

RESEARCH COMMUNICATION

Improving the specificity of indirect immunofluorescence for the serological diagnosis of bovine anaplasmosis

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ABSTRACT

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Fluorescing inclusion appendages were detected consistently in preparations of *Anaplasma centrale* and *Anaplasma marginale* when they were used as antigen in indirect immunofluorescence serological tests for the diagnosis of anaplasmosis in cattle. The presence of the inclusion appendages made it possible to confirm the specificity of the immunofluorescent reaction and to determine end-points with accuracy.

Keywords: Bovine anaplasmosis, diagnosis, indirect immunofluorescence, serological, specificity

Of the various serological tests routinely available for the diagnosis of bovine anaplasmosis, the indirect immunofluorescent test (IFT) has been regarded as the most sensitive (Gonzalez, Long & Todorovic 1978). However, it is not widely used, partly because the *Anaplasma* inclusion bodies which act as antigen, lack a morphological characteristic that makes them distinguishable from non-specific fluorescing particles when the test is read (Anon. 1993). We report here on the utilization of fluorescence of inclusion appendages of *Anaplasma* spp. when they react with antibodies in the IFT, making the test highly specific and reliable.

Anaplasma antigen slides for use in the IFT were prepared by infecting splenectomized haemoparasite-free calves with *A. centrale* Onderstepoort, obtained from the Tick Fever Research Centre, Queens-

land, Australia (TFRC), or *A. marginale* Dickson, 1991, isolated from the pooled blood of four healthy adult zebu cattle from a centre near Lilongwe, Malawi. In both cases, infected calves were bled into heparin as soon as the parasitaemia exceeded 5%. The infected blood was washed at least five times with phosphate-buffered saline at pH 7.2 (PBS), during which time all traces of buffy coat and serum were removed. The red cells were resuspended at a packed-cell volume of 30% in 3.5% bovine albumin fraction V in PBS, and 25 μ l was spread evenly across a microscope slide previously coated with 7% bovine albumin by spinning the slide at high speed around its own axis. The antigen slides were air dried, fixed at 55°C for 30 min and covered with masking tape (Parker 1971) before being stored at –70°C. Negative-control serum was supplied by the TFRC from an animal shown by the Centre to be negative for all tick-borne diseases, and positive-control sera were obtained from animals infected experimentally with the homologous parasite in Malawi.

The IFT was performed by conventional methods (Anon. 1984) with a variety of modifications. Blocking of free-antigen sites on the slide after reaction with the test sera by immersing the slide for up to 30 min in a suspension of spray-dried skimmed-milk powder (ASDA Stores Ltd, UK), 3 g in 150 ml phosphate-buffered saline at pH 7.4 with 150 µl sorbitan monolaurate ("Tween 20"—ACI Ltd), was found to reduce non-specific fluorescence. Examination for fluorescence was conducted by means of incident ultraviolet light under a x40 objective.

When the test was performed with positive-control sera for the homologous parasite, *Anaplasma*, inclusion bodies were detected as round fluorescing particles with irregular outlines and of variable size, up to a quarter of the diameter of the erythrocyte. A proportion of the bodies, usually less than 1/10, had fluorescing filamentous inclusion appendages projecting from them; the appendages were of variable length, up to the diameter of the erythrocyte (Fig. 1). Occasional bodies had two appendages, and appendages not attached to bodies were sometimes seen. A few bodies were also surrounded by small, fluorescing granules. Inclusion appendages were found consistently in three batches of *A. centrale* antigen prepared over a 5-year period and in one batch of *A. marginale* antigen. The detection of inclusion appendages was not affected by the various modifications that were investigated in the test.

When sera from experimentally infected animals were titrated in threefold dilutions against the homologous antigen, it was much easier to detect an end-point on the basis of presence or absence of fluorescence of inclusion appendages than on a subjective assessment of intensity of fluorescence of the inclusion bodies themselves. Sera from groups of cattle inoculated with live *A. centrale* vaccine (Anon. 1984) at fivefold dilutions from 1/10 to 1/6250, together with unvaccinated controls, were tested against the homologous antigen on day 56 after inoculation. When fluorescence of inclusion appendages at a dilution of 1/90 was used as the criterion of a positive serological reaction, results were correlated in 46/48 animals with microscopic detection of parasites in Giemsa-stained blood smears between days 35 and 56.

In cattle tested at intervals up to 98 d after infection with one parasite or the other, the titres to the homologous and heterologous antigens were usually identical, never differing by more than one threefold dilution. When sera from 98 cattle of unknown infection status from various centres in Malawi were tested against both antigens at 1/90, the results agreed in 96/98 animals, when fluorescence of inclusion appendages was taken as the criterion for a positive result.

The inclusion appendages of the two parasites utilized in this study appeared to be more filamentous



FIG. 1 Fluorescing inclusion appendages on *Anaplasma centrale* inclusion bodies reacting with homologous serum at 1/40 in the indirect immunofluorescence test (x100 objective, oil immersion)

and less frequent than those that have been described in other strains of *A. marginale*. In IFT preparations of such strains, the appendages may be found in up to 70% of the parasites and are robust, giving the organisms the shapes of commas, comets, matchsticks and dumb-bells (Madden 1962); ring forms have also been described (Kocan, Venable, Hsu & Brock 1978). The nature of the appendages has not been determined completely, but they are thought to represent organization of proteinaceous material within the parasitized erythrocyte (Simpson, Kling & Neal 1965). Kocan, Ewing, Hair & Barron (1984) suggested that the appendages might play a role in the entry of the parasites into the tick-gut epithelium after ingestion.

Inclusion appendages have not previously been described in *A. centrale*. Their detection in the IFT when both *A. centrale* and *A. marginale* are used as antigens, improves the specificity of the test. The use of fluorescence of inclusion appendages as the definitive criterion for interpretation of the test has demonstrated a high level of cross-reactivity between *A. centrale* and *A. marginale*.

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REFERENCES

- ANON. 1984. *Ticks and tick-borne disease control. II. Tick-borne disease control*. Rome. Food and Agriculture Organization of the United Nations.

- ANON. 1993. *Expert consultation on the use of applicable biotechnological methods for diagnosing haemoparasites*. Rome. Food and Agriculture Organization of the United Nations.
- GONZALEZ, E.F., LONG, R.F. & TODOROVIC, R.A. 1978. Comparison of the complement-fixation, indirect fluorescent antibody, and card agglutination tests for the diagnosis of bovine anaplasmosis. *American Journal of Veterinary Research*, 39: 1538–1541.
- KOCAN, K.M., VENABLE, J.H., HSU, K.C. & BROCK, W.E. 1978. Ultrastructural localization of anaplasma antigens (Pawhuska isolate) with ferritin-conjugated antibody. *American Journal of Veterinary Research*, 39:1131–1135.
- KOCAN, K.M., EWING, S.A., HAIR, J.A. & BARRON, S.J. 1984. Demonstration of the inclusion appendage of *Anaplasma marginale* in nymphal *Dermacentor andersoni*. *American Journal of Veterinary Research*, 45:1800–1807.
- MADDEN, P.A. 1962. Structure of *Anaplasma marginale* observed by using fluorescent antibody technique. *American Journal of Veterinary Research*, 23:921–924.
- PARKER, R. 1971. Masking tape as a protection for the cold storage of blood films for indirect fluorescent antibody tests. *Research in Veterinary Science*, 12:89–90.
- SIMPSON, D.F., KLING, J.M. & NEAL, F.C. 1965. The nature of bands in parasitized bovine erythrocytes. *Journal of Cell Biology*, 27:225–235.