CONTAGIOUS BOVINE PLEUROPNEUMONIA DIAGNOSIS AND PREVENTION

H.G. Jäger

Head, Research and Development and Quality Assurance, Ondersteopoort Biological Products
Private Bag X07, 0110 Onderstepoort, South Africa

Original: English

Summary: Reports of 20 Member Countries of the OIE in Africa are summarised for their methods of diagnosis and prevention of contagious bovine pleuropneumonia (CBPP). The current possibilities of diagnostic techniques and tests are discussed. Serological tests are evaluated. Methods to control CBPP are described and sanitary measures are compared with vaccination. The special conditions in African countries are emphasised and a solution to the problem is suggested.

1. INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is one of the major diseases threatening cattle production in Africa. The disease is also still present in some Asian countries and in Europe (24, 46).

It was widespread, earlier this century, in the pastoral areas of eastern and western subsaharan Africa, but control measures including vaccination campaigns successfully eradicated the disease from most of the continent. In 1970, CBPP was largely confined to the Horn of Africa, some parts of the West African Sahel and Angola. Since then, due to a number of reasons, the disease has spread back into Uganda, Rwanda, Kenya and Tanzania. From Angola it spread again into Namibia and finally into Botswana (20).

CBPP is a highly contagious disease causing damage not only by killing animals, but also by reducing fertility, milk, meat and manure production. It is an insidious disease that is not always detected immediately after infected animals have entered an area; in fact, it can take several months.

Diagnosis is based mainly on serology after the infection in an area has been confirmed.

Prevention is best achieved through intensive surveillance, establishment of buffer zones, quarantine, control programmes for the infected areas and disease preparedness for the threatened areas.

Vaccination is also reported to be a highly successful way of preventing CBPP if it is used correctly and consistently and potent vaccines are utilised.

2. CONTRIBUTIONS RECEIVED FROM OIE MEMBER COUNTRIES

Contributions were received from 20 of the 43 Member Countries of the OIE in Africa:

Algeria: CBPP has never occurred in this country, no national surveillance programmes exist.

Angola: CBPP is diagnosed in Angola by observation of clinical signs and macroscopical lesions during post mortem examinations. In the laboratory, the complement fixation test (CFT) is recommended for serology in suspect animals.

Benin: CBPP is regarded as one of the principal threats to livestock in Benin. Diagnosis is based mainly on epidemiological and clinical evidence and supported serologically by CFT. Because Benin is frequently visited by migrating cattle from the whole region, CBPP has spread despite repeated vaccination campaigns with a vaccine based on the strain T.1-44. This situation is explained by the fact that cattle breeders only vaccinate portions of their herds and the failure to apply a slaughter and compensation policy.
Botswana: CBPP was reintroduced into Botswana and confirmed in early 1995. During the first stages of the CBPP outbreak, diagnosis was based mainly on clinical and post-mortem examinations, as well as histopathology and bacteriology. Serology was done by other institutions until, with help from the FAO\(^1\), the complement fixation test (CFT) was introduced. This is at present the major serological test.

Prevention: the policy is to eradicate CBPP from Botswana completely. For this purpose, all cattle have been destroyed in the infected areas and restriction of cattle movement has been enforced by fencing the areas.

Burkina Faso: CBPP diagnosis is based on clinical signs, abattoir findings and in the laboratory, the immunodiffusion test is mostly used. The enzyme-linked immunosorbent assay (ELISA) and CFT test or isolation of the organism are for various reasons no longer in use. Prevention is mainly based on two principles: movement control and vaccination. The vaccine strain of choice was until now the T1-SR, but will be replaced by the T1-44 at the end of the 1996/1997 campaign.

Central African Republic: CBPP has been threatening cattle since 1975 in the north-west of the country and recently, since 1192, also the eastern regions. The spread of the disease has been controlled by the Veterinary Service of the Central African Republic with the assistance of the FAO and the World Bank. Diagnosis is based on clinical signs, post mortem and serology. Immunodiffusion and CFT are the serological tools of choice. Prevention is based on quarantine zones, movement control, systematic slaughtering of sick animals and obligatory vaccination in all herds of the region, using Perivax and Bivax since 1992.

Eritrea: CBPP has not been reported for three years; however, it is present in a neighbouring country. Surveillance activities in the field and at abattoirs have been increased.

Ghana: CBPP is reported to be spreading rapidly in West Africa and Ghana. Diagnosis in the laboratory is based on CFT. Prevention is based on vaccination, quarantine, movement control and slaughtering without compensation.

Guinea: CBPP has been present in Guinea for decades. It was recognised in 1936 in the north-east of the country and moved to the south-west in 1957, establishing an endemic zone which covers the whole of High Guinea and a part of the Guinea forest. Diagnosis is done clinically and serologically. CFT is the test of choice and the antigen is supplied by the CIRAD/EMVT\(^2\). Prevention is based on vaccination and slaughtering of sick animals.

Kenya: CBPP is diagnosed by clinical signs, isolation of the organism and CFT. Prevention is achieved by mass vaccination in endemic or border districts in the north. If CBPP is introduced into a non endemic area, all animals are tested with CFT, positives are slaughtered and the others are vaccinated. After 4-6 months, the animals are retested with the same consequences. Quarantine and strict livestock movement controls are also in place.

Malawi: CBPP has never occurred in Malawi, but as neighboring countries are infected, the Department of Animal Health and Industry has prepared a contingency plan including increased surveillance in border areas and training of veterinary personnel.

Mali: CBPP is reported to be the most important animal disease in Mali. Reports for 1996 showed 12 foci with 150 sick animals and 47 deaths. Diagnosis is based on epidemiological facts, clinical symptoms, post mortem findings and laboratory results. Laboratory tests include isolation and immunodiffusion as well as immunofluorescence, CFT and ELISA. Prevention is based on movement control, inspection at central points, inspection at abattoirs, branding of animals and isolation and slaughter of sick animals.

Namibia: CBPP is endemic in parts of northern Namibia and the infection is fed by illegal cross-border movements of cattle. Diagnosis is based on CFT. A polymerase chain reaction (PCR) test has been developed and is used to detect the organism in tissue samples. Prevention has been exercised in the

---

\(^1\) Food and Agriculture Organization of the United Nations  
\(^2\) Centre de coopération internationale en recherche agronomique pour le développement - Département d'élevage et de médecine vétérinaire
past by vaccination with the T1-SR strain based vaccine, but has been abandoned on the recommendation of the FAO. Ministerial approval is awaited for the importation of T1-44 vaccine for use in future campaigns.

Senegal: CBPP is diagnosed in Senegal by isolation and identification using classical bacteriological methods as well as PCR. Serology is based on the slide agglutination test (SAT), CFT and interfacial precipitation test (IFPT). ELISA is well-known and will be used as soon as the standard serum is evaluated. Prevention by sanitary measures, such as border surveillance and quarantine, is difficult for various reasons. By systematic annual vaccination of the total bovine population and minor sanitary measures such as isolation of the sick animals, remarkable success has been achieved. Senegal is on its way to declaring itself provisionally free CBPP, subject to improvement of sanitary measures.

Republic of South Africa: CBPP was eradicated from the RSA in 1924. A diagnostic centre exists at Onderstepoort, which has all the necessary equipment and helps with the laboratory diagnosis of CBPP in neighbouring countries. Prevention consists of import control of cattle and serological surveillance. South Africa also has the capacity to produce 900,000 doses of freeze-dried CBPP vaccine per week, if necessary.

Swaziland: CBPP has never been reported in Swaziland. For prevention, a surveillance system will have to be put in place, as well as links with diagnostic and training facilities established.

Tunisia: CBPP has never existed in Tunisia and no diagnostic test exists.

Zaire: CBPP was recently introduced into the country from a neighbouring country. The disease was suspected in 1991 and confirmed during the course of 1992. A systematic vaccination campaign was organised from 1993 to 1994, followed by a booster vaccination around the centre of the outbreak. Temporary economic problems did not allow the necessary steps to organise consolidation and control. More mortalities have been reported and it is feared that the disease will spread into neighbouring areas.

Zambia: Routine diagnosis is carried out in the Western Province using CFT. No positive case has been reported since 1976. In terms of prevention, vaccinations are carried out in a 10 km wide buffer zone adjacent to the Angolan border. Live animals from this zone are not permitted to enter other areas.

Zimbabwe: CBPP has not been diagnosed in Zimbabwe since 1904. Because of the increasing risks, however, staff have been sensitised and sent for training. Border surveillance in high risk areas has been increased and foreign cattle found within the borders are destroyed. The Veterinary Faculty is able to diagnose the disease and the Central Veterinary Laboratory is in the process of obtaining the necessary reagents.

3. DIAGNOSTIC TECHNIQUES

*Mycoplasma mycoides* subsp. *mycoides* SC (bovine biotype) and members of the *M. mycoides* and *M. capricolum* groups form the so-called *M. mycoides* cluster, which consists of six mycoplasmas originating from bovines and goats (9). The fact that they share serological and genetic characteristics causes taxonomic and diagnostic problems (9, 37). In natural conditions, *M. mycoides* subsp. *mycoides* SC affects only the ruminants of the *Bos* genus, i.e. mainly bovine and zebu cattle and, in some areas, domestic buffaloes (*Bubalis bubalis*) (41). Among wild animals, only yaks and American buffaloes (*Bison bison*) are probably susceptible, and not African buffaloes (*Syncerus caffer*) or other wild ruminants (42).

3.1. Clinical diagnosis

CBPP appears in different forms: the peracute form with sudden death, the acute form with symptoms of a serious febrile pleuropneumonia with subsequent death after five to eight days, and the subclinical form, characterised by a discrete pleuropneumonia which becomes chronic. The latter is the most common form. The animals become weak and the body temperature is around 40-41°C. Respiration is dyspnoeic and often jerky. Symptomless infections are common.

Under natural conditions, the incubation period is rarely less than three to six weeks, and may exceed three months. Under conditions of massive aerosol infection, the incubation period may be as short as five to 35 days. Acutely affected animals have an elevated body temperature, are listless and manifest signs of respiratory distress. They often grunt painfully and occasionally soft, moist coughs are heard, which become more frequent with exercise or percussion of the chest. As the disease progresses, coughing increases in frequency and in intensity. The animal is reluctant to move, and stands with its head extended, mouth open, tongue protruding and elbows turned out (photo
Contraction of the muscle of the abdominal wall occurs after each inspiration, while expiration is frequently followed by a characteristic groan. There is mucoid discharge from the nostrils and frothy saliva around the mouth.

In subacute cases, an occasional cough is the only clinical sign. The only clinical signs in chronic cases are emaciation and a cough when the animal rises. In calves up to six months old, only signs of arthritis may be seen.

Clinical diagnosis of CBPP is difficult. The disease must, therefore, be confirmed by laboratory examination.

3.2. Gross pathological signs

Pathological signs are confined to the thoracic cavity. Different degrees of pathological change may be found in one animal. The signs are usually unilateral without a preference for the left or the right side. Lesions are always localised in the diaphragmatic lobe. The cranial lobe is seldom affected. In the acute stage, many litres of serous fluid are usually present in the thoracic cavity. The fluid is clear yellow-brown and may contain pieces of fibrin. Frequently, a thick, caseous fibrinous deposit is present on the visceral and parietal pleura (photo 2), which is a pathognomonic sign (33). The lung shows another pathognomonic sign of CBPP: interlobular septa that are distended by amber-coloured, serous fluid in and around dilated lymphatics. The fluid separates the heptatised lung lobules that have different colours, varying from relatively normal to deep red or yellow-grey, due to acute fibrinonecrotic pneumonia. The lungs have a marbled appearance and are oedematous (photo 3).

In the chronic stage, adhesions connect the thickened visceral and parietal pleura. When the thoracic cavity is opened, pieces of lung adhere to the chest wall. The fluid in the thoracic cavity may have disappeared. Sequestra in the lung are characteristic lesions. Sequestra vary in size from 1 to 30 cm. A sequestrum may remain infected for years, even after the cow has recovered clinically. The necrotic contents of a sequestrum may be liquid to firm and yellow-green to brown. A sequestrum may discharge into a bronchus and infectious material may be released in the respiratory tract and coughed out as infectious droplets. This often happens when the animals are under stress.

After escape of the contents, a cavity may be left in the lung (33, 42). Hypertrophy of bronchial lymph nodes is usually observed. Additional lesions may be exudative pericarditis, exudative peritonitis, arthritis, bursitis or renal infarct (photo 4) (19, 33).

Lesions in calves are confined to fibrinous arthritis of carpal or tarsal joints.

3.3. Laboratory diagnosis by identification of the agent

The organism can be isolated from samples taken either from live animals or after necropsy. Nasal swabs or discharges, tracheal washings, pleural fluid taken by puncture or blood may be collected from live animals (30).

Samples taken after necropsy are lungs with lesions, pleural fluid, lymph nodes of the broncho-pulmonary tract and synovial fluid from calves. Samples should preferably be sent in transport medium and under cold conditions or frozen.

It must, however, be kept in mind that the presence of pathogens varies greatly with the development of the lesions, and a negative result is not conclusive!

a) Culture

*Mycoplasma mycoides* subsp. *mycoides* SC is best grown on a basic medium containing heart infusion or peptone, yeast extract and 10% (antibody free) horse serum. To avoid overgrowth of contaminants, thallium acetate should be added.

b) Biochemical tests

After two or three subcultures and the exclusion of bacterial L-forms the organism can be identified using biochemical tests (1). *Mycoplasma mycoides* subsp. *mycoides* SC is sensitive to digitonin, breaks down glucose, reduces tetrazolium salts, does not hydrolyse arginine, has no phosphatase activity, and has no or weak proteolytic properties.

c) Immunological tests

Immunological tests are necessary to confirm the identification. It is recommended to use both the direct fluorescent antibody test (FAT) and the growth inhibition test (GIT), since results may vary due to differences in sensitivity and specificity. The organism or its antigens can be demonstrated by FAT in infected tissues, organic fluids and/or in cultures.
Photo 1: Clinical case of contagious bovine pleuropneumonia: characteristic posture that is often seen in coughing animals* 

* All photographs are courtesy of Dr Leon Prozesky.
Photo 2: Lesions observed in animals that died from contagious bovine pleuropneumonia: pronounced fibrinous pleuritis

*Lésions observées chez des animaux morts de péripneumonie contagieuse bovine : pleurésie fibrineuse aiguë*
The two tests recommended are:

1. **Direct fluorescent antibody test**
   The FAT is commonly used and performed from broth and agar cultures and can be used on tissues if the level of infection is high. It uses a labelled hyperimmune serum, which is incubated on the methyl alcohol fixed antigen and then rinsed and read under an epifluorescence microscope.

2. **Growth inhibition test**
   The GIT is based on the direct inhibition of the growth of the organism on a solid medium by specific hyperimmune serum (13, 17). It is a specific and simple test to perform, but some results require experience to interpret.

Other tests: Indirect fluorescent antibody test (IFAT), Agar gel immunodiffusion test (AGID) (17, 32), Interfacial precipitation test (IFPT) (45), Dot immunobinding on membrane filtration (MF dot) (30, 31), Immunohistochemistry (11, 12), which, for different reasons (price, specificity, sophistication, equipment), are less common.

New tests, capable of detecting and identifying *M. mycoides* subsp. *mycoides* SC, are being developed, using nucleic acid recognition methods. A gene probe, CAP 21, which is capable of distinguishing the above mycoplasma from other closely related mycoplasmas, has been developed (45).

Also, a PCR has been used to detect and identify *M. mycoides* subsp. *mycoides* SC specific DNA in clinical material and field isolates (2, 3, 5, 10, 18, 21, 23).

### 3.4. Serology

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

**a) Serological tests that detect antigens in the serum of the infected host:**
- agar gel double immunodiffusion - only effective during clinical disease, cheap, simple (17)
- interfacial precipitation in liquid medium (47)
- counter current immunoelectrophoresis (29)
- competitive ELISA - still being tested, but should be good to distinguish vaccinated from infected animals (6, 15).

**b) Serological tests that detect antibodies:**
- slide agglutination on serum or blood - acute disease (48)
- passive haemagglutination - also detects vaccinated animals, can show non-specific reactions (8, 36)
- latex agglutination (28)
- CFT - vaccinated animals lose titre after 3 months (41)
- single reverse radial immunodiffusion (38)
- ELISA - indirect and sandwich - recent developments - need more development and are expensive, but would be able to distinguish between vaccinated and infected animals (4, 6, 22, 26).

The biggest problem is that there are many serological cross-reactions among the mycoplasmas belonging to the *M. mycoides* 'cluster', which provoke many false positive results (45).

A study was carried out recently comparing several serological tests and gross lung pathology for detecting CBPP in cattle (25). This study compared the rapid SAT, the CFT, an indirect ELISA, MF dot blotting and Western blotting with mycoplasma culturing and pathology.

The authors came to the conclusion that, at present, there is no single test which can detect all infected animals.

Abattoir surveillance by experienced meat inspectors, backed up by sensitive serological tests, provides the best chance of diagnosing CBPP.

The CFT is still the most reliable of the numerous serological tests and rarely gives false-positives (14). The limitations are well known. For groups of animals (herd or epidemiological unit), it is capable of detecting practically 100% of infected groups. Its specificity is greater than 99.5%. It is capable of detecting close to 100% of animals with acute lesions, and a rather smaller proportion of animals in the early stages of the disease and with
chronic lesions. Detection of chronic carriers having sequestered lung lesions remains a real problem (34).

The rapid SAT (with either serum or whole blood), however, is also very reliable early in the infection, when used on a herd basis.

Photo 3: Lesions observed in animals that died from contagious bovine pleuropneumonia: marble appearance of the lung, note the prominent interlobular septae

*Lésions observées chez des animaux morts de péripneumonie contagieuse bovine : aspect marbré des poumons, où l'on distingue bien les espaces interlobulaires*
Photo 4: Lesions observed in animals that died from contagious bovine pleuropneumonia: renal infarct

*Lésions observées chez des animaux morts de péripneumonie contagieuse bovine : infarctus rénal*
4. TRANSMISSION

CBPP is naturally only transmitted via live cattle. The disease can be produced experimentally, but this is a rather difficult exercise (7). There is no evidence to suggest that cattle can develop CBPP when exposed to dead animals or to diseased lungs or other organs from animals that have been slaughtered or died from the disease (19, 43).

5. CONTROL

5.1. Surveillance

The ideal surveillance programme should have a very high probability of detecting the disease in infected countries. Surveillance implies that official action will follow immediately from the discovery of evidence of CBPP - in contrast to monitoring, in which the gathering of data from the field takes place similarly, but no official action based on the findings is implied in the data gathering activity (16, 44).

No vaccination should be allowed in the surveillance zones, as this will mask the presence of the disease.

All abattoirs must have meat inspectors who are specially trained to detect lesions indicating CBPP.

5.2. Movement control

The most important factor to avoid spread of the disease is to achieve complete control of cattle movement. No animals can be allowed to be moved across international borders or from infected areas to surveillance zones without a movement permit issued at the point of departure, and declaring that the herd of origin has been inspected and is free of clinical CBPP and serologically negative (herd test).

The movement of cattle into or across the surveillance zone should be confined to stock destined for immediate slaughter. The movement of breeding cattle should be discouraged.

All consignments of animals crossing the zone must be examined for clinical evidence of disease. Where animals are moved in vehicles (road, rail or boat), they must be disembarked to allow for inspection.

All movements of cattle across a surveillance zone should be through recognised quarantine stations that have facilities (holding areas) for disembarked cattle.

'Cordon sanitaire' (buffer zones) must be established to separate infected and threatened territories.

Control programmes should be developed in the infected areas. Disease preparedness measures should be initiated in those areas that are presently clear of the disease but that are threatened (EMPRES3).

5.3. Slaughter and compensation

Because one animal can infect entire countries, it is important that infected animals are found and slaughtered. It is essential that owners are encouraged to indicate sick animals, and this is only possible on the basis of trust and immediate compensation.

The same holds true for vaccination. Livestock owners must trust the vaccine, and compensation for possible vaccination losses must be offered. One must keep in mind that possible losses due to one infected animal that forms new infection foci elsewhere is by far more costly than compensation.

5.4. Vaccination

Vaccination is a valuable tool in preventing CBPP (40, 44). This is even more important in countries where movement controls and abattoir surveillance are not possible.

In most African countries, livestock farming is extensive over wide areas and does not allow strict control of herds,
which makes serological surveillance rather difficult. For the same reasons, movement control is practically impossible.

Political realities do not allow, with one exception (35), a strict slaughtering-out policy, as most African governments are occupied with other priorities. It is therefore difficult to pay compensation to affected animal owners.

If the vaccination strategy is chosen, there are some very important points to be remembered. CBPP vaccination of individual animals will increase their resistance, but may not prevent them becoming infected. Vaccination of whole populations will gradually extinguish infection circulating in that population, but only if close to 100% of herds are vaccinated. Anything less means that there is a risk of susceptible cattle contracting the disease and, by close contact with vaccinates, infection may overcome vaccinal resistance. In such situations, livestock owners and authorities lose faith in the vaccination campaign and it fails (20).

It has been reported that just after a vaccination campaign some minor outbreaks may occur, due to the breaking of sequestra after handling stress. These outbreaks disappear, however, as vaccination takes effect (29).

The report from Senegal shows that it is possible to eradicate CBPP with vaccination and some additional minor sanitary measures. The following points are critical:

- The vaccination campaign must be well-planned and organised.
- The personnel must be well-trained, equipped and motivated.
- The vaccine must be of good quality.
- Neighbouring countries should be willing to co-operate.

Only the T1-44 strain should be used as seed strain for the preparation of the vaccine, until PANVAC\(^4\) has finished its comparison of the immunity of the T1-44 and that of the T1-SR, according to the recommendation of the FAO.

Problems encountered are the following:

- Field services are not always operational because of lack of funds or civil disturbances.
- The proper chain of command from the top to the field level is not guaranteed.
- Laboratories are not functioning due to lack of equipment, operating funds or properly trained staff.
- Data on cattle distribution and movements are not accurate.
- Vaccines are not properly handled.

**REFERENCES**


\(^4\) Pan African Veterinary Vaccine Centre


