A Comparison of Subcutaneous and Oral Administration of *Brucella abortus* Strain 19 in a Large Infected Dairy Herd

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Introduction

A previous experiment (3) showed that oral administration of *Brucella abortus* strain 19 provided good protection against an oral exposure with a pathogenic strain. None of the vaccinates aborted in contrast to 10 of 19 controls. Results of a subsequent experiment (4) to compare orally and subcutaneously vaccinated heifers with controls were not conclusive since few cattle became pregnant.

This is to report on a field study to compare oral vaccination with strain 19 and a low dosage administered subcutaneously in a large dairy herd. Prevaccinal and postvaccinal incidences of infection were compared among the 2 groups and among replacement cattle. Postvaccinal reactions to 3 serologic tests were also compared. The number and persistence of strain 19 infections following vaccination were also studied.

Materials and Methods

Vaccination

In the original herd, 358 adult cattle were subcutaneously vaccinated with approximately 1 x 10^9 strain 19 organisms. Replacement heifers, mostly homereared, were vaccinated by the same method during the middle to late pregnancy following a negative card test. These had been previously vaccinated as calves with a standard dose. A total of 340 cows were given approximately 500 x 10^9 strain 19 organisms mixed with a paste[1] and inoculated on the dorsum of the tongue.

Serologic Tests

The herd was tested at approximately 2 month intervals following vaccination and cattle considered to be infected were slaughtered. Serum samples were screened using the card test which was performed using standard procedures. Card test positive serums were further tested using the rivanol test. Equal parts of serum and rivanol solution were mixed and later centrifuged. The supernatant was tested using doubling dilutions of 1:25 to 1:200. Complete agglutination in the 1:25 or higher dilution was considered positive. Rivanol test positive serums were further tested by the complement fixation procedure using cold temperature incubation. Dilutions of serum and veronal buffer were made in microtiter plates beginning with the 1:10 dilution. A 2% suspension of sheep erythrocytes and 2 units of complement were used. A fixation of 25% or greater in the 1:40 dilution was considered positive.

Bacteriologic Methods

Most cattle with titers to the rivanol and complement fixation tests were serologically retested and bacteriologically examined. Udder secretions were collected, centrifuged, and the cream and sediment inoculated on medium containing a 1:1,000,000 solution of crystal violet. Colonies resembling *Brucella* sp. were typed using standard methods.

Classification of Cattle

Cattle were usually considered to be infected with a field strain prior to herd vaccination and on each herd test following vaccination are given in Table 1. There was little difference in the total numbers of infected cattle in the orally and subcutaneously vaccinated groups of the original herd. The number of infected cattle in the subcutaneous vaccinates was much higher on the first postvaccinal herd test. Five of the replacement heifers were found to be infected on the first test following vaccination and parturition.

Comparisons are made in Table 2 of the reactions on the 3 serologic tests among cattle considered to be noninfected

1) *Supplied by Merck and Co., Inc., Rahway, New Jersey*

TABLE 1. Incidence of Brucellosis in Original Herd Preceding and Following Subcutaneous (S) or Oral (0) Vaccination and in Subcutaneously Vaccinated Replacements (R).

		Cows at Risk					
Prevaccination* Group		Herd Test After Vaccination					Total Infected
		1	2	3	4	5	
Number in Herd - 735	S	302	254	204	177	155	
Number Infected - 27		16(5.3)	7(2.8)	3(1.5)	0(0)	3(1.9)	29
Percent Infected - 3.7	0	290	264	241	196	176	
		6(2.1)	10(3.8)	7(2.9)	4(2.0)	4(2.3)	31
	R	55	249	334	345	344	
		0	0	1	3	1	5
( ) = % infected		647	767	779	718	675	

* Mean of 3 herd tests

TABLE 2. Postvaccinal Titers Among Noninfected Cows.

	Months After Vaccination							
	1—3		4—6		7—9		10	
Vaccination Method	S	0	S	0	S	0	S	0
Test	Percent Positive							
Card	37.6	4.3	22.9	1.9	15.8	0.4	12.4	0.6
Rivanol	22.7	1.8	8.5	1.1	2.2	0.4	2.8	0.6
Complement Fixation	7.0	0.7	3.8	0.8	0.5	0	0	0

S = subcutaneous
0 = oral

TABLE 3. Number and Persistence of Strain 19 Infections.

Vaccination Group				Persistence		
	S	R	0	No. Infected/No. Cultured (%)		
No. Vaccinated	286	316	284	Months After Vaccination		
No. Infected	9	4	3	1-3	4-6	6-12
% Infected	3.1	1.3	1.1	16/16(100)	12/14(86)	5/11(45)

S = subcutaneous-original herd
R = subcutaneous-replacements
0 = oral

following vaccination by the 2 methods. There are large differences between the percentages of positive tests among the 2 groups of cattle. Only 12, 5, and 2 of the 290 noninfected oral vaccinates were positive to the card, rivanol, and complement fixation tests, respectively, approximately 2 months following vaccination. Among original herd and replacement noninfected cattle which were vaccinated subcutaneously, 161, 97, and 30 were positive to the same procedures at the same time period. By 7 to 9 months following subcutaneous vaccination, the number of false positive reactions to the complement fixation test was negligible in this vaccinal group.

The number and persistence of strain 19 infections in the udder were examined among the original and replacement cattle which were vaccinated subcutaneously and in the oral vaccinates. These results are shown in Table 3. The strain 19 culture positive cattle which remained in the herd were recultured at subsequent herd tests to examine the persistence of shedding. By 6 to 12 months, less than half the cattle remained culture positive and among culture negative cows the titers to rivanol and complement fixation tests were receding.

Discussion

Strain 19 has been given to cattle of various ages and by numerous routes and doses in efforts to increase resistence to brucellosis and reduce postvaccinal diagnostic problems. In recent years, a reduced dose given subcutaneously to adult cattle has been effective for both of these goals (4).

No previous studies have compared oral with subcutaneous vaccination in adult cattle. The data in Table 1 suggests there was little difference in infected cows removed among the 2 vaccinal groups during 1 year following vaccination. While the herd and replacement cattle vaccination did not eliminate brucellosis, there was approximately an 80% reduction in infected cattle. Following the fourth herd test, it appeared that the resistance from oral vaccination may be waning and a decision was made to revaccinate those cattle by the subcutaneous route at the next herd test. At that time, 3 additional infected cattle were found among the original subcutaneous vaccinates and 4 in the oral vaccinates.

There were 5 replacement heifers which were seronegative when vaccinated and found to be infected following parturition. This was surely important in perpetuation of the herd infection and emphasizes the problem of detecting infection prior to transmission.

No explanation is given for the larger number of infected cattle in the subcutaneous vaccinates (16) than oral vaccinates (6) on the first herd test. Crawford *et al* found that 54% of vaccinated cattle were culture positive 60 days after vaccination (subcutaneous) as compared with 16% in the nonvaccinated group. They suggested that vaccination of adult cattle would facilitate earlier identification of infected cattle in problem herds.

The percentages of false positive reactions following subcutaneous vaccination to card and rivanol tests was higher than in previous studies (4). They were lower with complement fixation tests. The same criteria for serologic classifications were used in all studies. A complete agglutination in the 1:25 dilution on the rivanol test for classification of positivity is too severe in vaccinated adult cattle. This study confirms others (3, 4) of the importance of the complement fixation test in vaccinated cattle.

The percentage of strain 19 infections following subcutaneous vaccination in adult cattle with a reduced dose in this study was greater than in a much larger population (2). No strain 19 infections were found in earlier studies on oral vaccinates (3, 4). Less than half the strain 19 infections

apparently persisted for more than 1 year in this and an earlier study (2). In the latter, only 9 of 50 (18%) of strain 19 shedders were sero-positive after 13 to 18 months.

Acknowledgements

The authors wish to thank the state-federal laboratory personnel in Jacksonville who performed the serologic tests and officials who permitted the studies to be conducted. We also wish to thank the dairy herd owners for their cooperation and Dr. F. Hanley, private veterinary practitioner, for his support. Finally, we wish to thank the large number of organizational and individual donors of funds for the research.

References

1. Crawford, R.P., F.C. Heck and J.D. Williams: 1979 J. Am. Vet. Med. Assoc., 173:1457. 2. Nicoletti, P.: 1981 J. Am. Vet. Med. Assoc., 178:143. 3. Nicoletti, P. and F. Milward: 1983 Am. J. Vet. Res., 44:1641. 4. Nicoletti, P.: 1984 Vaccine, in press.

Paper presented at the XIIIth World Congress on Cattle Diseases, Durban, S. Africa, Sept. 17-21, 1984.

Abstracts

Veterinary pathology, 5th ed.

Many new diseases are described in this edition. Emphasis is on anatomic pathology, clinical-pathologic correlations, and the basic mechanisms of disease. Individual disease states are discussed with reference to their clinical features followed by a description of gross, microscopic, ultra-structural, and biochemical lesions. Pathogenesis and etiology are critically reviewed as well.

By Thomas Carlyle Jones, D.V.M., and Ronald Duncan Hunt, D.V.M., both of Harvard Medical School, Southborough, Massachusetts, 1792 pp. (7 x 10) 626 illus. (two plates in color), 1983, $85.00. (Canada $113.00)

Techniques in large animal surgery

All drawings are based on sketches and photographs taken during actual surgery. Eight preliminary chapters discuss patient preparation, anesthesia and fluid therapy, instrumentation, suture composition and suture patterns, wound management, use of drains, and reconstructive surgery.

By A. Simon Turner, B.V.Sc., and C. Wayne McIlwraith, B.V.Sc., Ph.D., M.R.C.V.S., both of Colorado State University, College of Veterinary Medicine, and Biomedical Sciences, Fort Collins, Colorado. Illustrated by Tom McCracken. 333 pp. (8½ x 11), 87 full page line plates, 46 line drawings, 19 full pages of instruments, 1982, $49.50. (Canada $65.75)

A practical method of reducing spread of disease by hypodermic needles

A. H. Andrews, A. Lamport
Veterinary Record (1985) 116, 185-186

Transmission of infection between animals is possible when using multidose syringes. The possibility of infection spreading can be reduced by using a telescopic device which fits the syringe barrel and is topped by a cap containing plastic foam impregnated with biocide. Under laboratory conditions, and continually contaminating the needle with various bacteria, infection could not be cultured in broth until after at least 25 injections and in most cases contamination did not occur after 50 injections. When culture was undertaken on solid media breakdowns occurred more frequently, possibly because the agar became lodged in the needle and shielded the bacteria from the biocide.

Estimation of superovulation response in donor cows

L. E. Donaldson
Veterinary Record (1985) 117, 33-34

Estimates were made of the superovulation response in donor cows using ovarian size, the number of corpora lutea (palpated per rectum) and blood progesterone levels. Neither the estimated number of corpora lutea nor ovarian size gave a satisfactory prediction of superovulation response. There was a large discrepancy between the number of corpora lutea present on the ovaries of nine superovulated cows (166) and the estimated number (160). All the corpora lutea on superovulated ovaries were smaller than normal (1.95 g). Blood progesterone levels at the time of embryo recovery were correlated with both the number of corpora lutea on the superovulated ovaries ($r = 0.9$, P 0.001) and the weight of the corpora lutea ($r = 0.95$, P 0.001). There were 67 embryos and ova recovered from the nine donors, representing 40 per cent of the actual and 63 per cent of the palpated corpora lutea. However, neither ovarian size, the number of corpora lutea nor blood progesterone levels were correlated with embryo production.

Bovine leptospirosis: Some clinical features of serovar *hardjo* infection

W. A. Ellis, J. J. O'Brien, D. G. Bryson, D. P. Mackie
Veterinary Record (1985) 117, 101-104

During an investigation of natural in utero infection of cattle by *Leptospira interrogans* strains, infection (almost entirely caused by serovar *hardjo*) was diagnosed in 57 per cent of 505 calves (472 aborted fetuses, 20 stillborn calves and 13 perinatal deaths) examined over a six-year period. The prevalence of leptospire-infected fetuses showed a seasonal increase in September, October and December and was significantly higher in fetuses aborted by dairy cows than in fetuses aborted by beef cows. The majority of infected fetuses were aborted from the sixth month of gestation onwards. Cows which aborted infected fetuses had not previously exhibited overt signs of agalactia. There was an association between leptospiral infection and retention of fetal membranes.