Uses of serology for the diagnosis of contagious bovine pleuropneumonia *

E.R. RURANGIRWA **

Summary: A serological test involves detection of specific changes, induced by a pathogen, in the properties or actions of serum of an infected host. The test may detect the presence in serum of either antibodies to the pathogen (produced by the host) or antigens (i.e. the infecting agent itself and/or its components). The many serological tests which have been developed for the diagnosis of contagious bovine pleuropneumonia (CBPP) are classified into two groups on the basis of this distinction. To date, no single serological test is able to detect all stages of the disease. Thus the choice of serological test (or combination of tests) will depend on the specific aim of the investigation. Meanwhile, a sensitive, specific and simple 'pen-side' test for the diagnosis of all forms of CBPP is still lacking.

KEYWORDS: Antibodies – Antigens – Bovine – Contagious bovine pleuropneumonia – Diagnosis – Mycoplasma – Serological techniques.

INTRODUCTION

Webster's New World Dictionary defines 'serology' as: 'The science dealing with the properties and actions of serums'. Thus a serological test involves detection of specific changes, induced by a pathogen, in the properties or actions of serum of an infected host. Diagnosis can therefore be reached by detecting the presence in serum of either antibodies to the pathogen (produced by the host) or antigens (i.e. the infecting agent itself and/or its components).

Contagious bovine pleuropneumonia (CBPP) may be the only disease in veterinary medicine to which all forms of serology have been applied. This is primarily due to the need to develop a simple and quick, yet specific and sensitive test for use in the field and in relatively unsophisticated laboratories in countries where CBPP occurs. Also, there is a continuing need to develop a single test which will detect all of the various stages of CBPP infection in cattle.

SEROLOGICAL TESTS AVAILABLE

The many serological tests which have been developed for the diagnosis of CBPP fall into two groups, as follows:

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a) Serological tests which detect antigens in the serum of the infected host:
   i) agar gel double immunodiffusion (10, 24, 28, 30)
   ii) interfacial precipitation in liquid medium (27)
   iii) counter current immunoelectrophoresis (20)
   iv) enzyme-linked immunosorbent assay (ELISA) (2).

b) Serological tests which detect antibodies:
   i) slide agglutination on serum or blood (12, 14, 15, 29).
   ii) passive haemagglutination (4, 5, 19)
   iii) latex agglutination (18)
   iv) complement fixation (CF) (3)
   v) single reverse radial immunodiffusion (23)
   vi) ELISA (1, 2, 11, 17).

Limitations of the various serological tests as applied to the diagnosis of CBPP have been evaluated by several workers (6, 7, 8, 9, 21, 25, 26), and by far the biggest problem is the extensive serological cross-reactions among the mycoplasmas belonging to the *Mycoplasma mycoides* ‘cluster’, provoking false-positive results. It is hoped that this problem will be overcome in the near future through the use of more specific reagents (e.g. monoclonal antibodies). The other limitations of each of the commonly-used tests are outlined in Table I. The CF test was found to be the most reliable of the numerous serological tests and rarely gives false-positives, although false-negatives are frequent as the disease becomes chronic. The CF test is therefore recommended by the Office International des Epizooties as the standard serological test for the diagnosis of CBPP (16). Slide agglutination (with either serum or whole blood) is a rapid test which is frequently used, but only on a herd basis; it is most reliable early in the infection. Agar gel immunodiffusion is also used, especially for detection of antigens in tissues which are not suitable for *Mycoplasma* isolation. An indirect ELISA for the detection of antibody to *Mycoplasma mycoides* subsp. *mycoides* SC has been developed jointly by the Food and Agriculture Organisation of the United Nations and the International Atomic Energy Agency, and an ELISA kit is now available (1). No data were available on the sensitivity and specificity of this test in detecting CBPP cases at all stages of the disease.

To provide an insight into the reasons why so many serological tests are unreliable, the tests are categorised into primary and secondary binding tests.

**PRIMARY BINDING TESTS**

The primary binding tests are the most sensitive tests (in terms of the detectable amount of antibody or antigen). Primary binding tests are usually performed by allowing the reactants (antibody and antigen) to combine and then measuring the amount of immune complex formed using radio-isotopes, fluorescent dye and/or enzyme labelling.

Of the numerous serological tests for CBPP, only immunofluorescence and ELISA (indirect or direct) are primary binding tests.

The advantages of primary binding tests in the diagnosis of CBPP are as follows:

- All components of the organism may be used to detect antibody made to any component of the organism, although there is a chance of non-specific reaction.
### Table I

**Common serological tests for the diagnosis of contagious bovine pleuropneumonia**

<table>
<thead>
<tr>
<th>Test</th>
<th>Characteristics/limitations</th>
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<tbody>
<tr>
<td>Double agar gel immunodiffusion</td>
<td>Only effective during clinical disease; detects antigen</td>
</tr>
<tr>
<td>Interfacial precipitation in liquid medium</td>
<td>Requires clear samples; sensitivity diminishes rapidly as the disease approaches the chronic stage</td>
</tr>
<tr>
<td>Slide agglutination</td>
<td>Reliability declines rapidly as the disease approaches the chronic stage</td>
</tr>
<tr>
<td>Complement fixation</td>
<td>Not very sensitive; will not detect animals incubating disease; percentage of negative results increases as the disease becomes chronic</td>
</tr>
<tr>
<td>Passive haemagglutination</td>
<td>False-positives at low serum dilution</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>False-positives at low serum dilution</td>
</tr>
<tr>
<td>ELISA and its modifications:</td>
<td>May provide the answer to problems of specificity and sensitivity if well exploited</td>
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<tr>
<td>Indirect ELISA</td>
<td></td>
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<tr>
<td>Competitive ELISA</td>
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<tr>
<td>Immunocapture ELISA</td>
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<tr>
<td>Dot immunobinding assay (DIA)</td>
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ELISA: enzyme-linked immunosorbent assay

- Activities of all isotypes of host antibodies to the infecting organism are directly detectable without subjecting isotypes to secondary interactions which they may be incapable of effecting. For example, bovine IgG2 does not fix guinea-pig complement (13). Thus serum containing mainly IgG2 specific to *Mycoplasma mycoides* may give a false-negative result in the conventional CF test using guinea-pig complement. Indeed Queval et al. (22) increased sensitivity by performing CF on non-inactivated serum supplemented with normal bovine serum. The false-negative results obtained using CF in cases of chronic CBPP are probably due to the presence of IgG2 during this stage of the disease.

### SECONDARY BINDING TESTS

By contrast, secondary binding tests measure the consequences of immune-complex formation *in vitro*. These tests are therefore theoretically much less sensitive than the primary binding tests but are considerably simpler to perform. It is not surprising, therefore, that most of the serological tests for CBPP (except immunofluorescence and ELISA) fall into this category.

Secondary binding tests consist of a two-stage process. The first stage involves interaction between antigen and antibody. The second stage is determined by the
physical state of the antigen. Thus, if antibodies combine with soluble antigens in solution under appropriate conditions, the complexes precipitate. This forms the basis of the following tests for CBPP:

- agar gel immunodiffusion
- interfacial precipitation in liquid medium
- counter current immuno electrophoresis
- single reverse radial immunodiffusion.

If the antigens are particulate (e.g. whole organism or erythrocytes), they agglutinate. This forms the basis of the following tests for CBPP:

- slide agglutination (using serum and/or blood)
- passive haemagglutination
- latex agglutination.

Under other circumstances, the combination of antigen and antibody may lead to activation of complement. This can be measured and forms the basis of the CF test for CBPP.

Thus, should there be an imbalance in the process of secondary interactions, the test will be negative.

Various investigators have evaluated and re-evaluated the various secondary binding serological tests for their ability to detect CBPP at the various stages of development. All have come to the conclusion that no single test is capable of detecting CBPP at all stages (6, 7, 8, 9, 25, 26). The advent of ELISA (a primary binding test), however, and dot blot tests – in which most (if not all) components of *Mycoplasma mycoides* subsp. *mycoides* SC can be incorporated – coupled to the development of monoclonal antibodies, should enable the development of a test which will detect all stages of infection. Indeed, the potential for developing a very specific and sensitive pen-side diagnostic test is greater than ever. To achieve this goal, however, the following studies need to be conducted:

- Bovine humoral immune response to *Mycoplasma mycoides* subsp. *mycoides* SC should be delineated, from time of infection through all stages of the disease, including sequestration.
- Components of the *Mycoplasma* should also be dissected, and their role in the pathogenesis and diagnosis of the disease assessed.

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**APPLICATION DE LA SÉROLOGIE AU DIAGNOSTIC DE LA PÉRIPNEUMONIE CONTAGIEUSE BOVINE.** – F.R. Rurangirwa.

Résumé : L'épreuve sérologique permet de révéler les changements spécifiques que subissent, sous l'effet d'un agent pathogène, les propriétés du sérum d'un hôte infecté ou ses réactions. Elle décèle la présence, dans le sérum, d'anticorps vis-à-vis de l'agent pathogène (produits par l'hôte) ou d'antigènes (c'est-à-dire l'agent infectieux lui-même et/ou ses composants). Les nombreuses épreuves sérologiques mises au point pour le diagnostic de la péripneumonie contagieuse bovine se répartissent, selon cette distinction, en deux catégories. À ce jour, aucun test sérologique unique ne permet d'identifier toutes les étapes de la
maladie. Le choix d'une épreuve sérologique ou d'une combinaison d'épreuves dépendra donc de l'objectif spécifique de la recherche. En attendant, une épreuve sensible, spécifique et simple de type pen-side (« au pied de l'animal »), appliquée au diagnostic de toutes les formes de péripneumonie contagieuse bovine, fait toujours défaut.


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Resumen: Una prueba serológica entraña la detección de cambios específicos, inducidos por un agente patógeno, en las propiedades o acciones del suero de un huésped infectado. Tales pruebas pueden detectar la presencia en el suero ya sea de anticuerpos contra el patógeno (generados por el huésped) o de antígenos (esto es, el propio agente infeccioso y/o sus componentes). Sobre la base de esta distinción, las numerosas pruebas serológicas elaboradas para el diagnóstico de la pleuroneumonía contagiosa bovina se clasifican en dos grupos. Hasta la fecha, no existe ninguna prueba capaz de detectar todas las fases de la enfermedad. Por ello, la elección de una u otra prueba serológica (o de una combinación de ellas) dependerá del objetivo concreto de la investigación. Mientras tanto, se seguirá careciendo de una prueba de campo (pen-side), sensible, específica y sencilla que pueda practicarse para el diagnóstico de todas las formas de pleuroneumonía contagiosa bovina.


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REFERENCES


