Contagious bovine pleuropneumonia: a comparison between the passive haemagglutination test and the complement fixation test

J.C. CHIMA and G. PAM*

Summary: Five zebu cattle were vaccinated with T1 strain broth culture vaccine and the immune response elicited was measured with the passive haemagglutination test (PHA) and complement fixation test (CFT). It was found that the PHA was able to detect antibodies against Mycoplasma mycoides ssp. mycoides much earlier and for a longer period after vaccination than the CFT. All the animals were CFT negative 10 weeks after vaccination while they were still PHA positive after 24 weeks.

Also, in screening 26 animals involved in a natural outbreak of CBPP, the CFT detected nine positive cases while 14 animals, including all CFT positive cases, were PHA positive. These results are discussed in the light of the expected role of serological diagnosis in the current CBPP control programme.

KEY-WORDS: Cattle - Cattle diseases - Complement fixation - Laboratory diagnosis - Mycoplasma mycoides - Passive haemagglutination - Serological techniques - Zebu.

INTRODUCTION

Various serological methods (6, 9, 11, 3) have been used in measuring antibody response to Mycoplasma mycoides ssp. mycoides which is the cause of contagious bovine pleuropneumonia (CBPP) in cattle. Based on the comparison carried out by Gourlay (7), the complement fixation test (CFT) was considered the single most reliable test for the diagnosis of CBPP. While some of the tests used in the comparison have since undergone some modifications and improvements, new ones (10, 5) have also been developed.

It is, therefore, necessary to reassess the efficacy of the CFT in the light of available new techniques since the CFT has the disadvantage of being too cumbersome to perform and requires costly reagents and equipment that may not be easily available in some laboratories. A simple, fast and cheaper test as an alternative to CFT must, however, be comparable in terms of sensitivity and specificity.

In the present study, the complement fixation test (4) was compared with the passive haemagglutination test (5) in monitoring the immune response to T1 vaccine
in cattle and in screening an infected herd. The result obtained was assessed in the light of the expected role of serological diagnosis in the current CBPP control programme, code-named JP 28 in Nigeria.

MATERIALS AND METHODS

Vaccine

T1 strain broth culture vaccine produced essentially as described by Brown et al. (2) was used. A dose of 0.5 ml containing approximately $1 \times 10^8$ colony forming units (CFU) of *Mycoplasma mycoides* ssp. *mycoides* was given subcutaneously at the tip of tail.

Experimental animals

Five apparently normal zebu cattle ranging in age from two to three years were used. The animals were from the breeding herd of the National Veterinary Research Institute, Vom and were known not to have been previously vaccinated against CBPP. The animals were bled before and at weekly intervals after vaccination for two months and thereafter at monthly intervals. The sera collected were stored at $-20^\circ$C until required.

Also 102 animals having no prior contact with *Mycoplasma mycoides* ssp. *mycoides* and 26 animals involved in an outbreak were screened using the two tests.

Serology

The sera were assayed for antibody against CBPP using the passive haemagglutination test (PHA). The test was as described previously (5). Briefly, *Mycoplasma mycoides* ssp. *mycoides* antigen at a protein concentration of 7 mg/ml in 0.01M PBS of pH 7.0 was used to sensitize glutaraldehyde-fixed sheep erythrocytes. The titrations were made in V well microtitre plates (Linbro Scientific Co., Hamden, Connecticut) using a 2% suspension of the sensitized sheep red blood cells (SRBC). The test was read after incubation for two hours at room temperature.

The complement fixation test (CFT) was as previously described (4). The test was carried out on plastic haemagglutination plates (Baird and Tatlock, London) using test sera inactivated with 0.25% phenolised buffer.

RESULTS

Clinical observation following vaccination

There was no local or systemic reaction following vaccination, as was evidenced by lack of swelling at the site of vaccination, and no febrile response.

Serological response

The complement fixation test did not detect antibodies against *M. mycoides* ssp. *mycoides* at one week post-vaccination while the PHA gave titres between 1:40 and 1:80. However, both the PHA and CFT titres rose to a peak by the second week of vaccination (Fig. 1). By five weeks post-vaccination the mean CFT titre had
weeks post vaccination

FIG. 1
Measurement of antibodies against *Mycoplasma mycoides* ssp. *mycoides* with the complement fixation test (CFT) and passive haemagglutination (PHA) following the inoculation of animals with T1 strain broth culture vaccine dropped to less than 1:10 while that of the PHA was well above 1:80. In fact, while all the animals were CFT negative after ten weeks they were all PHA positive even at twenty-four weeks after vaccination.

Of the 26 animals involved in a field outbreak of CBPP, nine were CFT positive and 14 were PHA positive. The 102 samples collected from animals having no prior contact with *Mycoplasma mycoides* gave uniformly negative results with the CFT, but two of them were doubtful with the PHA.

In general, none of the CFT positive samples gave negative results with the PHA but two samples that were CFT negative gave PHA titres of up to 1:80. However, samples with high CFT titres invariably gave high PHA titres.

**DISCUSSION**

One of the important recommendations of the FAO/OIE/OAU Expert Panel on contagious bovine pleuropneumonia, which met to draw up proposals for the JP 28 vaccination campaign, was the establishment of at least one diagnostic unit in each country involved in the campaign (1). Apart from confirming clinical cases and detection of chronic and sub-clinical cases, the diagnostic units would also monitor the immune status of vaccinated animals.

The result of this study shows that PHA detects *M. mycoides* antibodies earlier and for a longer time than the CFT after vaccination. This is hardly surprising because the CFT is known to detect a minimum of about 0.05 μg antibody/ml,
while the PHA detects about 0.01 µg antibody/ml (12). This ability of the PHA to detect antibodies for a longer time after vaccination would be an asset in monitoring the immune status of vaccinated herds since nearly all such animals are CFT negative by two months post-vaccination. Hudson (8) also observed that most animals vaccinated with V5 M. mycoides, which is a more virulent strain than T1, were CFT negative after three months. Since such CFT negative animals are known to be protected against challenge it would appear that the PHA gives a better assessment of the immune status of a herd following vaccination.

Although there was no opportunity to relate the post-mortem findings with the result of serological screening of animals involved in the outbreak, the PHA proved to be more sensitive than the CFT. It is pertinent to note that the superiority of the CFT over most other tests (7) in the diagnosis of CBPP is usually based on its ability to detect most chronic cases and carriers. The present study showed that there were more PHA positive animals than CFT positive ones in the infected herd examined. It is also significant that none of the CFT positive animals were PHA negative. It is, therefore, likely that the PHA picked out more sub-clinical and chronic cases because of its ability to detect lower levels of antibody than the CFT.

It is, however, possible that the highly sensitive PHA test may be less specific, resulting in some false positives. This may be unavoidable because of the phenomenon of abortive infection. Even with the CFT, Hudson (8) reported that some CFT positive animals showed no evidence of infection at slaughter as a result of abortive infection. However, the consequences of false positives may be less than those of false negative reactions, which have the potential to completely jeopardize the eradication programme.

Finally, in addition to being more sensitive than the CFT, the PHA has the advantage of requiring very limited reagents and equipment and can easily be carried out by relatively inexperienced technicians. The PHA, therefore, has a lot of advantages to recommend it over the CFT as a valuable tool in the current effort to control CBPP in Nigeria.

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PÉRIPNEUMONIE CONTAGIEUSE BOVINE : COMPARAISON ENTRE LES TECHNIQUES D'HÉMAGGLUTINATION PASSIVE ET DE FIXATION DU COMPLÉMENT. — J.C. Chima et G. Pam.

Résumé : Cinq zébus ont été vaccinés contre la péripneumonie contagieuse bovine (PPCB) au moyen du vaccin de culture en bouillon de souche T1, et la réponse immunitaire induite a été mesurée par les techniques d'hémagglutination passive (HAP) et de fixation du complément (FC). Les auteurs ont constaté que la HAP permettait de détecter les anticorps contre Mycoplasma mycoides sous-espèce mycoides plus précocement et pendant plus longtemps après la vaccination que la FC. Tous les animaux sont devenus négatifs à la
FC 10 semaines après la vaccination alors qu’ils étaient encore positifs à la HAP après 24 semaines.

De même, lors d’examens pratiqués sur 26 animaux présents dans un foyer naturel de PPCB, la FC a permis de déceler neuf cas positifs, tandis que quatorze animaux, dont tous ceux positifs à la FC, se sont révélés positifs à la HAP. Ces résultats sont discutés à la lumière du rôle que le diagnostic sérologique est appelé à jouer dans le programme actuel de lutte contre la PPCB.


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PERINEUMONÍA CONTAGIOSA BOVINA : COMPARACIÓN ENTRE LAS TÉCNICAS DE HEMAGLUTINACIÓN PASIVA Y DE FIJACIÓN DEL COMPLEMENTO. — J.C. Chima y G. Pam.

Resumen : Cinco cebúes fueron vacunados contra la perineumonía contagiosa bovina (PNCB) con la vacuna de cultivo en caldo de cepa T1, midiendo la respuesta inmunitaria inducida con las técnicas de hemaglutinación pasiva (HAP) y de fijación del complemento (FC). Comprobaron los autores que con la HAP se detectaban los anticuerpos contra Mycoplasma mycoides subspecie mycoides con mayor precocidad y durante más tiempo después de la vacunación que con la FC. Todos los animales resultaron negativos en la FC 10 semanas después de la vacunación, mientras que seguían siendo positivos en la HAP 24 semanas después.

Asimismo, en los exámenes practicados en 26 animales presentes en un foco natural de PNCB, con la FC se descubrieron nueve casos positivos, mientras que catorce animales, incluidos todos los positivos en la FC, resultaron positivos en la HAP. Se glosan estos resultados a la luz del papel que el diagnóstico serológico ha de desempeñar en el actual programa de lucha contra la PNCB.


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REFERENCES


