Sensitivity and specificity of serological and bacteriological tests for contagious bovine pleuropneumonia

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Summary

In 1990 an outbreak of contagious bovine pleuropneumonia (CBPP) occurred in Italy. Subsequent surveillance for CBPP was based on random sampling in bovine herds, serological controls on all animals moved from the herd of origin and controls on slaughtered animals. Official tests employed were the complement fixation test (CFT) and bacteriological isolation and typing. A total of 33,856 serum samples collected from herds in CBPP-free regions were used to define CFT specificity, while samples from 595 animals from infected herds were employed to define the sensitivity. Ninety-nine animals from three infected herds were used to estimate the sensitivity of the isolation technique. Results showed the specificity of CFT (threshold +1:10) to be 98% and sensitivity to be 63.79%. The sensitivity of the test did not change significantly, regardless of whether the lesions were caused by acute or chronic infection. The sensitivity of the isolation technique was 54.1%.

Keywords


Introduction

Contagious bovine pleuropneumonia (CBPP) was endemic in the area of the Eastern Italian Alps up to the 16th Century and had spread throughout Europe by the 18th Century. Italy was free from CBPP from 1899 up to October 1990, when a new outbreak occurred in the Po valley (5, 7, 8).

Contagious bovine pleuropneumonia is considered an exotic disease in Italy, therefore eradication was deemed necessary. A surveillance plan was implemented by Ministerial decree (1) to determine infection occurrence and to plan the eradication programme. Surveillance was based on random sampling in bovine herds, serological controls on all animals moved from their herd of origin and controls on slaughtered animals.

Random herd sampling ceased after the end of the first phase of surveillance, while serological controls on animal movements and controls in slaughterhouses continued. In cases where positive serological results were obtained in a herd, one or more serologically positive animals were slaughtered and post-mortem histological and bacteriological examinations were performed to confirm suspicion.

Since October 1990, 94 outbreaks of CBPP have occurred in Italy. Of these, 47 have occurred in three circumscribed areas of northern Italy (Lombardy region), where the disease became endemic. The last outbreak in the endemic area was extinguished on 14 September 1993, while the last of the remaining 47 outbreaks (scattered in various other regions of Italy) was extinguished on 7 September 1993 (2).

In the course of surveillance, a number of false positive and false negative complement fixation tests (CFT) results were obtained. This was to be expected given the relatively low infection prevalence of CBPP infection observed in Italy and the low positive predictive value of diagnostic tests in such a situation.

Few quantitative data have been published on the sensitivity and specificity of diagnostic tests used for CBPP, and results of different studies are often contradictory. As far as CFT is concerned, some authors reported the test to be specific but
not very sensitive, unable to detect animals incubating the
disease and with percentages of false negative results that
increase as the infection becomes chronic (10). In a study
carried out in Portugal, CFT had 91% sensitivity in animals
with chronic lesions, 93% sensitivity in animals with acute
lesions and 28% in the presence of lesions at the initial stage
(9). In the same study, a number of non-specific reactions to
CFT was recorded, but the specificity of the test was not
estimated.

A comparative trial on the use of CFT for CBPP was
performed in the European Community in 1983 (4). The trial
was carried out by twelve laboratories in nine countries, using
20 bovine lyophilised sera from animals of known health
status and ranging from strongly positive to negative. The
sensitivity of the test performed in the various laboratories
varied from 50% to 100%.

Determination of the sensitivity and specificity levels of the
diagnostic tests becomes crucial for the planning and
evaluation of eradication activities. Therefore, a study to
determine the performance of official CBPP tests was carried
out.

Material and methods

Definition of a contagious bovine
pleuropneumonia-infected herd and case

The following definitions are given:

A 'CBPP-infected herd': a herd in which Mycoplasma mycoides
subsp. mycoides SC was isolated from at least one animal.

An 'acute case of CBPP': an animal from a CBPP-infected herd
in which pneumonia with acute pleurisy, without sequestra
and isolation of M. mycoides subsp. mycoides SC in the
absence of gross lesions, occurred, either alone or in
combination.

A 'chronic case of CBPP': an animal from a CBPP-infected
herd in which fibrinous pleurisy, sequestra and/or chronic
pneumonia occurred alone or in combination.

Animals and sera

A total of 33,856 serum samples collected from uninfected
herds in the Abruzzo and Molise regions, between
11 December 1991 and 31 December 1994 were used to
define CFT specificity. In the Abruzzo region, only one
outbreak occurred, in 1993, in a trader holding where a single
imported animal was found to be infected. In the Molise
region no outbreak occurred. A herd was considered to show
false positive results (and was included in the study) when it
gave positive reactions to serological testing and yet the
epidemiological investigation did not reveal the presence of
CBPP risk factors (animal introduction, contacts with infected
or suspected animals) and when either of the two following
events occurred:
a) seropositive animals were slaughtered and neither gross
lesions were observed nor isolation of Mycoplasma spp.
occurred, or
b) seropositive animals were not slaughtered, but all the
animals in the herd gave negative results to three subsequent
serological tests performed 21 days apart.

A total of 595 animals from 11 infected herds were used to
define the sensitivity of CFT. Following bacteriological
confirmation of the infection, all the animals in the herd were
slaughtered and examined by official veterinarians to detect
gross lesions and to collect further samples. Animals were
subdivided into two groups according to CBPP case definition
and cases were used to estimate CFT sensitivity. Typical CBP
lesions were observed in 301 animals (29 of the acute type
and 272 of the chronic type) and/or M. mycoides subsp.
mycoides SC was isolated.

A total of 99 animals from 3 infected herds were used to
estimate the sensitivity of the isolation technique in relation to
defined cases of CBPP.

Laboratory tests

Serum samples were analysed by CFT according to the
technique described by Campbell and Turner (3), slightly
modified according to the Office International des Epizooties
(OIE) Standards (6). The initial dilution of test samples was
1:10 and a serum was considered positive if at least a (+)
haemolysis inhibition was observed at the initial serum
dilution. Attempts to isolate M. mycoides subsp. mycoides SC
were performed on lung, lymph node and kidney, according
to the OIE Standards (6).

Results

Complement fixation test specificity

A total of 33,856 animals from CBPP-free herds were tested
and 33,162 (98%; 95% confidence interval = 97.8%-98.1%) gave
negative results (Table I). Increment of the minimal
positive titre increases CFT specificity (Fig. 1). As an example,
specificity increases to 99.52% if the minimum titre
considered positive was assumed to be (+++)1:10.

Complement fixation test sensitivity

A total of 192 out of 301 animals with specific lesions and/or
successful isolation (63.79%; 95% confidence interval =
58.0%-69.2%) showed positive CFT results at a titre of
(+1:10 or above (Table I).
Table I
Sero logical results of 33,856 sera from healthy herds and 595 sera from 11 contagious bovine pleuropneumonia (CBPP)-infected herds, by type of lesions and bacteriological results

<table>
<thead>
<tr>
<th>Herd status and animal lesions</th>
<th>Negative</th>
<th>+1:10 to +++1:10</th>
<th>1:10 to +++1:20</th>
<th>1:20 to +++1:40</th>
<th>1:40 to +++1:80</th>
<th>≥1:80</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBPP-free herds</td>
<td>33,162</td>
<td>533 (1.6%)</td>
<td>116 (0.3%)</td>
<td>31 (0.1%)</td>
<td>3 (0.0%)</td>
<td>3</td>
<td>33,856</td>
</tr>
<tr>
<td>CBPP-infected herds without lesions and without isolation</td>
<td>216 (6.4%)</td>
<td>4 (1.3%)</td>
<td>37 (12.5%)</td>
<td>14 (4.8%)</td>
<td>10 (3.4%)</td>
<td>3</td>
<td>294</td>
</tr>
<tr>
<td>CBPP-infected herds with lesions or isolation</td>
<td>105 (32.2%)</td>
<td>4 (1.3%)</td>
<td>40 (13.3%)</td>
<td>23 (7.9%)</td>
<td>27 (8.0%)</td>
<td>9</td>
<td>301</td>
</tr>
<tr>
<td>CBPP-infected herds with acute lesions or isolation</td>
<td>10 (34.5%)</td>
<td>1 (3.4%)</td>
<td>5 (17.2%)</td>
<td>1 (3.4%)</td>
<td>2 (6.9%)</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>CBPP-infected herds with chronic lesions</td>
<td>99 (36.4%)</td>
<td>3 (1.1%)</td>
<td>35 (12.9%)</td>
<td>22 (8.1%)</td>
<td>25 (9.2%)</td>
<td>9</td>
<td>272</td>
</tr>
<tr>
<td>Total from CBPP-infected herds</td>
<td>325 (54.6%)</td>
<td>8 (1.3%)</td>
<td>77 (12.9%)</td>
<td>37 (6.2%)</td>
<td>37 (6.2%)</td>
<td>111 (18.7%)</td>
<td>595</td>
</tr>
</tbody>
</table>

Fig. 1
Specificity of the complement fixation test in relation to different threshold titres

Chronicity and acuteness of lesions do not appear to influence CFT sensitivity: 65.5% of the animals with acute lesions and 63.6% of the animals with chronic lesions showed positive serology. As expected, increase of the minimal positive titre decreases CFT sensitivity (Fig. 2).

Fig. 2
Sensitivity of the complement fixation test in relation to different threshold titres

Sensitivity and specificity of the complement fixation test on a herd basis

Definition of the CBPP infection status of a herd depends on the test results of each single animal of the herd, e.g. the whole herd is considered to be infected if a single animal gives positive results. Effects of low sensitivity and specificity of tests, therefore, are amplified.

Table II
Comparison of anatomo-pathological and bacteriological findings of contagious bovine pleuropneumonia in 99 animals from infected herds

<table>
<thead>
<tr>
<th>Isolation of Mycoplasma mycoides</th>
<th>Lesions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>47</td>
</tr>
</tbody>
</table>

An attempt to estimate the probability of a false positive test result of a herd was made (Fig. 3). The probability of a false positive CFT result for the average-sized cattle herd in Italy (30 heads) is 45%, and this increases with the number of animals tested.

Fig. 3
Probability of obtaining false positive results for herds in relation to number of animals tested and threshold titre
animals tested. On the contrary, false positive CFT result probability decreases if the minimum titre considered to represent a positive result is increased. Probability, for example, becomes 13% if the minimum positive titre is (+++):1:10.

The probability of a false negative test result of a herd is 4.75% when morbidity rate is 10%. In CBPP-infected herds, increasing the minimum CFT titre considered to represent a positive result increases false negative result probability (Fig. 4). The probability increases rapidly with the increment of the minimum positive threshold titre: 5.29%, 13.13% and 19.99% probability in case of titres of (+++):1:10, 1:20 and 1:40, respectively.

Fig. 4
Probability of obtaining a false negative result for a herd in relation to number of animals tested and the threshold titre (Morbidity: 10%)

Conclusions

Observed CFT specificity is 98%, while sensitivity is 63.79%. In the present study the sensitivity of the test was not influenced by the acuteness of lesions. The sensitivity of CFT appeared to be significantly lower than that recorded in the study in Portugal (9), but within the limits of the European comparative trial (4). The difference between this finding and the results obtained in Portugal could be due to the different case definition in the two studies. In fact, animals in the pre-patent stage of infection were included in the acute group in the present study, whereas such animals were excluded in the study in Portugal. Given that animals in the initial stage of infection gave 28% sensitivity to CFT in Portugal, the use of the same case definition in both studies would have led to more similar values of sensitivity.

The specificity value observed caused the occurrence of numerous false positive herd results. The probability of obtaining false positive results increases with herd size (or the number of animals tested). For the average herd (30 heads) in Italy, the probability of a false positive herd result is 45% and that of false negative definition is close to 5% (4.75%), with a 10% morbidity rate.

CFT specificity could be increased by incrementing the threshold titre/cut-off titre, but specificity increase at the herd level would be minor in case of a large herd size (>50 heads). Furthermore, an increase in the probability of false negative results would also ensue.

During the CBPP eradication campaign, the majority of serological controls were linked to animal movement. Such controls aimed to prevent the spread of disease by animal trade, therefore only animals to be moved were tested. Whole herd testing was only performed when positive serology results in an individual animal, observation of suspect lesions at the slaughterhouse or trace-back activities from an outbreak, etc., induced suspicion. In such a framework, the only reasonable threshold to use was +1:10, and the results of this study confirm this to be a valid choice.

Results of the study show that an eradication plan for CBPP can be based on neither serological diagnosis nor bacteriological testing alone. In fact, the sensitivity of CFT is insufficient for individual case diagnosis. If positive serology is observed, shortcomings in CFT specificity in middle-sized and large herds is such that the true infection status of a herd should be determined by the following:

a) an in-depth epidemiological investigation
b) repeated whole herd serological testing, and
c) bacteriological examinations on selectively slaughtered animals, chosen from among the serologically positive. Indeed, since the sensitivity of bacteriological technique observed was 54.1%, negative bacteriological tests do not allow per se exclusion of the presence of infection.
Sensibilité et spécificité des épreuves sérologiques et bactériologiques pour la péripneumonie contagieuse bovine

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Résumé
En 1990, un foyer de péripneumonie contagieuse bovine est apparu en Italie. Depuis, un programme de surveillance a été mis en place, comprenant des analyses sérologiques aléatoires dans les élevages et une analyse systématique chaque fois qu'un animal quitte son élevage d'origine, ainsi que l'examen postmortem des animaux abattus. Les tests officiels utilisés sont l'épreuve de fixation du complément, l'isolement bactérien et le typage. En tout, 33 856 sérums prélevés dans des élevages de régions indemnes de la maladie ont été utilisés pour définir la spécificité du test de fixation du complément, tandis que les prélèvements effectués sur 595 animaux appartenant à des élevages infectés ont servi à en définir la sensibilité. Quatre-vingt-dix-neuf animaux provenant de trois élevages infectés ont permis d’estimer la sensibilité de la technique d’isolement. D’après les résultats obtenus, la spécificité de l’épreuve de fixation du complément (seuil de positivité : dilution au 1/10) est de 98 % et sa sensibilité de 63,79 %. La sensibilité du test ne variait pas significativement selon que les lésions résultaient d’une infection aiguë ou chronique. La sensibilité de la technique d’isolement était de 54,1 %.

Mots-clés

Sensibilidad y especificidad de pruebas serológicas y bacteriológicas para la detección de la perineumonía contagiosa bovina

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Resumen
En 1990 se declaró un brote de perineumonía contagiosa bovina (PCB) en Italia. Las subsiguientes medidas de vigilancia contra esa enfermedad instituyeron muestreos aleatorios en rebaños vacunos, control serológico de todos los animales separados de su rebaño de origen y control de los animales sacrificados. Las pruebas oficiales que debían aplicarse en esos controles eran la fijación del complemento y el aislamiento bacteriológico y posterior tipificación. Para determinar la especificidad de la prueba de fijación del complemento se emplearon un total de 33.856 muestras séricas tomadas de rebaños de regiones libres de PCB, y para fijar su sensibilidad se utilizaron muestras de 595 animales de rebaños infectados. Para estimar la sensibilidad de la técnica de aislamiento se emplearon 99 animales de tres rebaños infectados. Los resultados de la prueba de fijación del complemento pusieron de manifiesto una especificidad (umbral de positividad: dilución al 1/10) del 98% y una
sensibilidad del 63,79%. El origen de las lesiones, infección crónica o infección aguda, no alteraba significativamente la sensibilidad de la prueba. La sensibilidad de la técnica de aislamiento resultó del 54,1%.

Palabras clave

References


