

OBSERVATIONS ON SINGLE BRUCELLOSIS REACTORS IN LARGE HERDS

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SUMMARY: Over a 3 year period specimens were collected from single reacting cattle to the complement fixation test for brucellosis from large herds in north Queensland. Twenty-four such reactors were destroyed for culture, and *Brucella abortus* was isolated on 3 occasions. The existence of such low levels of *Br. abortus* in large herds should not be overlooked in the eradication campaign.

Explanations were found for 17 of the single reactors, and included introduction of cattle to the herd, exposure to adjoining infected herds or vaccination with Strain 19.

Introduction

Serological testing in North Queensland associated with the National Brucellosis Eradication Campaign, has revealed a number of herds containing a single reactor to the complement fixation test (CFT). The significance of these reactors is unknown, but their occurrence can be a cause of concern when classifying herds as infected or non-infected.

Materials and Methods

From January 1975 until December 1977 single CFT reactors were slaughtered and specimens taken for culture. Samples were received from 24 animals representing 23 properties. Other single reacting animals were located, but no specimens were received from these for culture. Only herds of at least 300 breeding animals, and in which the prevalence of CFT reactors was 0.33% or less, were considered. All serums from herd bleedings were subjected to the Rose Bengal test (RBT) (Morgan *et al* 1969), and any serums agglutinating with RBT antigen were then tested in the CFT. Until February 1976 the CFT was performed employing warm fixation in tubes. Serial dilutions from 1/5 to 1/80 were set up. Subsequently the micro CFT was employed with dilutions from 1/4 to 1/256. The Australian Standard Method (Anon. 1977) was followed. After slaughter at least 3 and up to 9 tissues were submitted on ice from each single reacting animal. Supramammary lymph nodes were always received from female cattle, and other specimens included retropharyngeal, ischiatic and iliac lymph nodes, spleen, uterus, udder, milk, foetus and any suspect lesion. From bulls the specimens included testes, seminal vesicles, iliac and superficial inguinal lymph nodes and spleen.

Tissues were alcohol flamed (Alton *et al* 1975) and then homogenised separately in serum broth. At all times individual samples were plated onto sheep blood agar and MacConkey agar, and incubated aerobically at 37°C for 3 to 5 days. Samples were also plated onto sheep blood agar which was incubated at 37°C in an atmosphere containing 10% CO₂. From December 1975 until December 1976 a selective Brucella agar with antibiotics (Nelson *et al* 1966) was included and was incubated in the same way. From January 1977 Brucella agars A and C and Brucella broth (Brodie and Sinton 1975) were inoculated and incubated at 37°C in an atmosphere containing

10% CO₂. For the biological test, homogenised lymph nodes were pooled, and other homogenised tissues were placed in a second pool. From each pool 1 ml was inoculated intraperitoneally into a separate guinea pig. After 6 weeks the guinea pigs were sacrificed, and serums collected for the RBT and CFT. A portion of the spleen was removed, and rubbed over a blood agar and a Brucella selective plate of the period and incubated. Colonies resembling *Br. abortus* by agglutination and staining techniques from either primary or biological methods were sent to the WHO Brucellosis centre at the Commonwealth Serum Laboratories, Parkville, Victoria, for biotyping.

Enquiries were conducted on each property in an attempt to explain the reason for the single reacting animals.

Results

From the 24 single reacting animals *Br. abortus* was isolated on 3 occasions (Table 1), all isolates being recovered only following guinea pig inoculation.

Pooled organs including foetal tissues yielded *Br. abortus* biotype 1 from the cow detected on property No. 1. No cattle had been introduced into the herd for the previous 15 years, apart from the possible exception of bulls. Cattle on surrounding properties were tested and no RBT reactors were found. *Br. abortus* biotype 1 was recovered from the supramammary lymph node of a cow from property No. 2. Animals from an adjoining infected property were known to get onto this property. The cow from property No. 3 had a swollen bursa under the ligamentum nuchae, and from the contents of the bursa pooled with udder tissue *Br. abortus* biotype 2 was isolated. No cattle had been introduced onto this property for several years. One adjoining property was found to be infected.

Of the remaining 21 single reactor animals 8 had been introduced to the herds (3 from properties subsequently found to be infected), 4 were on

TABLE 1

Single Complement Fixation Reactors from which *Brucella abortus* was Isolated

Animal and Property Number	No. of Breeders	No. of RBT 1975-77	No. RBT+	Age and Sex of CF Reactor	CF Titre	Gestation Status	Status of Adjoining Properties
1	1,800	1,505	1	4y ♀	80†	8m	tested negative
2	2,000	4,293	2	6y ♀	80†	Empty	1 infected
3	2,000	3,974	2	6y ♀	64*	Pregnant †	1 infected

† Warm fixation in tubes

* Warm fixation micro test

+ Length of Gestation not recorded

properties adjoining infected herds and 2 had been vaccinated with Strain 19. No explanation could be found for the other 7 single reactor animals.

Discussion

Nelson *et al* (1966) reported a recovery rate of 80% from the supramammary lymph nodes of cattle infected with *Br. abortus*, and with the inclusion of other nodes and tissues up to 93% could be expected. It is possible therefore that more of the single reactor cattle may have been infected, but were not detected by culture.

The use of selective media as described by Brodie and Sinton (1975) is reported to be equally as sensitive as guinea pig inoculation when examining tissues for *Br. abortus* (Robertson *et al* 1977). Our 3 isolates were made prior to using the media of Brodie and Sinton (1975), and this could explain the failure to detect *Br. abortus* on primary culture, although contaminating organisms were only a problem in a few instances.

Examination of the herd history often provided an explanation for the apparent exposure to *Br. abortus*. However, satisfactory explanations could not be found for some of the single reactors, including 1 of the 3 animals from which *Br. abortus* was isolated. It is possible that in some cases the serological titres may have been due to other factors such as cross reaction with *Yersinia enterocolitica* (Ahvonen *et al* 1969). Also as feral pigs are numerous on most of the properties concerned, exposure to *Br. suis* (Norton and Thomas 1976) may have been involved.

Prior to the eradication campaign, Rogers *et al* (1972) using the serum agglutination test, found the serological incidence of brucellosis in north Queensland was only 1.8%, and if 4 Barkly Tableland properties were excluded the overall incidence of reactors was reduced to 0.4%. Despite

the generally low incidence, it would seem unusual for *Br. abortus* infection to exist in single breeding animals without spread to susceptible, in-contact females. However, our 3 isolations of *Br. abortus* from 24 single serological reactors indicate that a very low level of infection can exist under the circumstances described. This being so, it is important to the eradication campaign that single reactors not be lightly dismissed or casually explained.

Acknowledgments

The considerable assistance given by staff of the Veterinary Services Branch in collecting specimens and obtaining herd histories is gratefully acknowledged. We are also grateful to the Commonwealth Serum Laboratories for typing the strains of *Br. abortus*.

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Addendum

Since submission of this article a further 1200 tests have been performed on property No. 1 and another CFT reactor detected (titre 4/128). This was a 6 year old homebred empty cow, and *Br. abortus* was isolated from the supramammary lymph nodes on primary culture. It is unknown if this cow had been previously bled for testing.