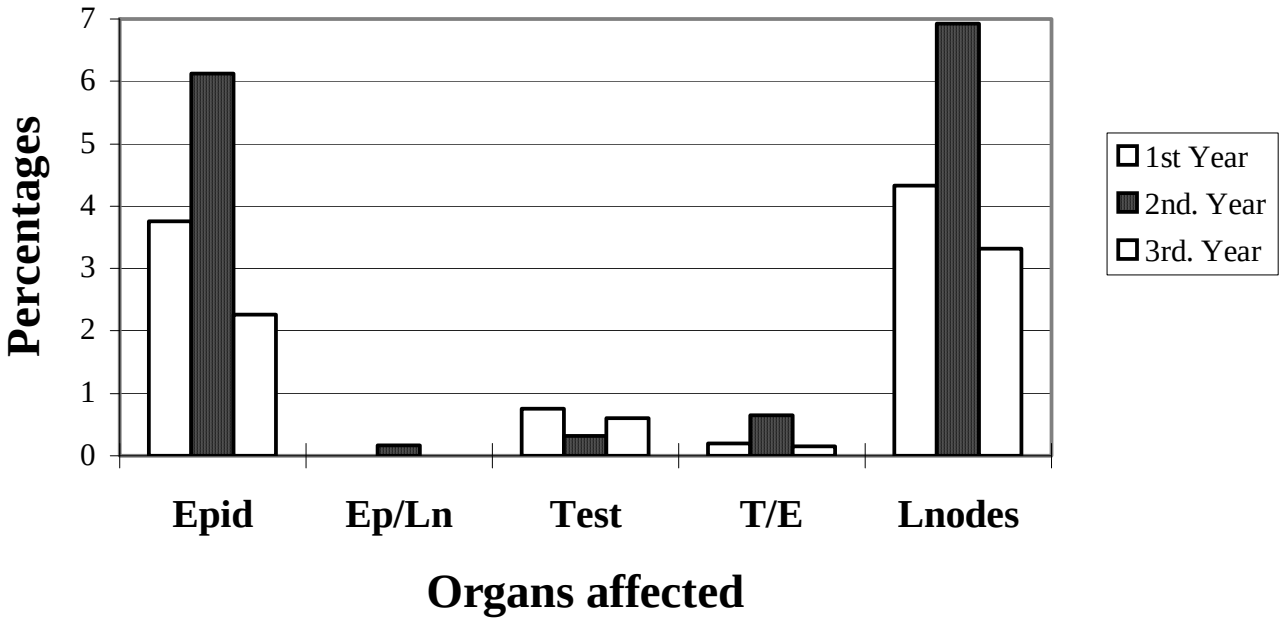
Table 1 : Results of the clinical examinations and *B. ovis* serology of the rams for the three years.

	1st Year	**2nd Year**	**3rd Year**	
				p value
Rams clinically examined	532	620	662	
Rams with any type of lesion	48 9 %	88 14.2 %	43 6.5 %	= 0.00077
Rams with epididymitis	20 3.8 %	40 6.45 %	15 2.3 %	= 0.00192
Rams positive to AGID	11 2.1 %	25 4 %	42 6.3 %	= 0.00131

Figure 1: Distribution of clinical lesions in the 179 affected rams.



Key
Epid: Epidydimis only - **Ep/Ln:** Epidydimis & Lymph nodes -
Test: testes - **T/E:** Testes & epidydimis - **Lnodes:** Lymph nodes only.

Table 2: Pattern of affection of the different parts of epididymis.

	1st Year	2nd Year	3rd Year	TOTAL	%	Conf. Interval
Head affected	0	1	0	1	1.3	0.03 to 7.21
Body affected	2	1	0	3	4	0.83 to 11.2
Tail affected	9	30	13	52	69.3	57.6 to 79.5
Head / Body	1	2	0	3	4	0.83 to 11.2
Tail / body	1	3	1	5	6.6	2.20 to 14.9
All epididymis affected	7	3	1	11	14.7	7.56 to 24.7
TOTAL	20	40	15	75		

Table 3: Number of rams with epididymitis according to the age during the three years.

	1st Year			2nd Year			3rd Year		
Age	Nº rams examined	Nº rams affected	%	Nº rams examined	Nº rams affected	%	Nº rams examined	Nº rams affected	%
Lambs	0	0	**0**	2	0	**0**	21	0	**0**
1 year	167	2	**1.2**	109	1	**0.9**	151	1	**0.6**
2 years	43	1	**2.3**	101	4	**3.9**	83	2	**2.4**
3 years	76	3	**3.9**	148	11	**7.4**	116	7	**6.0**
4 years	172	7	**4.1**	127	10	**7.9**	138	4	**2.8**
> 4 years	74	7	**9.4**	133	14	**10.5**	153	1	**0.6**
TOTAL	532	20	**3.8**	620	40	**6.4**	662	15	**2.3**

Table 4: Number of rams positive to *B. ovis* AGID test according to the age during the three years of study.

	1st Year			**2nd Year**			**3rd Year**		
Age	Nº rams examined	Nº rams positive	%	Nº rams examined	Nº rams positive	%	Nº rams examined	Nº rams positive	%
Lambs	0	0	**0**	2	0	**0**	21	0	**0**
1 year	167	1	**0.6**	109	0	**0**	151	3	**1.9**
2 years	43	0	**0**	101	3	**0.3**	83	3	**3.6**
3 years	76	1	**1.3**	148	4	**2.7**	116	17	**14.6**
4 years	172	3	**1.7**	127	7	**5.5**	138	9	**6.5**
> 4 years	74	6	**8.1**	133	11	**8.2**	153	10	**6.5**
TOTAL	532	11	**2.1**	620	25	**4**	662	42	**6.3**

Table 5: Relationship between rams with clinical epididymitis and positive to *B. ovis* serology.

		EPIDIDYMIS		
		Affected	***Unaffected***	Total
B. OVIS				
SEROLOGY	***Positive***	22	56	78
	Negative	53	1683	1736
	Total	75	1739	1814