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Brief Communication

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF ANTIBODIES TO MYCOBACTERIUM PARATUBERCULOSIS IN CATTLE

Paratuberculosis may be diagnosed by clinical, bacteriological and immunological methods, but so far only the demonstration of M. paratuberculosis is considered a definite proof of the infection. World-wide use is being made of the complement fixation (CF) test as a valuable immunological test for diagnosis of clinical cases, but its low specificity and sensitivity makes its value problematic in non-clinical cases.

ELISA (*Engwall & Perlmann* 1971) has been shown to be a sensitive technique for the detection of several antigens, including infectious agents (for a review, see: *Schuurs & Van Weemen* 1977). *Nassau et al.* (1976) found the ELISA to be a potentially useful test for diagnosis of tuberculosis in human beings.

In the present work the microplate ELISA has been compared with the CF test on 40 sera from diarrhoeic cows suspected of paratuberculosis. In 30 cases the diagnosis was confirmed bacteriologically (microscopy and/or culture). The assay was performed according to *Ruitenberg et al.* (1976) with some modifications. The solid phase was Nunc disposable plastic trays (96 U, 1182-1). Antigen, prepared from M. paratuberculosis by heat extraction at pH 11.5 followed by precipitation at pH 4.5 (*Jensen* 1956), was diluted 1:3000 with 0.1 M sodium carbonate buffer, pH 9.6, with addition of 0.02 % NaN_3, and applied as a coating on the plastic trays by incubation at 37°C for 30 min. The test sera in dilutions from 1:10 to 1:1280 with PBS containing 0.5 % rabbit serum albumin (RSA) and 0.05 % Tween 20, were placed in the wells of the plastic trays and incubated for 30 min. at 37°C under rotation. After rinsing, horseradish peroxidase conjugated rabbit anti-bovine IgG (H+L) (Nordic Immunological Laboratories, Tilburg) diluted 1:1000 with PBS with Tween 20 and 4 % RSA, was added, and the trays incubated for another 30 min. at 37°C under rotation. The substrate used was 5 amino salicylic acid (5AS), 80 mg per 100 ml distilled water adjusted to pH 6.0 with 1 M-NaOH. To 9 ml 5AS 1 ml

0.05 % H_2O_2 was added. The incubation time with substrate was 60 min. at room temperature under rotation. The ingredients were used in amounts of 100 µl, and after each step the trays were rinsed for 3×5 min. with PBS with Tween 20. The reaction was stopped by addition of 25 µl of 1 M-NaOH. Visual reading was performed by comparing colour intensity with that of known positive and negative sera. A score system of 0, 1, 2, 3 and 4 was used, 3 and 4 being considered positive.

Table 1. Results of bacteriology, complement fixation tests and ELISA, on 40 sera from cattle suspected of paratuberculosis.

Sample No.	Titer		Sample No.	Titer	
	CF	ELISA		CF	ELISA
Positive bacteriology					
771/76	20—40	80	1194/77	<10	40
803/76	20—40	160	1238/77	160	160
806/76	40	80	1565/77	0	20
1055/76	160—320	640	16/78	10—20	80
6/77	20—40	160	24/78	20—40	160
9/77	20—40	80	31/78	40—80	160
11/77	80	80	35/78	80	160
70/77	40—80	160	112/78	<10	80
101/77	80	320	294/78	20—40	160
112/77	80—160	160	310/78	10—20	40
122/77	40—80	80	318/78	10	40
1145/77	20—40	20	319/78	40	160
1161/77	160	320	331/78	<10	160
1162/77	0	20	405/78	40	320
1183/77	80—160	160	406/78	20—40	320
Negative bacteriology					
200/76	10—20	80	1187/77	0	0
1014/76	10	160	1190/77	0	0
69/77	<10	40	1191/77	0	0
1239/77	<10	40	1197/77	0	0
1240/77	<10	10	1237/77	0	0

The results are shown in Table 1. The ELISA was positive for all of 30 sera from cattle with bacteriologically confirmed paratuberculosis, with titers ranging from 1:20 to 1:640. The CF test was positive in 28 and negative in 2 cases. Generally ELISA titers were 2 to 4 times higher than CF titers, indicating that ELISA is more sensitive than CF. In animals with negative faeces culture and negative CF test, i.e. animals presumably free from paratuberculosis, the ELISA too, was negative, which indicates that ELISA is as specific as the CF test.

Cross reactions with other mycobacterial antigens have not been investigated. However, being apparently a sensitive test and easy to perform, the ELISA may be suitable for screening of different mycobacterial antigens for specificity and sensitivity, with a view to developing a serological test for paratuberculosis that may be used for diagnosis of the disease in its non-clinical stages.

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