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RESEARCH COMMUNICATION

LEPTOSPIRA INTERROGANS SEROVAR *HARDJO* ASSOCIATED WITH BOVINE ABORTION IN SOUTH AFRICA

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ABSTRACT

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Leptospira interrogans serovar *hardjo* was isolated from urine from dairy cattle in the Onderstepoort area. This was the first successful isolation of this serovar as sole agent causing an abortion storm in the Republic of South Africa. Abortions occurred as early as at 4 months' gestation.

INTRODUCTION

Serological evidence of *Leptospira interrogans* serovar *hardjo (L. hardjo)* infection in South African cattle has been available for some time (Herr, unpublished data, 1980). To date, however, attempts at isolation have been unsuccessful, except in one instance where *L. hardjo* was isolated together with *L. pomona* and *L. tarassovi* (Herr, unpublished data, 1980). Elsewhere in the world, *L. hardjo* is recognized as an abortifacient (Blood, Henderson & Radostits, 1979).

When high titres to *L. hardjo* were found in a dairy herd experiencing abortions, an attempt was made to culture the organisms. The abortions had occurred over a period of 6 months, with a gestational age varying from 4 months to a stillborn, full-term calf. A viable heifer was born at 8 months.

The herd was serologically negative for brucellosis, and no other cause of abortion could be found.

MATERIALS AND METHODS

Specimens were taken from cows forming part of a small dairy herd in the Onderstepoort area. Serum samples were taken from 4 cows, and subsequently serum and urine samples were collected from 9 and 10 cows on 2 occasions approximately 7 weeks apart.

Urine collection, bacteriological isolation and examination of formalinized urine were done as described by Herr, Riley, Neser, Roux & De Lange (1982). The amount of 5-fluorouracil[1]/mℓ culture medium was increased to 0,5 mg, as described by Herr & Winnen (1983). Isolations were made and maintained on semi-solid EMJH medium with EMJH enrichment[2]. *Culture* tubes from the 1st isolation attempt were kept for 2 months and those from the 2nd for 6 months.

Sera were tested, using the methods and antigens described by Herr *et al.* (1982).

Typing of isolates was done using the microscopic agglutination microvolume technique (Sulzer & Jones, 1978) with commercially available antisera[2] and antisera kindly supplied by the WHO Collaborating Centres for Leptospirosis in Australia[3] and in the USA[4]. No attempt was made by our laboratory to type the isolates beyond serogroup level.

The isolate from Cow 2K was sent to the WHO Leptospirosis Laboratory, Israel[5] for verification of the serotype.

RESULTS

The results of leptospirosis serology and isolation attempts are summarized in Table 1.

TABLE 1 Serological titres and results from culture of urine specimens

Cow	Titre* A	Titre* B	Titre* C	Isolation B	Isolation C	Comments
2K	—	—	2 560	—	+	P
3.2	640	320	NDX	—	NDX	Ab
6.1	—	—	640	—	—	Ab
1 N	1 280	320	80	—	—	Ab
2	ND	320	NDX	—	NDX	
11	ND	1 280	320	—	+	Ab
3	ND	1 280	40**	—	—	
12	ND	2 560	1 280	—	—	
8	ND	—	—	—	—	
1M	ND	ND	640	ND	+	Ab
2.1	ND	ND	320	ND	—	
1.1	ND	ND	160	ND	—	

* = titres to *L. hardjo* (reciprocal of 50 % end-point)
** = this animal also showed a titre of 40 to *L. canicola, L. pomona* and *L. icterohaemorrhagiae*
Ab = abortion within 6 months prior to the last samples being taken. One other cow, not tested, aborted 2 weeks after the last samples were taken
ND = not done
NDX = not done, died of causes unrelated to abortion problem
A = 1st serological test
B = 1st attempt at isolation
C = 2nd attempt at isolation
P = premature live calf

All the cows that aborted showed a high titre at some stage during the investigation. Cow M1, among the first to abort, had an initially high titre, which had dropped at the time of the first isolation attempt 2 weeks later. Of the cows which did not abort, only 1 showed no titre at all, and 1 had a high titre on 2 occasions.

The first attempt at isolation was entirely unsuccessful. Tubes were discarded as negative after 2 months, there being no leptospirae visible on direct microscopic (dark field) examination.

Three isolates were obtained from the 2nd attempt 7 weeks later.

Only 1 of these (2K) was also positive on examination of formalinized urine. Two culture tubes from 2K were positive by Day 60, and a 3rd by Day 90. The 2 tubes from Cow 1M to become positive were noted as such by Days 90 and 105 respectively. The only positive culture from Cow 11 took 150 days to be noted as such.

[1] Roche Products (Pty) Ltd, 4 Brewery St., Isando

[2] Difco Laboratories, Detroit, Michigan, USA

[3] WHO Collaborating Centre for Leptospirosis, Australia.

[4] WHO Collaborating Centre for Leptospirosis, Atlanta, Georgia, USA

[5] Israel Institute for Biological Research, Box 19, Ness-Ziona, Israel

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All 3 cows had positive titres at the time of isolation.

The results of the typing of an isolate from 2K are recorded in Table 2.

TABLE 2 Titre of isolate 2K against available antisera

Source of antisera	Serovar/serogroup	Titre*
Commercial	Canicola	—
	Icterohaemorrhagiae	—
	Grippotyphosa	—
	Hardjo	6 400
	Pomona	—
	Pyrogenes	—
	Tarassovi	—
WHO Lab, Australia	Hardjo	12 800
	Pomona	—
WHO Lab, USA	Australis	—
	Bataviae	—
	Autumnalis	—
	Canicola	—
	Celledoni	—
	Copenhageni	—
	Grippotyphosa	—
	Javanica	—
	Pomona	—
	Pyrogenes	—
	Sejroe	6 400
	Tarassovi	—

* Reciprocal of 50 % end-point

Typing placed the isolate in the *L. sejroe* serogroup, which includes *L. hardjo*. The WHO Collaborating Laboratory in Israel confirmed the isolate as serotype *L. hardjo*.

DISCUSSION

Although the isolation techniques used for the 1st attempt in no way differed from those used in the 2nd attempt, no isolations were made. This could have been due to the cultures' not having been kept for long enough, since *L. hardjo* is a fastidious grower (Ellinghausen, 1979). The length of time taken for some cultures to become positive lends credence to this assumption. Also, the excretion of leptospirae in the urine is probably intermittent (Faine, 1982).

Isolations were made only from cows that aborted or delivered a premature live calf. These were all serologically positive at the time of urinary excretion.

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