The diagnosis of bovine virus diarrhoea-mucosal disease in cattle

S. EDWARDS *

Summary: Laboratory diagnostic procedures for bovine virus diarrhoea and mucosal disease are reviewed. For serology, emphasis is placed on the virus neutralisation test and enzyme immunoassays. The techniques and the pitfalls which may be encountered in culture procedures for isolation of the virus are described, with special reference to the use of immunofluorescence and immunoperoxidase methods for the detection of non-cytopathogenic virus strains. Viral antigen may also be detected directly in clinical or pathological samples. Monoclonal antibodies are expected to play an increasing role in diagnostic tests, and the application of gene probes for virus detection is anticipated in future years. In the interpretation of diagnostic laboratory test results, the importance is stressed of reviewing them in the context of the herd disease and breeding history, and relating them to current understanding of the pathogenesis and epidemiology of the disease.

KEYWORDS: Abortion - Bovine virus diarrhoea - BVD virus - Cattle diseases - Congenital damage - Diagnosis - Mucosal disease - Pestivirus.

INTRODUCTION

As with any virus disease, specific diagnostic methods for bovine virus diarrhoea (BVD) virus can be divided into two major groups: the detection of the virus or viral components, and the detection of the host’s immune response to the infection. In order to interpret the results of diagnostic tests it is essential to have a full understanding of the biology of the virus, and the nature and duration of the host responses to different forms of the infection (62). In particular a clear distinction must be made between acute and persistent BVD virus infections, as well as the different expressions of disease which can result from each. This paper should therefore be read in conjunction with others in the present issue of the Review. Furthermore, mention will be made of special technical problems, unique to BVD virus, which can mislead the unwary into false diagnoses. This has often restricted BVD virus testing to specialist laboratories with facilities and expertise to overcome these difficulties, although a new generation of tests may soon emerge which will have a wider availability.

* Central Veterinary Laboratory, New Haw, Weybridge, Surrey, KT15 3NB, United Kingdom.
SEROLOGY

Although a number of workers have assayed components of the cellular immune system in the course of research studies, only the humoral response has been used for diagnostic purposes. Cattle undergoing acute BVD infection seroconvert within two to three weeks of exposure to the virus; the antibody titre may continue to rise for some weeks further. Paired acute and convalescent sera, taken three to four weeks apart, from the same individuals can therefore provide a most useful indication, albeit retrospective, of acute infection in a group of cattle. Antibody titres persist for years, probably for life, in recovered cattle (40). The finding of antibody in single serum samples thus indicates no more than infection at some time in the past and is of limited diagnostic value. Calves born to seropositive dams acquire antibodies from the colostrum. The titres decline steadily over the first few months of life (42, 46).

Serology may also be used for herd screening as part of a general health monitoring programme and, specifically, to identify seronegative cattle which can then be subject to further testing to determine if they are persistently infected virus carriers. Nevertheless it should not be assumed that all the persistent carriers are to be found among the seronegative population. It is known that a small proportion of persistently infected cattle also have BVD virus-specific antibody in their bloodstream (12).

Agar gel immunodiffusion test

Compared with neutralisation tests or enzyme immunoassays, the immunodiffusion in gel technique has a low sensitivity and, because it does not yield a quantitative result, interpretation of tests for seroconversion on paired sera is more difficult (23). Nevertheless it is cheap, easy to perform and, provided antigen can be obtained, offers a useful screening technique for laboratories lacking advanced technical and virological facilities. Although the antibodies detected are directed principally against a soluble, non-structural antigen (19, 49) they correlate better with neutralisation test results than with complement fixing (CF) antibodies (23).

Virus neutralisation test

Recovered cattle show a strong virus neutralising (VN) antibody response in their sera, and this is the accepted reference test for antibodies to BVD virus. Initially performed in test tube cell cultures (6, 23), it is now more commonly carried out in 96-well microtitre plates (17) which facilitate the economical testing of large numbers of sera. There is no international reference standard for BVD virus, and the results of VN tests can vary widely depending on the strain of virus used, the cell type and the test conditions. It is very important that the cell culture, whether primary, secondary or cell line, is screened for the absence of contaminating BVD virus (24); and that any serum supplement used in the culture medium is of a quality suitable for the growth of BVD virus (see below). Cells of bovine origin are preferred, for example testis, turbinate or kidney, the important principle being the establishment in any particular laboratory of a satisfactory combination of cell type and virus strain to give a clear and consistent distinction between wells where the virus grows, and those where it is neutralised. Variation in VN results between different batches of cell cultures is a well recognised problem (16) and can be reduced by the use of uniform cell passage levels from frozen stocks. For this reason, paired acute and convalescent sera being examined for seroconversion should always be tested together on the same day.
Although the distinction between cytopathogenic and non-cytopathogenic strains of BVD virus has been crucial to our understanding of the pathogenesis of the disease, and may also be relevant to diagnosis by virus isolation, it is of lesser concern in VN assays. Indeed whilst considerable antigenic variation has been shown between virus isolates from different outbreaks, using VN tests (16, 21, 27), cytopathogenic and non-cytopathogenic strains from individual cases of mucosal disease were indistinguishable by neutralisation reactions (30). Because it makes the test easier to read, most laboratories utilise highly cytopathogenic laboratory-adapted strains of BVD virus for VN tests, although immunoassay techniques are now available which enable simple detection of the growth or neutralisation of non-cytopathogenic strains where this is considered desirable (31, 35). No single strain is likely to be ideal for all circumstances, but for practical diagnostic applications one should be selected which detects the highest proportion of serological reactions in the local cattle population. The most widely used are the two American cytopathogenic strains “Oregon C24V” (18) and “NADL” (19) which appear to differ considerably (21). The viruses are not antigenically stable on repeated passage, and monoclonal antibody studies indicate that strains (such as NADL) bearing the same name but maintained in different laboratories are not in fact identical (7).

An outline protocol for a microtitre VN test is given below:

1. Heat inactivate the test sera for 30 min at 56°C.

2. From a starting dilution of 1/5 or 1/10, make serial two-fold dilutions of the test sera in a cell culture grade flat-bottomed 96-well microtitre plate, using cell culture medium as diluent. For each sample, two or four wells are used at each dilution depending on the degree of precision required. Control positive and negative sera should also be tested.

3. To each well, add an equal volume (e.g. 50 µl) of a stock of cytopathogenic strain of BVD virus containing 100 TCID₅₀. A back titration of virus stock is also done in some spare wells to check the potency of the virus.

4. Incubate for one hour at 37°C.

5. Trypsinise a flask of suitable cells (e.g. bovine turbinate, bovine testis) and adjust the cell concentration to 3 × 10⁵ per ml. Add 50 µl cell suspension to each well of the microtitre plate.

6. Either seal the plate, or incubate in 5% CO₂, for four days at 37°C.

7. Examine the wells microscopically for cytopathic effect. The VN titre for each serum is the dilution at which the virus is neutralised in 50% of the wells. This can be calculated by the Spearman-Karber method.

Enzyme-linked immunosorbent assay (ELISA)

ELISA is now established as a sensitive, rapid, reliable and economical test for BVD virus serology. The results correlate well with the VN test (8, 33) but less well with the CF test (44). ELISA can also be rendered immunoglobulin isotype specific (33) although this has yet not been applied to practical diagnosis. It is assumed that ELISA is less affected than VN by the strain of virus used in the test (62), but critical studies of the ELISA response in animals infected with different strains have not yet been published.
Both indirect and blocking types of test can be used (37, 41, 76). The chief difficulty in setting up the test lies in the preparation of a viral antigen of sufficient potency. The virus must be grown under optimal culture conditions using a highly permissive cell type. Any serum used in the medium must not inhibit growth of BVD virus. The optimum time for harvest should be determined experimentally for the individual culture system. The virus can be concentrated and purified by density gradient centrifugation (8, 44). Alternatively a potent antigen can be prepared by treatment of infected cell cultures with detergents such as Nonidet P40 (33), Mega 10 (37), Triton X100 or 1-octyl-beta-D glucopyranoside (OGP) (25). Some workers have used fixed infected whole cells as antigen (38, 41). An outline protocol of the ELISA as used in our laboratory is given below:

1. Inoculate roller cultures of secondary calf testis cells with a high multiplicity of infection (about 1) of BVD virus strain Oregon C24V. Overlay with serum-free medium. Incubate 24 hours at 37°C.

2. Scrape off and pellet the cells. Discard the supernatant medium. Treat the pellet with two volumes of 2% OGP in phosphate buffered saline for 15 min at 4°C. Centrifuge to remove the cell debris. The supernatant antigen is stored in small aliquots at −70°C, or freeze dried. Non-infected cells are processed in parallel to make a control antigen.

3. The antigen is diluted to a pre-determined dilution in 0.05M bicarbonate buffer, pH 9.6. Alternate rows of an ELISA grade microtitre plate are coated with virus and control antigens overnight at 4°C. The plates are then washed in phosphate buffered saline with 0.05% Tween 20 (PBST) before use in the test.

4. Test sera are diluted 1/50 in serum diluent (0.5M NaCl, 0.01M phosphate buffer, 0.05% Tween 20, 0.001M EDTA, 1% poly vinyl pyrrolidone, pH 7.2) and added to virus and control coated wells for one hour at 37°C. The plates are then washed five times in PBST.

5. Rabbit anti-bovine IgG peroxidase conjugate is added at a pre-determined dilution (in serum diluent) for one hour at 37°C, then the plates again washed five times in PBST.

6. A suitable enzyme substrate is added, such as hydrogen peroxide/tetramethyl benzidine. After colour development the reaction is stopped with sulphuric acid and the optical density read on an ELISA plate reader. The value obtained with control antigen is subtracted from the test reaction to give the final result for each serum.

Complement fixation (CF)

The CF test found application where a rapid serological test was required which did not depend on regular availability of cell cultures. In this respect it has largely been superseded by the ELISA. CF antibodies appear earlier than VN (20) and have a shorter duration (23). This could be of value in the interpretation of serological results, particularly where paired sera are not available.

Indirect immunofluorescence (IF)

The IF test provides a simple, rapid and highly sensitive test for the detection of BVD virus antibodies and is particularly useful for laboratories which are familiar with the technique from work with other viruses (50). It is also useful for laboratories
doing sporadic tests of small numbers of sera. Fixed slides can be stored at \(-70^\circ C\) and taken out as required. Like immunodiffusion and ELISA, it detects both group- and type-specific antibodies (51) reducing the problem of strain selection. Infected cell cultures are fixed to a multiwell glass slide with acetone, then reacted with a dilution of test serum. After thorough washing, specific binding of antibodies to viral antigens in the cells is detected by addition of anti-bovine IgG FITC conjugate. The slide is again washed and examined by fluorescence microscopy. Specific reaction is revealed as diffuse cytoplasmic fluorescence. Appropriate controls should always be set up. The IF test has been of particular value for detecting BVD virus antibody in fetal fluids (47, 55) which can give anomalous results with CF and VN tests.

**Peroxidase linked assay (PLA)**

PLA is similar in concept to IF, but uses a peroxidase anti-species conjugate. Subsequent addition of insoluble chromagen (e.g. 3-amino 9-ethyl carbazole) produces a visible colour reaction. This can be read by light microscopy, which is less tiring than fluorescence and enables the test to be performed easily in microtitre plates. Although originally devised for screening pig sera for antibodies to hog cholera and BVD viruses (28), PLA can also be used for serum from cattle.

**VIRUS ISOLATION**

**Sampling and interpretation**

The most useful sample for BVD virus isolation is blood (12). Virus may be found free in serum, or be released by grinding up blood clots in the laboratory, but the highest sensitivity can be obtained by culture from leukocytes in the buffy coat of EDTA or heparinised samples. Faeces is not a good source of virus. At necropsy, the virus may be found in many organs, particularly of the lympho-reticular system (55).

Persistently infected cattle, including those suffering from mucosal disease (MD), have continuously high titres of virus (54) in the blood and other tissues. Isolation of the virus in cell culture is a very useful and sensitive diagnostic technique. In young calves, maternal antibody may interfere with virus isolation, so that they should be re-examined later in life. Acutely infected animals have a low level viraemia detectable only sporadically over a two to three week period. In this case negative virological findings mean little, and the diagnosis needs to be supported by the demonstration of seroconversion. The isolation of BVD virus from a single blood sample from a live animal may indicate either acute or persistent infection. Differentiation is achieved by taking a second sample three to four weeks later. A persistently infected animal will remain virus positive and (generally) seronegative, whereas acutely infected cattle will have seroconverted and be virus negative by the time of the second sample. Where virus is isolated from tissues at necropsy, the result must be related to the pathology to determine whether acute BVD or MD has occurred. In some cases it may not be possible to be certain, for example where persistently infected cattle die from intercurrent diseases rather than MD.

Special consideration should be given to cattle used in artificial breeding programmes. Persistently infected bulls shed virus continuously in semen (63). They
may be identified most readily by blood sampling as discussed above. Virus isolation tests may also be carried out on semen if blood is not available, and this would also reveal any transient virus contamination due to acute BVD infection of the bull (59). Extended or diluted semen may be a better sample for BVD virus detection than raw semen (63). In embryo transfer work, recipient females should be screened for BVD virus because if persistently infected they would be expected to give birth to persistently infected calves. Acute infection of recipients in early pregnancy can also result in persistently infected calves (45). The virological status of embryos from persistently infected donors has not been determined. It would be a wise precaution to do a virus isolation test on donors with a view to avoiding use of such animals. Flushing and washing fluids used in embryo transfer can be tested for virus, as can a representative sample of embryos (including degenerate or non-transferable ones); however, negative results should be interpreted with caution in view of the dilution factors involved and the question of test sensitivity (70). Fetal calf serum used in embryo transfer medium should be considered as a potential source of BVD virus infection.

**Cell cultures and media**

It is widely recognised that fetal calf serum used as a culture medium supplement has a high risk of contamination with BVD virus (27, 43, 65). It is less well known that many batches also contain inhibitory factors which can reduce the titre of BVD virus harvests by 10 or 100 fold, even though they may be seronegative by conventional serological tests. Commercial batches of fetal calf serum must therefore be carefully screened before use in BVD virus work. At our laboratory we grow roller bottles of calf kidney cells in medium containing 25% of the serum under test. These are passaged three times using fresh medium with the same serum addition. At each passage, subculture is made to Leighton tubes for fluorescent antibody testing for the presence of BVD virus antigen. Only when all passages are negative is the serum accepted for use. In addition, virus titrations are performed of known BVD virus stocks in the presence of the serum, to identify any inhibitory activity. Alternative approaches are to use calf or adult bovine serum from a known BVD-free donor, or to use serum from non-ruminant species such as horse. These are available commercially. It is important to ensure that the batch used is satisfactory for supporting adequate cell growth.

Cell cultures, both primary and established lines, may also be contaminated, usually with non-cytopathogenic BVD virus (27, 65, 73), and must be carefully screened by immuno-labelling methods before use in BVD diagnosis (24, 65, 73). Individual virus strains vary in their permissive cell range, but only bovine cell cultures are considered suitable for general diagnostic use. Primary cells such as testis, kidney or turbinate are the most sensitive; they can be sub-passaged at least six times without significant loss of sensitivity. Where established bovine cell lines are to be used, the individual cell strain should be checked for its sensitivity to BVD virus.

Standard virological procedures can be followed for the preparation of samples and the inoculation of cell cultures (6). After incubation for three to five days they should be harvested by the freeze-thaw technique and passed to fresh cultures for the maximum sensitivity of virus detection. Cultures should be grown in a system such as Leighton tubes with flying coverslips which enables an immuno-labelling test to be carried out after incubation.
**Virus identification**

Both cytopathogenic and non-cytopathogenic isolates of BVD virus will be encountered in the course of routine diagnostic work (2). The former are most likely to be found in association with intestinal tissue from cases of MD (9), where the presence of cytopathogenic virus provides a useful diagnostic indicator. Because MD is associated with both cytopathogenic and non-cytopathogenic forms of the virus, it is likely that many isolates will in fact be mixed. The degree of expression of cytopathogenicity varies in different cases, and if it is desired to identify the biotype, the isolate should be passaged and the monolayers examined very carefully for cytopathic effects. The cytopathic effect should be specifically neutralised by BVD virus antiserum to confirm the presence of a cytopathogenic strain of the virus.

For general diagnosis the biotyping of virus is often unnecessary, and it is simpler to establish a routine procedure whereby cultures are stained after three to five days incubation by an immuno-labelling method for BVD virus antigen, such as fluorescence (51) or peroxidase (67, 72). Viral antigen will be found in a diffuse form, confined to the cytoplasm of infected cells.

No serotyping scheme exists for BVD virus. Isolates can be partially characterised by their neutralisation reactions with antisera raised against specific strains, but there is likely to be considerable overlap between viruses. A number of attempts have been made to group virus isolates by their reaction with panels of monoclonal antibodies (4, 14, 48, 60) but there is as yet no agreed system of classification.

**Herd screening**

It is often desirable for herd health control purposes to screen the whole or large portions of herds in order to identify persistently infected cattle. To facilitate processing of the large numbers of samples involved, and to minimise costs, a batch test based on the isolation of virus from sera in microtitre plate cell cultures has been devised (53, 54). Virus growth is detected by an immunoperoxidase system. The sensitivity is equivalent to tube culture combined with immunofluorescence (53, 54), and the test has proved robust and adaptable to routine use for diagnosis or surveillance (13, 31, 32).

**ANTIGEN DETECTION**

BVD virus antigen can be detected directly in tissue samples, the most widely applied technique being immunofluorescence on acetone-fixed frozen sections, or smears of nasal epithelial cells (15, 52, 64, 66). It has also been applied to buffy coat smears (3). The technique is rapid, and can be done in laboratories lacking cell culture facilities. Nevertheless it is less sensitive than virus isolation in cell culture (68) and is more likely to be successful in persistently infected cattle and mucosal disease cases than in acute infections. Good labelling is generally found in sections of thyroid and salivary glands, spleen, lymph nodes and around the site of lesions in the intestine (12).

Directly conjugated antisera are generally used, to reduce the level of background non-specific staining. Fluorescent counterstains such as Evans blue or naphthalene black enhance the contrast with specific FITC fluorescence and also enable
visualisation of the tissue architecture which can aid interpretation. The quality of
the antiserum is crucial for successful immunofluorescence. Generally speaking, only
cattle or other ruminants produce a high titre response free from non-specific activity
against cell proteins. Good results have been obtained in colostrum-deprived calves
by intranasal inoculation of the virus, prolonged rest (two to three months) then
intranasal challenge. The intensity of fluorescence varies with different strains of the
virus (27). This problem can be partly overcome by the use of a mixture of antigenically
diverse strains in the virus inoculum. The best “signal over noise” discrimination
is achieved with sera prepared by immunisation of gnotobiotic calves (31) or by intra­
uterine inoculation of bovine fetuses (71). Success has recently been reported in pigs
by giving primary immunisation with intranasal BVD virus, followed eight weeks later
by heterologous intranasal challenge with hog cholera virus (34).

Immunoperoxidase labelling can also be applied to frozen tissue sections, leukocyte
smears and alcohol-fixed paraffin-embedded sections (56, 57, 58). BVD virus antigen
has recently been demonstrated in conventional formalin-fixed, paraffin-embedded
sections by a combination of protease XIV digestion of the sections and biotin-
streptavidin amplification of the labelling signal (1).

ELISA for BVD virus antigen detection in body fluids or secretions is not yet
generally available, but it is anticipated that this technique will come into diagnostic
use as soon as sufficiently sensitive protocols are developed, probably based on
monoclonal antibodies. Yolken et al. (77) used such a method to detect pestivirus
antigen in human faeces and could also detect BVD virus antigen in experimentally-
infected cows.

APPLICATION OF MONOCLONAL ANTIBODIES

Murine monoclonal antibody panels with pestivirus reactivity have been developed
at various laboratories (4, 7, 11, 14, 37, 48, 60, 74). Those of diagnostic value may
be divided into two groups: genus-specific, non-neutralising, usually directed against
the p125/80 non-structural protein; and species-specific, often neutralising, directed
against the gp53 envelope glycoprotein (11). The genus-specific group includes a
number of monoclonals which react with all strains of pestivirus, including BVD,
Border disease and hog cholera viruses (7). These could be useful substitutes for
polyclonal sera in general screening tests for BVD virus or pestivirus antigen, for
example in testing cell cultures for viral contamination, detection of non-
cytopathogenic virus in virus isolation tests (67), and direct detection of BVD virus
antigen in tissues of affected animals. This has particular appeal in view of the
difficulties, mentioned above, of obtaining good quality antisera for BVD virus antigen
detection tests. Our experience suggests that when these reagents are used to stain
cell cultures, the soluble antigen is expressed most strongly in early cultures, i.e. after
two to three days incubation.

The virus species-specific group of monoclonals is of particular value in porcine
diagnostic work, to distinguish between hog cholera and BVD viruses (26, 74, 75).
The BVD virus reactive monoclonals may usefully be combined with the genus-specific
group to broaden the antigenic specificity of a general purpose diagnostic reagent.
No single monoclonal, or panel, has yet been found which reacts exclusively with
all strains of BVD virus, or which can distinguish between BVD and Border disease viruses. Panels can be assembled which enable the rapid antigenic classification of BVD virus isolates (4, 10, 14, 48), reducing the need for laborious cross-neutralisation studies. Such procedures may be of value in epidemiological work, or in vaccine evaluation, but will have little place in the routine diagnostic laboratory. Most monoclonals do not distinguish between cytopathogenic and non-cytopathogenic strains (4, 10) although one monoclonal is reported (14) which binds to hog cholera virus and to cytopathic strains only of BVD virus.

Monoclonal antibodies are also useful in certain configurations of ELISA for antibody detection in cattle sera, particularly blocking or competitive tests (37, 41, 76). It is important to select monoclonals which are directed towards epitopes relevant to the immune response in infected cattle.

NUCLEIC ACID DETECTION

Diagnostic DNA gene probes are in their infancy as far as pestiviruses are concerned. Nevertheless the high specificity and sensitivity of the technology, especially through the application of the polymerase chain reaction, hold out great promise for its future diagnostic potential. Brock et al. (5) found that a cloned complementary DNA sequence from the BVD virus genome could detect all strains of BVD virus tested (cytopathogenic and non-cytopathogenic) and was 100 times more sensitive than infectivity assays. Before routine application becomes possible it will be necessary to develop non-radioactive labels giving satisfactory “signal to noise” ratios.

RELATIONSHIP OF DIAGNOSTIC TEST RESULTS TO CLINICAL DISEASE AND DIFFERENTIAL DIAGNOSIS

In the individual animal, mucosal disease is clinically and pathologically distinctive and should be readily confirmable by antigen detection tests on tissue sections and the isolation of both cytopathogenic and non-cytopathogenic BVD viruses from appropriate tissues (9). Although it has its origins in fetal life, MD sometimes occurs in apparently epidemic form among a cohort of calves, a result of acute infection among their dams in early gestation resulting in a crop of persistently infected calves. It is therefore worth examining the rest of a group for viraemia following a diagnosis of MD in an individual animal. MD is an important differential diagnosis from rinderpest in countries where the latter occurs. A number of cases have also been encountered (Edwards, unpublished data) which clinically and pathologically resemble MD, but from which neither BVD virus nor any other virus can be isolated. The cause of these remains unknown. BVD should be considered as a possible diagnosis in outbreaks of diarrhoea, especially in yearlings or adults; of pyrexia, possibly associated with diarrhoea and reduced milk yield; and of general reproductive wastage (infertility, embryonic loss, abortion) especially where there is evidence of congenital abnormality in calves. In interpreting laboratory test results, due cognisance should also be taken of the fact that many acute BVD infections are subclinical.
Because BVD virus may be implicated as a component of a wide variety of diseases of multifactorial origin, for example pneumonia, it is a good practice routinely to screen tissues from carcasses presented for necropsy for BVD virus. Some such cases can be very complex and may require the analysis of herd histories over long periods and extensive sampling in order to unravel the epidemiology of the disease (2, 61).

It can be put forward as a general principle that BVD virus diagnosis should be approached at herd level (12). A confirmed diagnosis of mucosal disease should always lead on to the taking of a full herd history covering at least two years, and encompassing animal movements, purchases, breeding records, births of congenitally damaged calves, poor thrift in calves, and any unexplained disease or deaths in calves and growing stock. Such an analysis frequently reveals that the problem is more extensive than first envisaged (64).

The diagnosis of BVD virus-associated abortion is particularly difficult (12, 22, 69). Maternal serology is rarely helpful even when paired sera are taken since seroconversion usually precedes the abortion (39). Single sera taken at the time of abortion are of little value in view of the high prevalence of BVD virus antibody in the adult cattle population (29). In some aborted fetuses BVD virus antigen may be detected by immunofluorescence, or virus isolated in cell culture; in others exposure to BVD virus may be demonstrated by the presence of specific antibody in fetal fluids (e.g. by IF test) (47). In all these cases it may be difficult to prove that BVD virus was actually responsible for the abortion (36, 69), while in other cases it may prove impossible to demonstrate virus or antibody even though it is strongly implicated by circumstantial evidence (22).

**CONCLUSIONS**

With the development of modern techniques in immunoassay, the laboratory diagnosis of BVD virus is now relatively straightforward for a virological laboratory, provided due attention is paid to quality control of the cells, culture media and test performance. Further developments should soon bring many of the tests within the capability of general diagnostic laboratories without a need for cell culture facilities. Our understanding of the pathogenesis and epidemiology of the disease has also advanced greatly in the past decade. This enables a much more meaningful interpretation of diagnostic test results, particularly when they are taken within the context of a herd diagnosis.

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**LE DIAGNOSTIC DE LA DIARRHÉE VIRALE BOVINE-MALADIE DES MUQUEUSES CHEZ LES BOVINS. – S. Edwards.**

*Résumé : L'auteur passe en revue les techniques du diagnostic expérimental de la diarrhée virale bovine et de la maladie des muqueuses. En sérologie, l'accent est mis sur la séro-neutralisation et les dosages immuno-enzymatiques. Les techniques utilisées et les pièges à éviter dans les méthodes de culture pour l'isolement du virus sont décrites, notamment en ce qui concerne l'utilisation.*
de l'immunofluorescence et de l'immunoperoxydase pour la détection des souches virales non cytopathogènes. L'antigène viral peut aussi être détecté directement dans des prélèvements effectués sur l'animal vivant ou post mortem. On prévoit que les anticorps monoclonaux joueront un rôle de plus en plus important dans les épreuves de diagnostic et que, dans les années à venir, on pourra appliquer des sondes de gènes à la détection du virus. Pour l'interprétation des résultats des épreuves du diagnostic expérimental, il est essentiel de les étudier dans le contexte des antécédents de maladie et de reproduction du troupeau, et de les relier aux connaissances actuelles sur la pathogénie et l'épidémiologie de la maladie.


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EL DIAGNÓSTICO DE LA DIARREA VIRAL BOVINA-ENFERMEDAD MUCOSA EN LOS BOVINOS. — S. Edwards.

Resumen: El autor pasa revista a las técnicas de diagnóstico experimental de la diarrea viral bovina (virus BVD) y de la enfermedad mucosa. En serología, se pone el acento en la seroneutralización y los inmunoensayos enzimáticos. Se describen las técnicas utilizadas y las trampas a sortear en los métodos de cultivo para aislar el virus, en particular la utilización de la inmunofluorescencia y de la inmunoperoxidásis para la detección de las cepas virales no citopatógenas. El antígeno viral puede también detectarse directamente en muestras obtenidas del animal vivo o post mortem. Se prevé que los anticuerpos monoclonales desempeñarán un papel cada vez más importante en las pruebas de diagnóstico y que en los próximos años podrán aplicarse sondas de gènes para la detección del virus. Con respecto a la interpretación de los resultados de las pruebas de diagnóstico, es esencial estudiarlas en el contexto de los antecedentes de enfermedad y de reproducción del rebaño, así como de vincularlos con los conocimientos actuales acerca de la patogenia y la epidemiología de la enfermedad.

PALABRAS CLAVE: Aborto - Diagnóstico - Diarrea viral bovina - Enfermedad mucosa - Enfermedades de los bovinos - Lesiones congénitas - Pestivirus - Virus de la diarrea viral bovina.

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