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Brief Communication

DIFFERENTIATION OF CROSS-REACTING ANTIBODIES AGAINST BRUCELLA ABORTUS AND YERSINIA ENTEROCOLITICA BY ELECTROIMMUNO ASSAY *

The serological cross-reactions between different species of the genus Brucella and Yersinia enterocolitica O-group V (formerly serotype 0—9) seriously complicate the serodiagnostic work associated with brucellosis and yersiniosis (for references see *Hurvell* 1973). These cross-reactions make the serological differential diagnosis impossible by the routinely used serological tests, such as agglutination and complement fixation tests.

The object of the present investigation was to describe the electroimmuno assay as a method of differential diagnosis for cross-reacting antibodies of Brucella abortus and Yersinia enterocolitica O-group V.

Material and Methods

Preparations of antigens. The employed strains of Y. enterocolitica O-group *V* (Y.e.) and Brucella abortus (B.a.) have been described earlier (*Hurvell et al.* 1971). Subcultures of smooth Y.e. and B.a. strains were grown in Roux flasks, acetone-killed, and dried, as described earlier (*Hurvell* 1972). Acetone-dried bacteria, 3.5. g, were suspended in 50 ml of barbital buffer, pH 8.2. Four ml of this stock suspension was mixed with 8.5 g of glass beads (size 0.10—0.11 mm) and treated for 3 min. in a Braun disintegrator Modell MSK (B. Braun, Melsungen, West Germany). The temperature of the cell suspension was maintained at 0—5°C throughout the disintegration. After centrifugations (43,000 × g, 20 min. at 4°C) the supernate was decanted and used as antigen at the electroimmuno assay.

Antisera. Antisera to whole acetone-killed B.a. and Y.e. bacteria were produced in rabbits by injection twice weekly for 7 weeks as described earlier (*Hurvell* 1972). Four rabbits were immunized with each strain and the sera were pooled. The used pooled antisera gave equal homologous and heterologous titres 1:2,560) at the crosswise agglutination test with B.a. and Y.e. antigen.

Electroimmuno assay. The electrophoretic equipment, including all accessories, was obtained from Svenska Hoechst AB,

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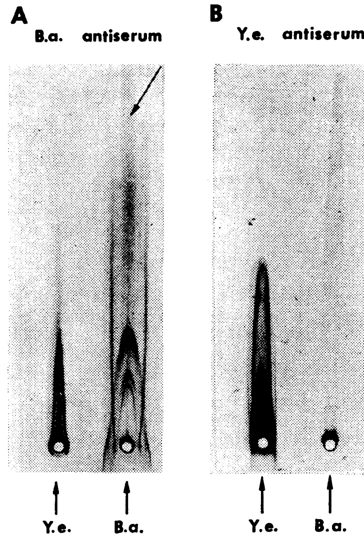

Figure 1. Electroimmuno assay on Yersinia enterocolitica O-group V (Y.e.) and Brucella abortus (B.a.) antigen with antiserum against A: Brucella abortus and B: Yersinia enterocolitica O-group V.

Stockholm, Sweden. The electroimmuno (e.i.) assay was performed essentially as described by *Laurell* (1972). Fifteen ml of a 1 % (w/v) agarose (Behringwerke, Marburg-Lahn, West Germany) in barbital-acetate buffer, pH 8.2, ionic strength 0.05, containing rabbit antiserum corresponding to 3.0 $\mu l/cm^2$ gel was poured on to a 10 $\times$ 10 cm glass plate. Five μl of the antigen was applied in wells in the agarose with a diameter of 2.5 mm. The electrophoresis was carried out at pH 8.2 using barbital-acetate buffer, ionic strength 0.1, with 10 v/cm in the gel at 15°C for 2 hrs. The washing, drying, and staining with Coomassie Brilliant Blue R were performed essentially as described by *Laurell* (1972).

Results and Discussion

The results from the e.i. assay of antigens from B.a. and Y.e. tested with antisera in agarose gel against B.a. and Y.e., respectively, are shown in Fig. 1 A and B.

There was a distinct difference in the appearance of the formed rockets when homologous antigen and heterologous antigen, respectively, was tested against the antiserum used in the agarose gel. With Y.e. antigen, tested against B.a. antiserum, a rocket was obtained which differed both in size and in shape from that obtained with homologous B.a. antigen (see Fig. 1 A).

Fig. 1 B also illustrates the difference in appearance between the rockets formed at e.i. assay when antigen from Y.e. and B.a., respectively, was tested against Y.e. antiserum. The homologous Y.e. precipitate gives a rocket with multiple distinct precipitation lines. With B.a. antigen, a long diffuse precipitate was obtained whose appearance is identical with that of the precipitate formed with homologous B.a. antigen and antiserum (see the arrow in Fig. 1 A). These "false rockets" with B.a. antigen could also be reproduced if normal rabbit serum was used instead of immunsera in the agarose. The cause of this ill-defined appearance of these "false rockets" is probably that a formed unspecific precipitate between rabbit sera and components in the disintegrated B.a. antigen migrates through the agarose gel towards the anode during the electrophoresis.

The present experiments show that it is possible by e.i. assay to differentiate in a simple and quick way if antibodies derived from B.a. and from Y.e. immunizations in the experimental system used, where crosswise agglutination tests of the sera gave the same titres (1:2,560) against both B.a. and Y.e. antigens.

Studies on patient sera both from man and from cattle are in progress in order to examine the possibility to applicate the method as a qualitative differential diagnostic test of brucellosis and yersiniosis in the case of "field sera". By carrying out the e.i. assay with antibody-containing agarose on microscope slides (85 × 25 mm) several sera can be tested simultaneously and a small amount of each serum will be required for study (*Hurvell*, unpublished data).

Bengt Hurvell
The National Veterinary Institute,
Stockholm, Sweden.

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Reprints may be requested from: Bengt Hurvell, National Veterinary Institute, S-104 05 Stockholm 50, Sweden.