DETECTION OF ANTIBODY TO MYCOPLASMA F38 IN GOAT SERA BY AN ENZYME-LINKED IMMUNOSORBENT ASSAY

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SUMMARY

An indirect enzyme-linked immunosorbent assay (ELISA) was developed to screen goat sera at a single dilution for antibody to mycoplasma F38. Antibody was detected in sera of six convalescent goats following experimental infection. Antibody was also detected in 34 sera three to four weeks after vaccination. No antibody was detected in 164 sera from goats without a history of vaccination or infection with contagious caprine pleuropneumonia. The ELISA was more sensitive than the complement fixation test in detecting antibody in vaccinated goats.

INTRODUCTION

Mycoplasma F38 causes a severe and often fatal contagious caprine pleuropneumonia (CCPP) in Kenya (MacOwan and Minette, 1976, 1977) and Sudan (Harbi, El Tahir, MacOwan and Nayil, 1981). The disease causes great economic losses wherever it occurs (McMartin, MacOwan and Swift, 1980). To date the assessment of the antibody response in CCPP depends mainly on the complement fixation (CF) and passive haemagglutination (PHA) tests (Muthomi and Rurangirwa, 1983). Results of a recent study suggested the ELISA was the more sensitive in detecting antibody in infected and vaccinated goats (Bari, 1984). In the study only a limited number of sera were examined. All sera were serially diluted and their titration end-points used to distinguish positive and negative reactions. This procedure like the CF and PHA tests would be tedious and expensive to employ when examining a large number of sera. The availability of a more sensitive test requiring only a single dilution of serum would make such testing quicker and less tedious thus enabling laboratories with limited financial and manpower resources to conduct large scale serological surveys for CCPP efficiently. This paper describes studies carried out in an attempt to evaluate the possible use of the ELISA in serodiagnosis of CCPP and for the routine assessment of antibody response in vaccinated goats using a single dilution of serum.

MATERIALS AND METHODS

Mycoplasma F38 originally isolated from field cases of acute CCPP (MacOwan and Minette, 1976) and preserved by lyophilisation was obtained from the Veterinary Research Laboratories, Kabete, Kenya. Cultures grown in Newing’s tryptose broth (Gourlay, 1964) at 37°C for 10 days were passed through a cream separator and washed with three changes of physiological saline. The organisms were resuspended in physiological saline to one tenth of the original culture volume. The suspension was sonicated on ice for 40 min in alternating cycles of two min. The protein content of the sonicated antigen was determined.
as described by Watters (1978). Antigen was stored at $-20^\circ\text{C}$ in aliquots of 1.0 ml in screw-capped glass bottles. The optimal dilution of antigen for use in the test was determined by chequerboard titration against known positive and negative sera.

Sera were obtained from the following groups of goats:

1) Four goats infected by the intratracheal-endobronchial route and another four infected by contact exposure when housed in close confinement with the first group of goats. Two contact-infected goats died before the second bleeding.
   a) The eight goats experimentally infected with mycoplasma F38 were bled three days after the onset of pyrexia.
   b) The six surviving goats were bled two weeks later.
2) Thirty four goats vaccinated three to four weeks previously using inactivated monovalent mycoplasma F38 vaccine (Rurangirwa and Muthomi, 1984).
3) One hundred and sixty four sera from farms without histories of vaccination or infection with CCPP.

The single dilution of serum for use in the test was selected by titrating serial two-fold dilutions of three known positive sera and a known negative serum against the optimal antigen dilution. Rabbit anti-goat IgG peroxidase conjugate was obtained as a 2.0 ml liquid product (Zymed Labs Inc., USA). It was distributed in volumes of 100 $\mu$l in 1.0 ml screw-capped glass bottles and stored at $-20^\circ\text{C}$. The optimal dilution of conjugate for use in the test was determined by chequerboard titration against two-fold dilutions of a crude preparation of ammonium sulphate precipitated goat IgG (Lucas and Naphine 1971). For use in the test conjugate was diluted in phosphate buffered saline (PBS) containing 3% skim milk powder and 0-05% Tween 20 (Polyoxyethylene (20)-sorbitan monolaurate). Skim milk powder was added to this buffer to prevent the non-specific attachment of other reagents added subsequent to the antigen.

An indirect micro-ELISA was developed using rigid polystyrene ELISA grade plates (Nunc). Various conditions for coating plates with mycoplasma antigens and periods of incubation at 37$^\circ\text{C}$ for the various steps were examined. The conditions described here were those found optimal. Mycoplasma F38 antigen was diluted appropriately in 0-01 M phosphate buffered saline pH 7.2 to 7.4; 100 $\mu$l of antigen were put into each well of the microtitre plates. They were covered and incubated at 37$^\circ\text{C}$ for two hours and then kept at +4$^\circ\text{C}$ overnight. The plates were washed in three changes of PBS containing 0.05% Tween 20 (PBST); 100 $\mu$l volumes of goat sera diluted 1 in 100 in PBST with 3% skim milk powder were added to the wells in triplicate. The plates were covered and incubated stationary at 37$^\circ\text{C}$ for an hour; they were then washed as before and 100 $\mu$l of rabbit anti-goat IgG conjugate diluted to 1 in 4,000 were added to each well. The plates were incubated at 37$^\circ\text{C}$ for another hour and then washed.

The substrate orthopenylene diamine (OPD) was prepared and used as described by Anderson, Rowe, Taylor and Crowther (1982). The optical density (OD) was read at a wave length of 492 nm using a multichannel spectrophotometer (Titertek Multiskan, Flow Laboratories). Control wells for plate background levels were set up as follows:

1) Wells coated with antigen, serum not added but all other reagents added.
2) Wells without antigen but with all other reagents. The mean OD for plate background was calculated using values from these wells. The activity of antigen was checked on each plate using a known positive control serum from a vaccinated goat. Three wells containing 100 $\mu$l of this serum diluted 1 in 100 were included in each test.