

BRUCELLA SUIS INFECTION IN PREGNANT CATTLE

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SUMMARY: Six pregnant, *Bos taurus* cows with stages of gestation ranging from 11 to 33 weeks were each inoculated into the right conjunctival sac with 0.2 ml of a smooth culture of *Brucella suis* type I containing 27×10^6 viable organisms. The 6 cows produced 7 calves of which one single calf and one twin calf were stillborn, the cause of which was not determined. *Br. suis* was not isolated from any of the cows or calves using either special media or guinea pig inoculation. No abnormality was found in any of the cows or calves at autopsy. Microscopic examination of placentas and tissues from stillborn calves revealed no abnormality. Serologically, 2 weeks after inoculation all 6 cows had positive reactions to the Rose Bengal Test (RBT) and serum agglutination (SAT) titres of 25 iu to 116 iu. However, these reactions disappeared within 11 weeks. Only 2 cows had a complement fixation (CFT) titre which lasted a maximum of 5 weeks and reached a titre of 4/4.

Following the anamnestic use of *Br. abortus* strain 45/20 vaccine on 3 of the cows, positive RBT reactions, SAT titres of 33 iu, 29 iu and 58 iu and CFT titres of 4/16, 1/8 and 3/8 respectively were recorded 6 weeks after vaccination.

Introduction

Cattle may become infected with *Br. suis* when they live in close association with infected pigs and especially aborting sows (Elder 1946). Natural exposure to *Br. suis* infection may produce serum agglutination titres of up to 1/200 in cattle for 4 to 6 weeks (Cotton *et al* 1938). No clinical signs including abortion have been reported in cattle (Meyer 1966) as a result of natural exposure to *Br. suis*. Nevertheless *Br. suis* has been found to localise in the udder of naturally infected cows and to be excreted in the milk (Beattie and Rice 1934; Horning 1935; Borts *et al* 1943).

In northern Queensland cattle are known to graze pastures where *Br. suis* has been isolated from feral pigs (Norton and Thomas 1976), and this was suggested by Trueman *et al* (1979) as a possible cause of single CFT reactors. It was considered desirable to know what effect *Br. suis* infection might have on the serological tests for brucellosis used in the National Tuberculosis and Brucellosis Eradication Scheme (NTBES). Other information also sought in the experiment included whether *Br. suis* would readily produce abortion, whether localisation of infection would occur in cattle and whether *Br. suis* might also sensitise cattle to the *Br. abortus* strain 45/20 anamnestic test.

Materials and Methods

Animals

Six pregnant *Bos taurus* cows from a brucellosis free herd known also to be free from infection with *Campylobacter fetus*

subsp. *venerealis* and *Tritrichomonas foetus* were used. The cows numbered 1 to 6 were aged 2.5 years to 5.5 years, and the stage of gestation ranged from 11 to 33 weeks (Table 1) as estimated by rectal examination. The cows were kept in pairs that is 1 and 2, 3 and 4, 5 and 6.

Inoculum

The inoculum containing 27×10^6 organisms was a smooth culture of *Br. suis* type I stabilate Oonoonba-12 previously isolated from a feral pig (Norton and Thomas 1976).

Experimental Procedure

Prior to the commencement of the trial, a vaginal swab and a pooled sample of mammary secretion from all 4 quarters of each cow were cultured for *Brucella* spp. The cows were bled weekly, commencing 2 weeks prior to the date of inoculation until the time of slaughter. The cows were inoculated via the right conjunctival sac.

Calves were killed within 24 hours of birth and autopsied. The following samples including a vaginal swab, colostrum from each quarter and foetal liver, kidney, spleen, lung, heart and abomasal contents were cultured and pools of these samples homogenised and injected into 4 guinea pigs. Because of contamination the cotyledon samples were cultured only. Where stillbirths occurred, foetal abomasum, liver, kidney, spleen, lung, heart and cotyledon were collected for histopathology.

Six weeks after calving, cows 1, 4 and 5 were killed and autopsied. The following samples including pooled mammary secretion, pieces of uterus, spleen, supramammary, ischiatic, internal iliac, right parotid, right mandibular and retropharyngeal lymph nodes were cultured and pools of the samples homogenised and injected into 4 guinea pigs.

Six weeks after calving, cows 2, 3 and 6 were inoculated subcutaneously with 2 ml of *Br. abortus* strain 45/20 vaccine*. After a further 6 weeks, these cows were killed, autopsied and samples collected as for the other 3 cows.

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TABLE 1

Details of Foetuses Following Inoculation of Pregnant Cows with *Br. suis*

Cow No.	Length of Gestation at Inoculation (wks)	Viability at Birth
1	24	alive
2	21	alive
3	11	alive
4	32	stillborn†
5	33	alive
6	19	* { alive stillborn†

* twins † fullterm foetuses

Serology

Serums were stored at -10°C and serological tests including the Rose Bengal plate test (RBT), the microcomplement fixation test (CFT) (Anon 1977) and the serum agglutination tube (SAT) test for brucellosis were performed using standard *Br. abortus* antigen* on each serum.

Bacteriology

All samples were macerated with normal saline and aliquots streaked on to sheep blood agar, MacConkey agar (Oxid)†, and the selective media A and C of Brodie and Sinton (1975). A brucella selective broth (Brodie and Sinton 1975) was also inoculated, and after 3 to 4 days this was used to streak each of the above media also.

Results

All 6 cows remained healthy throughout the trial and 4 of them produced normal healthy calves. Cow 4 had a stillborn calf. Cow 6 had twins, one of which was stillborn, while the other was normal and healthy. Both stillborn calves were fully developed, full term foetuses. No lesions were seen in any of the cows, calves or stillborn foetuses. Microscopic examination of placentas and tissues from stillborn calves revealed no abnormality.

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†Oxoid Australia Pty Ltd. Hurstville, New South Wales 2220

No *Brucella* spp were isolated from any of the samples. All guinea pig serums were negative serologically to the CFT and RBT.

Two weeks after inoculation, all 6 cows had a 2+ or 3+ reaction to the RBT which turned negative over the following 3 to 11 weeks, and a SAT titre of 25iu to 116iu which decreased to less than 25iu over the following 3 to 11 weeks. Some cows had sporadic, low RBT reactions of 1+ and SAT titres of up to 58 iu over the remaining duration of the experiment (Table 2). Only cows 1 and 4 had CFT titres which were detected within 4 weeks and 2 weeks respectively of inoculation and which lasted 4 weeks and 6 weeks respectively. The maximum titre reached in either case was 4/4 (Table 2).

Following calving, all serological tests remained negative or continued to decline following the initial response to inoculation.

Six weeks after the anamnestic use of *Br. abortus* strain 45/20 vaccine, cows 2, 3 and 6 gave RBT reactions of 2+, 2+ and 3+, SAT titres of 33 iu, 29 iu and 58 iu and CFT titres of 4/16, 1/8 and 3/8 respectively.

Discussion

Although the conjunctival route was successfully used by Cotton *et al* (1938) to infect cattle and to produce abortion, we were unable to achieve a similar result.

The low transient SAT titres to brucellosis which we found were similar to those obtained by Cotton *et al* (1938) in cattle naturally exposed to *Br. suis*. However, Washko and Hutchings (1951) were able to produce SAT titres well in excess of 1/400 (672 iu) following intramammary inoculation which produced acute mastitis.

The important finding is the strong RBT reactions which could be of importance in the NTBES, although the CFT appears to remain negative or

TABLE 2

Serological Reactions in 6 Pregnant Cows Following Inoculation with *Br. suis* type 1 Oonoomba-12 into the Conjunctival Sac

Cow No.	RB		SAT		CF	
	Weeks Positive	Maximum Reaction	Weeks Positive (> 25 iu)	Maximum Titre	Weeks Positive	Maximum Titre
1	1-9, 11, 17 19-21	3+	2-11, 13, 15, 17 19-22	66 iu	4-7	4/4
2	2-4	2+	2-9, 20	42 iu	—	—
3	2-5, 8	2+	2-4	29 iu	—	—
4	2-12	3+	2-12	100 iu	2-6	4/4
5	2-8	2+	2-10	58 iu	—	—
6	2-8, 17, 18	3+	2-8, 17, 18	66 iu	—	—

reach only low titres. However, *Br. suis* could be responsible on occasions for single CFT reactors in north Queensland as suggested by Trueman *et al* (1979).

Our data suggest that animals exposed to *Br. suis* and then tested anamnesticly may give positive CFT titres and be classified as brucellosis infected. This is reasonable since *Br. abortus* and *Br. suis* share a common surface antigen (Wilson and Miles 1964). This could in part explain some of the apparent anomalous reactions that have been recently observed in north Australian herds undergoing eradication.

Twinning and lack of exercise are associated with an increased number of stillbirths (Roberts 1971) and this could explain the loss of 2 of our calves.

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