Brucellosis surveillance and control in Zimbabwe: bacteriological and serological investigation in dairy herds

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ABSTRACT

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Brucellosis in dairy cattle is endemic in Zimbabwe. The prevalence continues to be monitored intensively. Only milk and serum samples are routinely screened. Attempts to culture *Brucella* spp. from clinical specimens are seldom made. Consequently, incidence of various *Brucella* spp. within Zimbabwe is virtually unknown, despite the high serepositivity reported. This information is paramount in understanding the transmission cycle and is also significant to public health; particularly as *B. melitensis* infects humans more often than do the other brucellae.

This paper describes the results of bacteriological and serological investigations of brucellosis in a dairy from near Bulawayo. The said farm was selected for the present pilot study because of the high incidence of reported abortion.

The milk ring test was employed to test the bulk pooled milk samples once a month for 14 months. The test was recorded highly positive on all 14 occasions. To locate reactors, milk samples from 36 individual cows were similarly tested. Of these, 21 (almost 59 %) were found to be reacting positively. One hundred and seventy-seven animals were marked for serotesting. Of these, 40 (approximately 25 %) showed quite high serum titres (> 1:360) in both the STT and the Rosebengal test. The farmer was advised to havet all abortions fully investigated. However, all the clinical material from cases of abortion, except one, were received in an advanced state of putrefaction. From this, *Brucella* was isolated on culture from stomach contents and cotyledons. The isolates from both the sites were characterized in detail, employing dye inhibition, phagetyping; the oxidative metabolic test and agglutination with monospecific sera. Both the isolates belonged to *B. abortus* biovar I, which was confirmed by the Central Veterinary Research Laboratory, Weybridge.

The significance of isolation and the need to intensify similar studies have been discussed.

Keywords: Bovine, brucellosis, control, surveillance, Zimbabwe

INTRODUCTION

Brucellosis in dairy herds in Zimbabwe was reported as early as 1913, when serologically positive animals were identified, following abortion storms around Harare (Bevan 1931). Since then, brucellosis has constantly been monitored serologically as well as by use of the milk ring test (MRT) (Bryant & Norval 1985; Manley 1969; Swanepoel, Blackburn & Lander 1976). The results of these investigations led to the introduction of calf vaccination with strain 19 (Onderstepoort vaccine), on most commercial farms. Following the recent introduction of the Accreditation Scheme, seromonitoring has been made compulsory

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for the commercial sector. Consequently, a great deal of data confirming endemicity of brucellosis in Zimbabwe have accumulated, but reports of attempts to culture *Brucella* are scanty. Madsen (1989), however, reported that 74 isolates of *B. abortus* were cultured from aborted foetuses during 1981 to 1987, but the procedures followed to culture and confirm the isolates were not described, nor do the isolates appear to have been characterized in detail.

In this paper we report on the seroprevalence of brucellosis in dairy herds in Zimbabwe from 1992 to 1995, the culture of *Brucella* from aborted foetuses and detailed characterization of the isolates. The results are discussed in the light of the current brucellosis-control programme in Zimbabwe.

MATERIALS AND METHODS

The antigens from Onderstepoort were used in the standard tube test (STT) and the Rosebengal test (RPT) for the seromonitoring, and FAO/WHO-recommended procedures were followed (Alton, Jones & Pietz 1975). The procedures followed to set up and read the tests, were essentially the same as those Williamson & Herr (1987) described for canine-brucellosis testing. From each of the serologically positive dairy herds of two commercial farms reporting frequent abortions, one near Bulawayo and the other near Chinhoyi, two full-term aborted foetuses were investigated. The fifth foetus was received from a new farm near Mazoe. The stomach contents, spleen and liver of each foetus were cultured on *Brucella* agar enriched with the recommended supplements (Oxoid). Cultures were set up for other aerobic bacteria, as well as for *Campylobacter*, on 10% sheep-blood agar, chocolate agar and MacConkey agar (Oxoid). Selective medium (Oxoid) and recommended procedures for incubation were used. Cold enrichment technique as described by Carter & Chengappa (1991) was employed for the isolation of *Listeria*. The isolates of *Brucella* were identified at the Faculty of Veterinary Science, University of Zimbabwe. The identification was based on Gram-stained morphology, cultural characteristics and the results of the following biochemical and physiological tests: Growth in air, in $C0_2$ and anaerobically; catalase; oxidase; motility; O/F glucose; urease; H_2S with lead-acetate paper; nitrate and nitrite reductase; and growth in the presence of basic fuchsin, thionin, safranin and agglutination in the polyvalent *Brucella* anti-serum. The isolates were further characterized in detail, and confirmed at the FAO/WHO Reference Centre of the Central Veterinary Laboratory, Weybridge, by means of the oxidative metabolic test, with the recommended range of sugars, amino-sugars, and amino acids; and lysis with six different bacteriophages, and agglutination with mono-specific antisera.

RESULTS

Details of serology are shown in Tables 1, 2 and 3. Results of the identification tests are shown in Table 4. Stomach contents from all five foetuses yielded pure culture of *Brucella*, while the cultures from the spleen and liver of three foetuses also grew post-mortem contaminants. No other bacteria of pathological significance were isolated.

All five isolates have been identified as *B. abortus* biotype I, but the C1 and C2 strains differed from those of B1 and B2 strains in their $C0_2$ requirements and their sensitivity to thionin (Table 4).

Besides the results of characterization detailed in Table 4, the oxidative metabolism of the four strains for which the tetrazolium-microplate assay technique was used, was studied at the Reference Laboratory with the following substrates: L-alanine; L-asparagine; L-glutamic acid; L = arginine; L = ornithine; L=

Table 1 Bovine brucellosis serology on commercial farms

Region	Year	No. of farms tested	No. of sera tested	Total no. of sera tested	% positive
Mashonaland Central	1992	12	1 815	11	0,60
	1993	29	1 450	11	0,75
	1994	8	2 139	27	1,26
Mashonaland East	1992	106	39 585	301	0,76
	1993	157	25 765	262	1,01
	1994	61	23 087	246	1,06
Mashonaland West	1992	24	13 183	106	0,80
	1993	81	8 319	84	1,00
	1994	13	4 783	31	0,64
Manicaland	1992	41	9 056	18	0,19
	1993	37	7 569	22	0,29
	1994	7	6 912	22	0,31
Midlands	1992		12 768	114	0,89
	1993	65	10 239	283	2,76
	1994	16	2 603	27	1,03
Masvingo	1992	32	13 183	66	0,50
	1993	13	898	2	0,22
	1994	4	1 513	18	1,18
Matebeland	1992	45	9 463	69	0,72
	1993	35	4 314	50	1,15
	1994	6	1 465	60	4,09

lysine; D = galactose; D = ribose; D = xylose; erythritol and urocanic acid. These results also confirmed our identification; but the people at Weybridge have yet to publish the microplate assay technique, hence the results are not reproduced here.

TABLE 2 Small-scale or communal farms

Region	Years	No. of farms tested	No. of sera tested	Total no. positive	% positive
Mashonaland Central	1992	5	253	2	0,79
	1993	14	40	0	0
	1994	1	34	1	2,94
Mashonaland East	1992	25	352	4	1,13
	1993	54	552	2	0,36
	1994	4	420	2	0,47
Mashonaland West	1992	7	50	0	0
	1993	27	104	0	0
	1994	3	96	3	3,12
Manicaland	1992	6	59	0	0
	1993	15	523	11	2,10
	1994	1	183	0	0
Midlands	1992	2	344	2	0,58
	1993	29	486	61	12,55
	1994	1	5	0	0
Masvingo	1992	0	0	0	0
	1994	0	0	0	0
	1993	5	57	1	1,75
Matebeleland	1992	12	27	0	0
	1993	24	127	5	3,93
	1994	2	42	9	21,40

DISCUSSION

Certain countries, other than those from Africa, appear to have eradicated brucellosis in dairy cattle. Zimbabwe initiated control measures aiming at possible eradication by introducing compulsory calf vaccination on commercial farms. This has been reinforced by legislating an Accreditation Scheme whereby a dairy herd which passes three consecutive MRTs and STTs within a year, and the fourth test a year later, is accredited. It is too early to assess the success of this scheme, but the percentage of seropositive animals in the country does not appear to be declining; instead it showed an upward trend during the 3-year period from 1992 to 1994 (Tables 1 and 2). Calf vaccination has an inherent disadvantage related to interpretation of the results of STT, which currently happens to be the main method of sero-monitoring. Several dairy farmers have felt uneasy about the results of STT and MRT, suspecting that the positive reaction might be attributable to strain 19 vaccine, the use of which is not regulated by the Veterinary Surgeons Act.

The Accreditation Scheme also does nothing to solve the problem of brucellosis in small ruminants kept together with dairy herds, as found on a number of farms throughout Zimbabwe. Although *Brucella* spp. tend to discern host predilection in causing overt disease, cross-infection between cattle and small ruminants is not uncommon (Verger 1985). In a survey on communal land in Zimbabwe, goats have been found to be seropositive (Halliwell, Honhold & Schlundt 1987). It is worth mentioning that, over the past 20 years, only *B. abortus* was thought to be present in South Africa, but recently, *B. melitensis* was isolated from goats (Ribeiro, Herr, Chaparro & Van der Vyver 1990).

A well-drawn brucellosis-control programme should interrupt the transmission cycle as well as cross-infection between different livestock species on farms, and it should effectively differentiate between the infected seroreactors and those which might have *Brucella* agglutinins due to factors other than actual infection. Attempts at bacteriological isolation from seropositive animals and a detailed characterization of the isolates would provide convincing evidence on the status of the seroreactor, and might lead to identification of the possible source of infection. We want to point out that the current surveillance and control programme in Zimbabwe needs to be critically reviewed with a view to extending it to small ruminants kept along with the dairy herd, and we also suggest that the Accreditation Scheme should include a bacteriological investigation of all cases of abortion.

Although we investigated only five foetuses, the Bulawayo and Chinhoyi farms have reported abortion storms with a high percentage of serepositive animals (Table 3), recording serum titres as high as 1 in 10 240 (2 500 IU) in some cases. The new farm near Mazoe does not appear to have been serologically monitored for brucellosis. All five isolates cultured have been identified as *B. abortus* biotype I. Although these isolations are from three geographically different regions, they are too few for any definite conclusion to be drawn as to the prevalence of *Brucella* biotypes in Zimbabwe. It might well be that with more isolations, bovine brucellosis in Zimbabwe will eventually prove to be similar to that in South Africa, where over 90% of the isolates have been found to be *abortus* biotype I (Herr, Lawrence, Brett & Ribeiro 1991). It is noteworthy that *abortus* biotype

TABLE 3 Details of serology in *B. abortus* biotype 1 positive, as tested on Bulawayo and Chinhoyi farms

Period	Test	No. tested		No. positive		% positive	
		Bulawayo	Chinhoyi	Bulawayo	Chinhoyi	Bulawayo	Chinhoyi
January 1994	STT	58	5	15	2	26	–
May 1994	STT	39	–	9	–	23	–
June 1994	STT	41	–	11	–	27	–
July 1994	STT	39	–	10	–	26	–
January 1994–June 1995	MRT Bulk samples	14	–	14	5	100	100
January 1994–June 1995	MRT Individual samples	36	20	21	14	58	70
January–August 1995	STT	–	112	–	16	–	14

TABLE 4 Detail results of culture identification

Tests	Isolates				
	B1	B2	C1	C2	MI
Gram–negative coccobacilli	+	+	+	+	+
Motility at 37 °C and 22 °C	–	–	–	–	–
Modified Z.N. stain	+	+	+	+	+
H_2S lead acetate	+	+	+	+	+
Urease**	+	+	+	+	+
CO_2 for growth	–	–	+	+	+
Anaerobic growth	–	–	–	–	–
Simon's citrate	–	–	–	–	–
Indole	–	–	–	–	–
O/F glucose	NR	NR	NR	NR	NR
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Nitrate and nitrate reductase	+	+	+	+	+
Growth in the presence of basic fuchsin	+	+	+	+	+
and Thionin: 1:50,00 concentration	–	–	–	–	–
Safranin: 1:10,000 concentration	+	+	+	+	+
Lysis with Phages at RTD					
Tb	CL	CL	CL	CL	NT
Wb	CL	CL	CL	CL	NT
BK_2	CL	CL	CL	CL	NT
Iz	CL	CL	NT	NT	NT
Fi	CL	CL	CL	CL	NT
R/C	NL	NL	NL	NL	NT
Agglutination with monospecific sera					
Abortus type	+	+	+	+	+
Melitensis and rough type	–	–	–	–	–

B1, B2: Bulawayo isolates 1 and 2
C1, C2: Chinhoyi isolates 1 and 2
MI: Mazoe isolate
** Reaction within 2 h for B1 and B2, and within 3 h for C1 and C2
A very poor growth in one out of five streaks was recorded with thionin for C1 and C2 strains
CL: Confluent lysis

I infection has been reported to persist in cows for 7–9 years (Lapraik & Moffat 1982; Herr, Ribeiro & Chaparro 1990). Incidentally, this is the first report of detailed characteristics of *Brucella* isolated in Zimbabwe.

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