Field diagnostic kits:
a solution for developing countries?

P.-C. LEFÈVRE *, J. BLANCOU **, L. DEDIEU *, A. DIALLO *, G. LIBEAU * and F. THIAUCOURT *

Summary: An exact assessment of the animal health situation in a country is an essential element in formulating eradication and control programmes, and in regulating international trade in animals and animal products from that country. Due to a lack of human and technical resources, Veterinary Services in developing countries often lack precise knowledge on disease occurrence. Since the collection and transmission of reliable information on animal diseases in developing countries are major concerns of the Office International des Epizooties (OIE), a project aimed at improving this situation was implemented with international financial support. This project involved the development by the Centre for the Application of Methodology for the Diagnosis of Animal Diseases (CAMDA) of field kits for the diagnosis of the main diseases present in tropical Africa: rinderpest, peste des petits ruminants (PPR), contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP).

Several tests already exist, such as complement deoxyribonucleic acid (cDNA)-specific probes and polymerase chain reaction (PCR) for rinderpest and PPR, DNA probes and PCR for CBPP, capture enzyme-linked immunosorbent assay, the agglutination test and the immunobinding peroxidase test for CCPP, etc. With specific reference to these examples, the various problems faced by the OIE and CAMDA are reviewed.

KEYWORDS: Contagious bovine pleuropneumonia – Contagious caprine pleuropneumonia – Field diagnosis – Field kits – Monoclonal antibody – Nucleic acid probe – Peste des petits ruminants – Rinderpest.

INTRODUCTION

An accurate assessment of the animal health situation of the livestock in a country is a necessary condition for implementing disease eradication or control programmes, as well as for international trade in animals and animal products.

In many developing countries, it is difficult for Veterinary Services to establish a full list of the diseases prevalent in the country, due to constraints such as:

- insufficient numbers of field veterinarians or veterinary technicians and a lack of incentives for such technicians

* Laboratory for Tropical Animal Diseases ("PATHOTROP"), Centre de coopération internationale en recherche agronomique pour le développement – Département d'élevage et de médecine vétérinaire (CIRAD-EMVT), Office International des Epizooties Centre for the Application of Methodology for the Diagnosis of Animal Diseases, 10 rue Pierre-Curie, 94704 Maisons-Alfort, France.

** Director General, Office International des Epizooties, 12 rue de Prony, 75017 Paris, France.
- scarcity of well-equipped diagnostic laboratories with well-trained technicians
- use of extensive husbandry systems and distribution of animals over large areas with poor communication and transport facilities
- inadequate or non-existent cold chains.

Many other factors also impede the gathering of information on disease prevalence; these factors vary from country to country.

As a result of these difficulties, Veterinary Services in developing countries are often aware of only a small proportion of disease outbreaks. This knowledge is generally based on clinical reports alone, with few (if any) laboratory confirmations. It is therefore difficult to make an accurate assessment of the geographical distribution and relative importance of diseases.

While epidemiological surveillance is very important for any country, it is even more so for developing countries, for at least two reasons:

a) a lack of confidence on the part of potential importing countries may lead to exclusion from international trade

b) appropriate disease control programmes cannot be instituted without a realistic assessment of disease prevalence in the country.

FIELD DIAGNOSTIC KITS: A POSSIBLE SOLUTION

Recent advances in molecular biology and more generally in biotechnology may help to resolve these problems through the development of field diagnostic kits (1, 6). It seems unlikely, if not impossible, that the number of laboratories and field technicians will be increased in the near future, and one possible solution would therefore be to produce and distribute reliable tools which would enable field veterinarians and/or technicians to obtain a rapid confirmation of clinical diagnoses. Moreover, these field kits could help diagnosticians to make rapid and appropriate decisions in the event of a disease outbreak.

The ideal field diagnostic kit should, of course, be sensitive and specific (like any other laboratory technique) but should also be easy to use by unskilled persons and resistant to rough climatic conditions.

In January 1991, the Office International des Epizooties (OIE) appointed the Laboratory for Tropical Animal Diseases ("PATHOTROP") of the Centre de coopération internationale en recherche agronomique pour le développement – Département d'élevage et de médecine vétérinaire (CIRAD-EMVT) to be its first Centre for the Application of Methodology for the Diagnosis of Animal Diseases (CAMDA-OIE) in order to develop field kits for the following diseases:

- rinderpest
- peste des petits ruminants (PPR)
- contagious bovine pleuropneumonia (CBPP)
- contagious caprine pleuropneumonia (CCPP-F38)
- contagious agalactia.

Rinderpest and peste des petits ruminants are two Morbillivirus infections widespread throughout tropical Africa, the Middle East and India. Two specific probes
for rinderpest and PPR along with a panel of monoclonal antibodies are now available for the development of kits. Moreover, the gene coding for the nucleocapsid protein (N) has now been inserted into the baculovirus and large amounts of protein can be obtained.

CBPP, caused by *Mycoplasma mycoides* spp. *mycoides* small colony type (Mmm SC) is enzootic in Southern Europe (Italy, Spain and Portugal), tropical Africa, probably in some areas of India (e.g. Assam), in China and several countries of South-East Asia. The main difficulty in eradication of CBPP involves the existence of latent carriers (10-15% of recovered animals) which may excrete mycoplasmas while apparently healthy.

The geographical distribution of CCPP (caused by *Mycoplasma* sp. F38 biotype) is not known, as the pathogen requires special media for growth. To date, *Mycoplasma* sp. F38 biotype has been isolated only in Africa (Kenya, Ethiopia, Sudan, Chad and Tunisia) and the Middle East (Oman and Yemen). The presence of this pathogen is suspected in Turkey.

The three characteristic lesions of contagious agalactia are mastitis, arthritis and keratitis. This disease is caused by *M. agalactiae*, *M. capricolum*, *M. m. mycoides* large colony (LC) type and *M. putrefaciens*.

Probes have been developed to detect some of these mycoplasmas (*M. capricolum*, *Mmm SC* and *M. agalactiae*) and others are being developed (*M. putrefaciens* and *M. bovis*). Panels of monoclonal antibodies have also been obtained against *Mmm SC*, *M. capricolum* and *Mycoplasma* sp. F38 biotype.

**DEVELOPMENT OF FIELD KITS**

**Probes**

Although there were few arguments in favour of using probes in field diagnostic kits, trials were conducted with probes for the diagnosis of several diseases.

*For rinderpest and peste des petits ruminants*

Complementary deoxyribonucleic acid (cDNA) clones specific for the nucleocapsid genes of the rinderpest and PPR have been used as radio-labelled probes to detect unequivocally these two viruses (4). When this test was performed on lymphocytes from cattle experimentally infected with rinderpest in Chad, it was possible to detect the virus ten days before the onset of disease.

To promote wide use of the probe technology in Africa, efforts were made to develop non-radioactive oligonucleotide probes. Two 20-mer oligonucleotides (specific for the variable 3' end of the N genes) were synthesised and labelled with biotin or digoxigenin. Unfortunately, the use of such probes on biological samples was disappointing: low sensitivity and considerable non-specific background led to unreliable results.

Tests are being carried out to simplify the extraction of ribonucleic acid (RNA) before the use of the probe, by adding guanidium thiocyanate and glass milk (RNA Matrix, BIO 101). Samples collected in the field could be preserved in a guanidium thiocyanate solution (a strong nuclease inhibitor) kept at ambient temperature. Once the samples have reached a regional laboratory, the RNA extraction step takes no more than half an hour.
For mycoplasma infections

Probes are obtained after extraction and purification of the total DNA and digestion by various restriction enzymes (mainly HindIII, RsaI and AluI).

The length of the different available probes is 305 bp for Mmm SC, 922 bp for M. capricolum and approximately 1,400 bp for M. agalactiae (2, 3; L. Dedieu, unpublished findings).

These were tested as "cold" (digoxigenin-labelled) probes on various biological samples. Unfortunately, it again seems that the DNA must first be extracted from the biological sample (especially when dealing with milk) by addition of guanidium thiocyanate and glass milk followed by centrifugation. The pre-hybridisation, hybridisation and washing steps also have to be performed under highly stringent conditions (65°C) and within 24-48 h.

The specificity of the probes varies according to the species. While the probe for M. agalactiae does not cross-react with any other mycoplasma, the probes for M. capricolum and Mmm SC can react with other mycoplasmas of the mycoides cluster but only when high quantities of heterologous DNA are involved.

The M. capricolum probe, for instance, may detect some strains of Mycoplasma sp. F38 biotype (actually, a biovar of M. capricolum) in the presence of 500 ng DNA per spot, i.e. $5 \times 10^7$ mycoplasmas per spot.

These probes are considered to be highly sensitive, as the cold probe can detect 1 ng DNA per spot, i.e. $10^5$ mycoplasmas.

Polymerase chain reaction

While sequencing the DNA fragments corresponding to the probes, it was possible to develop the polymerase chain reaction (PCR). At present, primers have been synthesised for rinderpest, PPR, M. capricolum and Mmm SC. It is obvious that this technique (especially for rinderpest and PPR) is of little interest in developing countries. Rinderpest and PPR are caused by RNA viruses, which require a preliminary step using Moloney murine reverse transcriptase (MoMu RT) in order to obtain cDNA.

Apart from some rare exceptions, this technique remains inaccessible to most tropical countries due to the need for expensive equipment, maintenance, etc. But even if PCR is introduced, it will not circumvent the need to send samples to a laboratory.

Kits with monoclonal antibodies

For rinderpest and peste des petits ruminants

Using a variety of monoclonal antibodies (MAbs), an immunocapture enzyme-linked immunosorbent assay (ELISA) was developed for diagnosis of rinderpest (7, 8). The microplates are coated with a non-specific anti-N MAb which can capture the N protein of the rinderpest or PPR virus. The sample and all the reagents – i.e. phosphate-buffered saline-Tween (45 µl), the two biotin-labelled specific MAbs (IV B2-4 for rinderpest and 38-4 for PPR) and peroxidase-streptavidin – are added in a single step and incubated for 1 h. After washing, orthophenylenediamine (OPD) is added, and 10 min later the reaction is blocked and a reading can be made.
This test is very specific (there are no cross-reactions between the two viruses) and sensitive, since it can detect 10 ng of N protein per sample, i.e. $10^2-10^3$ TCID$_{50}$ per ml.

Improvements should be made to this test in the near future to increase sensitivity and enable use with strips rather than microplates.

_For mycoplasma infections_

Initially, an immunobinding peroxidase test was developed for the detection of _Mycoplasma_ sp. F38 biotype in pleural fluids using strips and a polyclonal antiserum. However, while it is sensitive ($10^6-10^7$ mycoplasmas/ml), it often cross-reacts with _M. capricolum_ or _Mycoplasma_ of the _mycoides_ cluster (9).

Five µl of pleural fluid collected during necropsy from a goat suspected of having CCPP are spotted onto a nitrocellulose strip. The sample can be dried, inactivated and processed immediately or easily transported. The strip is then washed and saturated with Tween-tris-buffered saline and 10% horse serum before rabbit antiserum is added. After 30 min, the strip is washed and a peroxidase anti-rabbit IgG conjugate is added. The strip is then washed again and the reaction is revealed with a solution of diethylamino-azobenzene (DAB), NiCl and H$_2$O$_2$. MAbs were produced to avoid the cross-reactions, but though the specificity of the test has improved, the selected MAbs may still detect some other mycoplasma strains. Several MAbs may therefore be necessary and a “bar code” type strip is being developed.

Protein expression by a vector

Since the genes of the PPR virus N protein were inserted into the baculovirus, high quantities of N protein can easily be made available to coat latex beads (0.8 µ) and develop an agglutination test for antibody detection (5).

In countries where animals are not individually identified, it is very useful to have a pen-side test to check if the animal has been vaccinated or not, even if the sensitivity is in no way comparable to that of the ELISA.

**PROBLEMS LINKED TO FIELD TESTS**

General problems

The continual increase in human and animal populations places an obligation on those responsible for food production to improve the control of animal diseases. This obligation is all the more significant in that trade in animals and animal products increases the risk of spreading disease.

One of the primary objectives of the Food and Agricultural Organisation of the United Nations (FAO), OIE and World Health Organisation (WHO) is the surveillance and control of animal diseases. Surveillance would be greatly facilitated by the development of methods for rapid diagnosis in the field. The OIE considers the development of such methods a priority, and encourages all of its collaborating laboratories and centres – and CAMDA in particular – to perform research and develop practical applications in this area.

At the same time, however, national and international officials are also concerned to avoid any undesirable effects which may arise from the distribution of field tests. For
example, some farmers could conceal or dispose of diseased animals in an improper way. Therefore, not only the quality of the kits, but also their distribution and use and the exploitation of results, must continue to be officially controlled.

**Technical problems**

*For kits using probes*

“Hot” probes cannot be used in developing countries for various reasons (short half-life of \(^{32}\)P, lack of storage facilities, human health risks, etc.). Only cold probes, labelled with biotin or digoxigenin, should be developed since they are easier and safer to use under field conditions.

Unfortunately, at present it seems difficult (if not impossible) to overcome a number of technical problems:

- There are considerable non-specific background reactions. Treatment of the sample is required before performing the test in order to extract and purify the RNA or DNA. This is not especially difficult under laboratory conditions but it may render the test too complicated for field use.

- While cold probes are safe, they are not very sensitive for viruses and mycoplasmas due to the rare repetitive sequences in the genomes. With mycoplasma probes, a longer probe will be more sensitive and specific; however, more time will be required to perform the test. On the other hand, a shorter probe (oligonucleotide) will provide results in a few hours and at rather low temperatures (40-50°C), although specificity will be reduced.

- It is impossible, given the current state of the art, to carry out diagnostic tests using probes without a certain amount of equipment: water, centrifuge, water bath, etc. A pen-side test using a probe is therefore feasible only if mobile laboratories or well-equipped four-wheel drive vehicles are available.

*For kits using MAbs*

Immunocapture ELISA or immunobinding assays may be more useful in the near future since specificity can easily be improved using a panel of MAbs which will give a specific “pattern”, especially when dealing with pathogens such as mycoplasmas.

Sensitivity is more difficult to assess, as it depends on the excretion of the pathogen by the infected animals and the fact that different types of biological samples (pleural fluid, milk, nasal discharge, etc.) may have to be processed in different ways.

The sensitivity of the tests may also be increased if low concentration equipment is available. This is the case for the immunobinding assays which can detect \(10^7\) mycoplasmas/ml of pleural fluid without previous concentration and as few as \(10^5\) mycoplasmas after concentration.

**Difficult conditions of use**

In many developing countries, particularly in tropical regions, climatic conditions impose severe constraints on field work. Therefore, when cold chains are non-existent or limited, it is necessary to develop kits which can withstand high temperatures (35°C or more) and transport over long distances. The kits must not only be reliable but must also tolerate such conditions.
This is not the case at present, since the reagents (coated plates, probes, MAbs, etc.) and the chemicals (DAB, OPD, etc.) need to be kept at 4°C.

**Human and commercial problems**

**Training of technicians**

Except for the agglutination test, most of these techniques require some degree of skill. As field technicians are usually not trained in laboratory work, training courses are needed before these tests can be employed in the field.

In the past, kits have sometimes been distributed and never used because the field technicians had not been provided with the necessary training. A training component was therefore included in the initial CAMDA project.

**Distribution**

Obtaining probes or monoclonal antibodies is not a major problem for a veterinary research laboratory. However, the development of kits is more problematic as it requires industrial expertise which most research laboratories are incapable of providing. For the final development and large-scale production of the kits, the research laboratory must seek collaboration with a private industrial company. Unfortunately, the diseases prevailing in developing countries (such as rinderpest and PPR) are not of great interest for these companies, since the market is rather limited. Moreover, the kits cannot be bought by the developing countries themselves and the producers have to rely on international aid.

In collaboration with the OIE, the International Atomic Energy Agency in Vienna (Austria) is carrying out a world-wide programme for the production and distribution of some kits (e.g. ELISA and agglutination tests) and the training of the necessary technicians.

**CONCLUSION**

To overcome the very specific difficulties of animal disease diagnosis in developing and/or tropical countries, it is obvious that many technical problems have still to be solved. On the one hand, cold probes have not proven very successful for use in the field. On the other hand, kits based on MAbs (immunocapture ELISA, immunobinding assays, etc.) may be usable in the present state of development, although their sensitivity should be improved. To resolve this problem, samples should come from several animals and field kits should be used only in case of severe outbreaks.

At present, the main conclusions are:

- Field kits should always be used for diagnosis at the herd level rather than on single animals. Negative results should be confirmed by other techniques.

- Field kits should be used in severe outbreaks to confirm the clinical diagnosis. In the case of mycoplasma infections, for instance, kits cannot be used to detect latent carriers on account of the inadequate threshold of sensitivity.

- Field kits can be used on samples which have not been kept at -20°C and from which the pathogens are no longer viable and cannot be isolated.
Little equipment is necessary and the test can easily be performed in small regional laboratories or well-equipped vehicles.

Regional laboratories or mobile teams could perhaps provide an alternative solution while genuine pen-side tests are under development.

While the present field kits available for diagnosis of rinderpest, PPR, CBPP and CCPP are not entirely satisfactory, they nevertheless represent a number of major improvements compared to the classical isolation techniques or more sophisticated techniques such as PCR. Only very simple laboratory equipment is required (water baths, bench centrifuges, etc.) and the tests can be performed in a few hours. It should therefore be possible to decentralise (and increase the number of) diagnoses performed, by either of the following means:

a) developing networks of regional laboratories with a minimum of equipment and one or two trained technicians, or

b) creating mobile teams with well-equipped four-wheel drive vehicles which could be present on site in a few hours or days.

**Résumé :** Un bilan précis de la santé animale dans un pays donné est un élément indispensable à l'élaboration de programmes de prophylaxie ainsi qu'à la réglementation du commerce international des animaux ou des produits d'origine animale en provenance de ce pays. Étant donné l'insuffisance de leurs ressources humaines et techniques, les Services vétérinaires des pays en développement manquent souvent de données précises sur l'incidence des maladies. Comme la collecte et la transmission d'informations fiables sur les maladies animales dans les pays en développement comptent parmi les sujets de préoccupation majeurs de l'Office international des épizooties (OIE), un projet visant à améliorer cette situation a été mis en œuvre grâce à une aide financière internationale. Dans le cadre de ce projet, le Centre pour l'application de la méthodologie au diagnostic des maladies animales (CAMDA) a mis au point des trousses (kits) pour le diagnostic sur le terrain des principales maladies présentes en Afrique tropicale : peste bovine, peste des petits ruminants, péripneumonie contagieuse bovine et pleuropneumonie contagieuse caprine.

Plusieurs techniques sont d'ores et déjà disponibles : les sondes spécifiques utilisant un acide désoxyribonucléique complémentaire (c-ADN) et l'amplification en chaîne par polymérase (polymerase chain reaction : PCR) pour la peste bovine et la peste des petits ruminants, les sondes à ADN et la

Resumen: Poder establecer un balance preciso de la situación de la sanidad animal en un país determinado es un factor indispensable tanto para la elaboración de programas de control como para la reglamentación del comercio internacional de animales o de productos pecuarios provenientes de ese país. La insuficiencia de recursos humanos y técnicos de los países en vías de desarrollo hace que sus Servicios veterinarios no cuenten a menudo con datos precisos sobre la incidencia de las enfermedades animales. Como la colecta y transmisión de informaciones fiables sobre estas enfermedades en los países en vías de desarrollo es uno de los temas de preocupación más importantes de la Oficina internacional de epizootias (OIE), se ha implementado un proyecto tendiente a mejorar esta situación, con apoyo financiero internacional. Como parte de este proyecto, el Centro para la aplicación de metodologías para el diagnóstico de las enfermedades animales (CAMDA) ha creado kits para el diagnóstico en el terreno de las principales enfermedades presentes en África tropical: peste bovina, peste de pequeños rumiantes, pleuroneumonía contagiosa bovina y pleuroneumonía contagiosa caprina.

Varias técnicas son actualmente disponibles: sondas específicas que usan ácido desoxirribonucleotico complementario (c-ADN) y la amplificación en cadena por polimerasa (PCR) para la detección de la peste bovina y la peste de pequeños rumiantes, sondas de ADN y la técnica PCR para la pleuroneumonía contagiosa bovina, una prueba de inmunocaptura enzimática, la prueba de aglutinación, la prueba de inmunoperoxidasis para la pleuroneumonía contagiosa caprina, etc. El artículo pasa revista a los diferentes problemas encontrados por la OIE y el CAMDA durante este trabajo.

REFERENCES


