Monitoring of dairy herds for *Brucella abortus* infection when prevalence is low

D C ROLFE* and W E SYKEST†

SUMMARY: A total of 2,698 dairy herds were surveyed in 1981-1982 in New South Wales and north eastern Victoria in a review of the methods used to monitor them for the presence of *Brucella abortus*. The methods used to monitor dairy herds were testing of all breeding cows over 1 year of age using the rose bengal test (RBT) and complement fixation test (CFT), the bulk milk ring test (BMRT), and testing of blood samples collected at abattoirs using the RBT and CFT. The surveyed herds had at least one whole herd test, and BMRT was done at regular intervals in the period of the survey.

Of the 99 (3.7%) herds that reacted to the BMRT, 91 (3.4%) herds had false positive reactions and 8 (0.3%) herds were declared infected on follow-up herd testing. False-positive reactions were obtained in 22 herds on more than one occasion. Common causes of false positive reactions to the BMRT were thought to be previous vaccination with Strain 19 and sampling in very early or late lactation.

Of the 98 (3.63%) herds that reacted to the whole herd serological tests, 80 (2.96%) herds had false-positive reactions and 18 (0.67%) herds were declared infected. Strain 19 vaccination was thought to be an important cause of false-positive reactions. Fifty-three (2.0%) herds showed suspicious reactions on abattoir monitoring but none was declared infected on follow-up testing.

Of the 18 herds with infected or equivocal status, the BMRT identified 8. In a further 6 herds, the infected cattle were not in the milking herd. Four other herds had milkers with high CFT titres which could not be confirmed as infected on culture. In no herds were culture positive RBT or CFT reactors from the milking herd detected without the BMRT being positive. The proportion of false-positive reactions to the BMRT was high but the BMRT proved very useful in identifying dairy herds infected with *B. abortus* when the prevalence of brucellosis was very low.

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Introduction

The National Brucellosis Eradication Campaign started between 1975 and 1977 in south eastern Australia, and a variety of methods were used in the campaign (Anon 1979a). Heavy reliance was placed on whole herd serological tests to confirm that herds remained free of brucellosis. While this was costly, the serological tests used were known to be highly accurate (Nicoletti 1969). Alton *et al* (1975a) found that the complement fixation test (CFT) identified all of 79 culture positive cattle at a serum dilution of 1/4 or above. The rose bengal test (RBT) identified 78 of the same cattle.

The bulk milk ring test (BMRT) is a highly sensitive test for detecting brucellosis in an infected dairy herd (Pietz 1977). Reopke *et al* (1950) found the BMRT to be positive for 88% of the herds in which infected animals were in production. The BMRT identified 68% of infected dairy herds overall but 32% of herds with positive BMRT reactions were later classed as negative on herd test. Reopke and Stiles (1970) estimated that on a single sampling of a herd there was a 65% probability of detecting one reactor cow in > 95% of herds. This probability would increase if sampling were done on a number of occasions throughout the year.

There is little published information on the performance of abattoir monitoring in Australia and there are great difficulties in sampling at a sufficient level to ensure reliable detection of infected herds when herd prevalence is low (Rolfe 1984).

As the Brucellosis Eradication Campaign enters the final monitoring phase, there is a need to review the performance of the available monitoring procedures. Use of these procedures can then be related to cost effectiveness and ability to detect infected dairy herds when prevalence is very low.

Dairy herds are of special interest in the campaign because prevalence of brucellosis has been high in them in the past,

the incidence of new infection has been higher than in beef herds, there is regular trading in cattle and these are usually heifers, the enterprises are intensive in nature, the environment favours survival of the organism on the ground, and many management practices favour spread from one animal to another.

This survey in south eastern Australia was carried out to review the performance of the whole herd serological tests, abattoir monitoring and BMRT in identifying infected herds and monitoring non-infected herds when the herd prevalence of brucellosis was known to be very low, 0.67% in the survey period. Additionally, the usefulness of results of monitoring procedures to veterinarians supervising the eradication campaign were reviewed.

Materials and Methods

A total of 1,442 dairy herds in New South Wales and 1,256 dairy herds in north eastern Victoria were surveyed in 1981 and 1982 out of a population of about 3,000 dairy herds in New South Wales and 2,140 in north eastern Victoria. To be included in the survey a herd was required to have been regularly tested using the BMRT and to have had at least one whole herd test using the RBT and the CFT. The BMRT was carried out on all registered dairy herds in New South Wales on a monthly basis because of all year round calving. Allowance was made for milking herd size by varying the quantity of milk tested (Anon 1979b). Individual milk ring tests (IMRT) and culture of milk for *B. abortus* were done using the methods described by Anon (1979b). Mastitis examination was carried out using the methods described by Hoare (1985).

Dairy production in north eastern Victoria was mainly seasonal. The BMRT was carried out 6 times each lactation and was timed to ensure that each herd had at least 4 tests per lactation.

In both states, whole herd serological testing using the RBT and CFT was done every 2 to 3 years. The RBT and CFT

* Division of Animal Health, Level 8, McKell Building, Rawson Place, Sydney, 2000

† Regional Veterinary Office, Box 388, Benalla, Victoria, 3672

were carried out using methods described by Anon (1979b). The indirect haemolysis test (IHLT) was used as a supplementary test in some herds (Plackett *et al* 1976). Culturing of lymph nodes was done as described by Corner *et al* (1985) in New South Wales and Farrell (1974) in Victoria on cattle with suspicious or positive reactions in the serological tests.

Breeding cattle from all herds were sampled on slaughter at an abattoir in Australia and the serums were tested using the RBT and CFT.

Data for the survey was collected by veterinarians responsible for the supervision of the National Brucellosis Eradication Campaign. Using the results from monitoring procedures and local knowledge, they decided whether a herd was infected.

Results

Herds Classed as Not Infected

Monitoring by the BMRT — 2,698 herds were sampled and 2,680 (99.3%) were classed as not infected during the period by their supervising veterinarians. Table 1 shows the number of herds in each BMRT classification and their status after follow-up investigations. Ninety-one herds (3.4% of those surveyed) were considered to show false-positive reactions to the BMRT (92% of herds with BMRT reactions). Veterinarians used whole herd serological testing and herd history to decide on the status of a herd with a positive BMRT.

TABLE 1
BMRT reactions and subsequent classification of herds as infected, not infected or equivocal status.

BMRT reaction	Number of herds with status assessed as			Total
	Not infected	Equivocal	Infected	
0	2589	5	5	2599
1	30	0	2	32
2	41	0	1	42
3	15	0	5	20
4	5	0	0	5
TOTAL	2680	5	13	2698

In New South Wales the causes of false positive BMRT were largely unknown (in 37 herds of 44 herds). Mastitis was thought to be the cause in 2 herds, *B. abortus* strain 19 vaccination in 2 herds; and a combination of these factors in 3 herds.

In north eastern Victoria sampling in the late lactation of seasonally calving herds was believed responsible for most of the false positive BMRT reactions in 1981/82 (26 of 46 herds — 54%). In 1982/83, testing only in early and mid lactation resulted in only 8 herds giving BMRT false-positive reactions. Other reasons cited for false-positive reactions were sampling in very early lactation, mastitis and strain 19 vaccination.

Twenty-two herds, 18 of which were in Victoria, showed multiple BMRT reactions. The causes of multiple BMRT reactions in Victoria were thought to be infection with *B. abortus* (1 herd), sampling in late lactation (5 herds), sampling in early lactation (1 herd), mastitis (2 herds), Strain 19 (3 herds), and unknown (6 herds). On follow-up testing 3 herds had what were considered to be false-positive reactions to the CFT. The change in sampling policy in 1982/83 resulted in only 3 herds with multiple BMRT reactions compared with 15 in 1981/82.

Monitoring at abattoirs — Monitoring at abattoirs resulted in 53 (2%) of the surveyed herds coming under suspicion; 51 herds had one traceback animal and 2 herds had 2 tracebacks. CFT titres ranged from 4 to > 32; 47 herds had a subsequent herd test with negative results. No action was taken in 3 herds (because they had been disbanded), and 3 herds had reactors on herd test but where later classed as not infected. BMRT was positive in only 2 of the 53 herds, but both were resolved

as being non-infected on subsequent whole herd serological testing.

Monitoring by serological testing of whole herds — 80 herds (2.96% of surveyed herds) were considered to have false-positive reactions to the CFT. Of these herds, 8 also had false-positive BMRT reactions. The majority of herds had only one reactor with a CFT titre of 4 or 8. However, CFT titres of up to 128 occurred. The methods used to resolve the status of these herds were, in order of frequency of use, retest of reactor, review of herd history, culture of reactor and retest of the herd. Slaughter and culture of the reactor was used to decide the herd's status in 46% of herds with false-positive CFT reactors.

Herds Classed as Infected

Thirteen herds were classed (Table 1). These herds had BMRT readings from 0 to 3. In 8 herds the presence of cattle classed in infected was associated with positive results in the BMRT and positive whole herd CFT (Table 2). Culture was not done in all herds and was not always positive for a herd to be classed as infected. Three herds were known to be infected at the time of testing, and 2 were thought to have become infected from a neighbouring herd. Three herds had no history of brucellosis and no known contact with infected herds. The BMRT was successful in identifying infected herds with a great variety of milking herd sizes from (46 to 930), and with a prevalence of brucellosis in the herd ranging from 0.6 to 4.5%. Most of the reactors had been vaccinated with Strain 19, either as calves or adults. The latter were vaccinated with 1/400 the usual calfhood dose or approximately 1.3×10^7 viable cells.

In 5 herds, BMRT results were at variance with CFT results when the whole herd was tested (Table 3). In each herd a

TABLE 2
Data on infected herds that had a positive BMRT

Herd information				Infected milkers		
Herd number	BMRT result	Herd size	Total reactors*	Vaccination status	CFT titre	Culture results
1	3	110	5	NV†	8	
				S19‡	128	Positive
2	3	46	1	S19	128	Not done
3	1	930	6	S19	128	Positive
				AS19§	16	
				AS19	128	
				AS19	128	
4	3	50	1	S19	16	Aborted (culture not done)
5	2	100	1	S19	8	Positive
6	3	170	1	AS19	8	Positive
7	1	61	2	S19	32	Negative
8	3	82	1	S19	32	Positive

* All reactors to whole herd CFT, and includes milkers and dry cattle

† NV = Non-vaccinated

‡ S19 = calfhood strain 19 vaccination

§ AS19 = adult 1/400 strain 19 vaccination

TABLE 3
Data on infected herds that had a negative BMRT

Herd information		Reactor information*		
Herd number	Herd size	Vaccination status	CFT titre	Culture results
1	82	NV†	32	Not done
2	150	NV	64	Not done
3	970	S19‡	128	Positive
4	106	NV	64	Not done
5	60	S19	32	Not done

* All were heifers and not lactating

† NV = Non vaccinated

‡ S19 = calfhood strain 19 vaccination

single CFT reactor was found which was not in the milking herd.

The ability of the BMRT to detect herds classed as infected is shown in Table 4. The sensitivity of the BMRT was 44% (8/18). If herds with infected cattle not in the milking herd are excluded and herds with milkers whose status is equivocal are included, the sensitivity of the BMRT is 66% (8/12). The specificity of the BMRT was 97% (2589/2680.)

Abattoir monitoring in both New South Wales and north eastern Victoria failed to identify any of the herds classed as infected.

TABLE 4
Classification of herds using the bulk milk ring test

BMRT	Herd classification		Total
	Brucellosis infected	Brucellosis Not infected	
Positive	8	91	99
Negative	10*	2589	2599
Total	18	2680	2698

* Includes herds with equivocal status

Herds with Equivocal Status

The status of 5 herds could not be resolved. None had a BMRT reaction, but reactors were found on a routine whole herd (CFT) test. One herd had 5 reactors (which were not lactating) with low CFT titres, all of which were negative when cultured. All reactors had been vaccinated against leptospirosis using *Leptospira hardjo/pomona* vaccine* 6 weeks previously. There was no evidence to suggest that Strain 19 contaminated syringes were used.

The remaining 4 herds had CFT reactors in the milking herd. In 3 herds culture results were negative, and in the other culture was not done. CFT titres ranged from 4 to 128.

All herds had CFT reactors and other findings which suggested brucellosis. The findings in a herd included some of the following; confirmed brucellosis in the past, a history of abortions and/or stillbirths, a BMRT reaction, an infected neighbouring herd.

Discussion

Methods Used to Classify Herds

The main methods used to resolve a BMRT reaction as false-positive were a whole herd test using the RBT and CFT, and a review of the herd history. Other techniques such as repeated BMRT, IMRT using a dilution of 1/10 (Anon 1979b) or doubling dilutions (Alton *et al* 1975b), milk culture or mastitis examination were little used. This could lead to cows infected with brucellosis in the udder but with no serological titre, being left in the herd (Roepke *et al* 1950).

The follow-up procedures after a positive CFT were more extensive and included retest of the reactor or the herd, a review of the history, and cultural examination of selected tissues at slaughter. The use of the culture method described by Corner *et al* (1985) in New South Wales has significantly improved the quality of data available to the veterinarian.

Regular testing of dairy herds using the RBT and CFT stopped in both states in July 1986. The BMRT is now the most effective monitoring method. We believe that all recognised methods should be used to investigate BMRT reactions, despite the high proportion of false-positives, because of its ability to identify herds with infected milkers.

Effectiveness of Monitoring Methods

Monitoring at abattoirs was not useful for dairy herds. It failed to identify any of the infected herds and placed 53 herds under suspicion which were not confirmed as infected.

* Commonwealth Serum Laboratories, 45 Poplar Road, Parkville, Victoria 3052

This was because the level of sampling at abattoirs was not high enough to detect an infected herd. In 77% of surveyed herds in New South Wales, up to 7 reactors could have been present and not detected at the 90% confidence level, using the model of Cannon and Roe (1982).

The BMRT was effective in identifying herds infected with *B. abortus* at a low herd and animal prevalence. In both states it identified infected herds where the infected animal was in the milking herd. In no herds were culture positive cows from the milking herd detected without the BMRT being positive.

Whole herd serological tests identified all herds considered infected, including those where infected cattle were not in the milking herd. This method helps detect brucellosis before it spreads to the milking herd or to neighbouring herds, but is much more expensive than the BMRT. This advantage is also largely dependent on the timing of the whole herd test. It could be argued that it is now not so imperative to identify infected herds early when effective methods exist for the eradication of brucellosis even when the disease is spreading, such as use of 1/400 Strain 19 vaccine in adult cattle (Nicoletti 1979) and the ELISA test (Cargill *et al* 1985; Hornitzky and Searson 1986).

If brucellosis is in a milking herd, BMRT and whole herd serological testing appear to identify the herd as infected with similar efficiency. If brucellosis is only in the non-milking portion of the herd, the BMRT will give a false negative result and whole herd serological tests are likely to be more efficient. The advantage of the BMRT is that it is cheap, sample collection can be regular and does not interfere with herd management, and it can be rapidly performed in the laboratory. As a result of the findings of this survey, routine herd testing in north eastern Victoria ceased in July 1984, except for nominated high risk herds.

Difficulties in Classifying Herds

Given that the aim of the survey was to classify herds using the available monitoring procedures and that the decision of the supervising veterinarian was regarded as final, it is not unexpected that some equivocal results were obtained. One herd best represents all of the elements that make classification difficult. There was no confirmed history of brucellosis, the BMRT was periodically positive, there was one cow with a history of abortion and stillbirth, 2 cows had high titres to serological tests and culture results were inconclusive.

The main difficulty was deciding whether a serological reaction was a false-positive or not. Residual titres to Strain 19 are believed to be the major cause of false positive reactions to the CFT in Victoria, either from calfhood vaccination (W E Sykes, unpublished data) or vaccination for leptospirosis using syringes contaminated with Strain 19 vaccine, as described by Beck *et al* (1964) and Cullen and Corbel (1970).

The problem of false-positive reactions to the BMRT was largely solved in Victoria by changing the time of sampling. The level of false-positives was high in late lactation and, to a lesser extent, in very early lactation. Changing the time of sampling to early to mid lactation reduced the false-positive rate substantially. This was done in the knowledge that infections in late pregnancy may not be detected but to date no such problem has occurred. This approach is not feasible in New South Wales where herds calve all year round.

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Virus and virus-like particles in the faeces of cats with and without diarrhoea

J A MARSHALL*, M L KENNETT*, S M RODGER*, M J STUDDERT†, W L THOMPSON* and I D GUST*

SUMMARY: Negative staining electron microscopy was used to identify viruses in 166 normal and 62 diarrhoeal faecal samples from 208 cats admitted to an animal shelter during a 16-month period (March 1984 to June 1985). On the basis of size and shape 7 distinct viral types were detected: 24 nm parvovirus-like particles, 30 nm astrovirus, 30 nm picornavirus-like particles, reovirus, rotavirus, coronavirus and a 75 nm "togavirus-like" particle. The incidence of these particles in the 208 cats was 11%, 7%, 6%, 0.4%, 5%, 1% and 1% respectively. Virus isolation studies using 40 of the faecal samples succeeded in isolating reovirus 1 in 2 cases. Immune electron microscope studies demonstrated the presence of antibody in a human serum to cat astrovirus, but failed to clarify the identity of the parvovirus-like particles and picornavirus-like particles, other than showing that some of the parvovirus-like particles were not related to feline panleukopenia virus. It was found that parvovirus-like particles, astrovirus, picornavirus-like particles, reovirus and rotavirus could be excreted by cats with normal faeces as well as cats with diarrhoeal faeces. Parvovirus-like particles, astrovirus, picornavirus-like particles and rotavirus could be excreted in high concentration in normal faeces. There was no simple relationship between age and diarrhoea in the population of cats studied. Age was not a critical factor in the excretion of parvovirus-like particles, astrovirus, picornavirus-like particles and rotavirus. The incidence of diarrhoea was not clearly associated with the seasons.
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Introduction

Viral gastroenteritis has emerged in the last decade as a major cause of morbidity and mortality in many animal species including man. So far, 7 main groups of virus or virus-like particles have been associated with the cat intestinal tract or cat faeces. These include parvovirus (Johnson 1965; Langheinrich and Nielsen 1971; Studdert and Peterson 1973; Siegl 1984), astrovirus (Hoshino *et al* 1981b), calicivirus (Wardley 1976; Studdert 1978), reovirus (Scott 1971), rotavirus (Chrystie *et al* 1979; Hoshino *et al* 1981a), coronavirus (Pedersen *et al* 1981) and coronavirus-like particles (CVLP) (Hoshino and Scott 1980; Stoddart *et al* 1984). In many cases, information on these particles in cats is scant and there appears to have been no major survey of viruses in cat faeces. In this study the nature and frequency of excretion of virus and virus-like particles in a large sample of diarrhoeal and non-diarrhoeal cats were examined, and the significance of the findings to cat and human health briefly reviewed.

Materials and Methods

Collection of Specimens

Faeces were collected, usually on a weekly basis, from March 1984 to June 1985, from the Royal Society for the

Prevention of Cruelty to Animals (RSPCA) Centre, Burwood, Victoria. The cats were either strays or boarding and all were thought to be of domestic origin. Faeces were only collected from the cages of cats housed singly so that a given faecal sample could be ascribed to a particular cat.

On admission to the RSPCA Centre cats were routinely vaccinated against feline panleukopenia virus and treated for common parasites. The cats were fed twice a day and their enclosures cleaned at least once a day.

Faeces were classified as normal or diarrhoeal according to their appearance at the time of collection. Faeces that were predominantly firm and well formed were classified as normal and faeces that were predominantly soft, moist and poorly formed were classified as diarrhoeal. A total of 228 faecal samples (166 normal and 62 diarrhoeal) were collected from 208 cats. Details such as age, sex, date of admission and clinical signs were recorded. Apart from minor clinical symptoms all cats sampled appeared healthy. The cats ranged in age from 6 weeks to adults.

Preparation of Faecal Samples

Faeces were processed as described by Oliver *et al* (1985). Briefly, faecal specimens were prepared as a 20% (wt/vol) suspension in Hank's complete balanced salt solution, vigorously shaken, then centrifuged twice at low speed to deposit debris. The clarified supernatant fluid was then concentrated and further purified by ultracentrifugation through a sucrose cushion.

* Virology Department, Fairfield Hospital, Yarra Bend Rd, Fairfield, Victoria 3078 (address for correspondence)

† School of Veterinary Science, University of Melbourne, Parkville, Victoria 3052