Pestivirus infection of ruminants in Australia

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Summary: Pestivirus infections are commonly diagnosed in cattle but are relatively uncommon in other ruminant species in Australia. Virus isolation is a very reliable technique for detecting pestivirus in specimens, especially when group reactive monoclonal antibodies are used with immunoperoxidase staining to detect non-cytopathogenic virus. Care must be taken to prevent adventitious pestivirus contamination of serum or cells used for cell culture. A recently developed antigen capture enzyme linked immunosorbent assay has been extensively evaluated and found to be extremely accurate. This test is also much quicker and less expensive than virus isolation. Procedures are outlined to reliably certify animals to be free of pestivirus infection for export or as donors of semen or embryos.

KEYWORDS: Antigen capture ELISA - Artificial breeding Diagnosis - Pestivirus.

INTRODUCTION

In Australia, infection of cattle with bovine virus diarrhoea (BVD) virus is the most commonly diagnosed pestivirus infection of ruminants. Border disease (BD) virus isolates have also been made from the sheep population but infection of pigs with classical swine fever or hog cholera (HC) virus has not occurred for over 30 years. It is likely that there are occasional cross species infections between cattle and sheep with BVD and BD viruses.

BVD virus infection in domestic cattle is associated with a variety of clinical syndromes, including reproductive wastage (embryonic death, abortion, congenital malformations, stillbirths and perinatal mortality), the mucosal disease syndrome and respiratory disease. In sheep, infection is not widely recognised and the BD syndrome is uncommon. Pestivirus has also been isolated from goats in instances of reproductive disease. Significant populations of feral ruminants (cattle, goats, buffalo) and farmed deer exist, but their pestivirus status is unknown. The incidence, epizootiology and control of pestivirus infection of sheep and cattle in Australia has been described in detail in a recent issue of the OIE Scientific and Technical Review and will not be covered further here (3).

DIAGNOSIS

Diagnosis of pestivirus infection usually depends on the demonstration of persistent infection by the isolation of the virus from affected animals.

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As congenitally deformed calves develop specific antibody when infection occurs in the latter half of gestation, it is also necessary to test for antibody in these cases. Virus neutralisation and agar gel immunodiffusion are the most commonly used serological tests.

**Cell culture requirements**

A prerequisite to accurate diagnosis is the availability of a sensitive, reliable cell culture system. The most important consideration is avoidance of adventitious contamination of cells and bovine serum used for medium supplementation with either virus or specific antibody. Generally, bovine serum from commercial sources contains either pestivirus or antibody, which in turn may mask the presence of virus. Such adventitious contaminants can seriously affect the results of both virus isolation and neutralisation tests. Australian State diagnostic laboratories are fortunate to have access to supplies of bovine cells and both foetal and adult bovine serum derived from pestivirus free stock, alleviating the risks of such contamination.

**Virus isolation**

As most isolates of pestivirus are non-cytopathogenic in cell culture, antigen detection methods are required to screen cell cultures. The method most commonly in use is the immunoperoxidase staining technique employing a pestivirus antiserum and a peroxidase conjugated second antiserum (4). As originally described, the method involved the preparation and use of a polyclonal hyperimmune antiserum (4) but this has been largely replaced with a pestivirus group-reactive monoclonal antibody (MAb) or a mixture of several group MAbs. Ideally, the broad reactivity of the monoclonal antibody should be confirmed by testing against a panel of BVD, BD and HC virus isolates before being introduced to routine use. The use of MAbs selected for high affinity in the immunoperoxidase test is now recommended in preference to polyclonal sera because of high specificity combined with virtually undetectable background staining. Although these reagents are equally suited to screening by immunofluorescence, it is generally acknowledged that the peroxidase method has higher sensitivity, can be examined by light microscopy and gives permanent staining, which allows stained cultures to be stored for future reference if necessary.

A range of cell cultures are in use for pestivirus isolation. Testis, kidney and lung cells from the bovine foetus or neonate are regularly used either as primary cells or limited passage (usually up to passage 6) secondary cultures. Continuous bovine cell lines (mainly bovine turbinates) have been used in some laboratories, but the susceptibility of these cells is marginally less than low passage cultures from the foetus or neonate. For identification of persistently-infected carrier animals by examination of either blood or tissue samples, a single passage in a susceptible cell culture, followed by peroxidase staining five to seven days after inoculation, is sufficient in most cases. However, to ensure the diagnosis of all cases, a second passage is required. Additional passages have not been shown to result in a higher virus isolation rate. The only justification for more than two passages is examination of biologicals such as foetal calf serum and vaccines where the titre of virus may be extremely low or be suppressed by the co-existence of specific antibody. In these circumstances, it is essential to passage the specimens for a total of five passes at five to seven day intervals, preferably with peroxidase staining at the end of each pass.
Antigen capture

An enzyme linked immunosorbent assay (ELISA) for the detection of pestivirus antigen has recently been developed and evaluated at the Elizabeth Macarthur Agricultural Institute, Camden, New South Wales, Australia (5). The test is rapid, highly specific and inexpensive. It employs a hyperimmune goat polyclonal antiserum to capture the antigen from a specimen treated with detergent (NP40). A mixture of three group-reactive MAbs followed by enzyme-labelled antiserum to mouse immunoglobulins detects the bound antigen. The polyclonal antiserum is broadly reactive, having been raised against seven cattle, sheep and goat isolates of pestivirus.

This ELISA will detect antigen in a wide range of specimens. The essential requirement is that the specimen contains either tissue or blood cells. The test will not detect antigen in serum or biologicals where the antigen content is low. From the live animal, antigen can be detected in leucocytes from theuffy coat of unclotted blood treated with either ethylenediaminetetraacetic acid (EDTA) or heparin, or in cells entrapped in blood clots. Antigen has also been detected reliably in the semen of carrier bulls. At post-mortem, antigen can be detected in virtually any tissue, but the preferred specimens are spleen, lung, intestinal mucosa, mesenteric lymph nodes and salivary gland.

In a comparison with virus isolation conducted on over 1,500 diagnostic specimens, the antigen capture ELISA has been shown to have high sensitivity and specificity. All specimens from which pestivirus has been isolated, including a number from calves with maternal antibody, have been positive in the ELISA. There have been no false positive results, i.e. samples positive by ELISA but no virus isolated. In addition to the large number of bovine specimens examined, the ELISA has been shown to be highly sensitive in detecting antigen in porcine tissues and blood during the examination of material from over 20 pigs experimentally infected with 3 strains of HC virus and a further 40 field isolates of HC virus. Although a limited quantity of ovine material has been examined (about 30 BD virus isolates), both the MAbs and polyclonal antiserum have been shown to react strongly with all of the BVD (110 isolates), BD (30) and HC (45) virus isolates examined. There is therefore a high level of confidence in this test having application to the diagnosis of pestivirus infections in all species. The ELISA should prove to be a major breakthrough in the certification of livestock for international export.

**DISEASE CONTROL AND ARTIFICIAL BREEDING**

In general terms, there has been no change to the control measures described by Littlejohns and Horner (3). In Australia, there are no commercially available vaccines, and efforts to control infection are applied only in the cattle industries. In recent years there has been increasing awareness of the need to prevent pestivirus carrier animals becoming involved in artificial insemination (AI) or embryo transfer (ET) programmes. Testing of bulls prior to admission to AI centres is compulsory in two states, is recommended elsewhere and is carried out in the preparation of export consignments as required. Recent research has shown that even bulls undergoing transient BVD virus infection excrete virus in their semen for a period of seven to ten days (2). It is therefore essential to test all livestock in AI centres to ensure that virus is not spread in semen. Substantial losses from pestivirus induced disease have
been observed in ET programmes (1), supporting the need for both donor and recipient cows to be free of pestivirus infection and, if other stock on the property are not tested, for cattle in the ET programme to be held in isolation from all other livestock. It has been shown that infection of susceptible cows seven to ten days before joining will result in a decrease in conception rate of up to 50%; and the virus can persist in the reproductive tract for at least one cycle, providing the potential to infect the conceptus at successive cycles. Therefore, the period of isolation of ET cattle from potential sources of infection must commence well before the programme begins and must extend to the end of the second trimester if reproductive losses are to be minimised.

**DISEASE CERTIFICATION PROTOCOLS**

The need to prevent the introduction of pestivirus infection in susceptible ruminant populations is now widely acknowledged. This is desirable both in order to minimise the loss of valuable genetic material and to prevent the introduction of strains of virus which may be antigenically different to endemic strains. These objectives can clearly be achieved by preventing the movement of carrier animals. There are several strategies which will assist the efficient and effective achievement of these objectives.

In the past, some importing countries have adopted expensive measures which have been fundamentally flawed, through a lack of appreciation of the epidemiology and pathogenesis of pestivirus infection. For example, it has not been uncommon to test breeding females to ensure that they are not carriers, and yet these animals are often pregnant. There is currently no way of certifying that the foetus of a seropositive cow is not infected with BVD virus. Conversely, these shipments often contain seronegative pregnant animals which become infected on arrival in the importing country. The subsequent birth of a carrier calf is then taken as proof that the original testing was inadequate, when in fact this simply indicates that pestivirus is endemic in the importing country. The most satisfactory way of overcoming such difficulties is to screen ruminants for export for active pestivirus infection and to prevent the shipment of pregnant animals.

For the certification of semen and embryos, the most reliable and most sensitive method remains the certification of the donor bull or cow, and ensuring that there is no exposure to pestivirus infection in the period immediately before and during collection. These objectives can easily be satisfied by certifying that the donors are not persistently-infected and that their serological status does not change within a specified time after collection.

Finally, the antigen capture ELISA test (5) should be adopted as a matter of priority for the certification of animals for export and the screening of semen or embryo donors. The test overcomes the problems and costs associated with virus isolation, can be conducted quickly at low cost and can be highly standardised, particularly if reagents for the test are prepared against a well-controlled set of reference reagents. This test will allow all non-pregnant animals in a shipment to be reliably tested regardless of their serological status and without access to cell culture and virus isolation facilities.
LES MALADIES A PESTIVIRUS CHEZ LES RUMINANTS EN AUSTRALIE. – P.D. Kirkland.

Résumé : Les infections à pestivirus sont courantes en Australie chez les bovins mais sont relativement rares dans les autres espèces de ruminants. L'isolement du virus est une méthode très fiable pour déceler les pestivirus dans les prélèvements, notamment lorsqu'on associe aux anticorps monoclonaux à réactivité de groupe, la coloration à l'immunoperoxydase, pour déceler les virus non cytopathogènes. L'examen doit être réalisé avec précaution pour éviter de contaminer accidentellement par des pestivirus le sérum ou les cellules employées pour la culture cellulaire. Une technique ELISA à capture d'antigènes, récemment mise au point, s'avère, après de multiples évaluations, extrêmement précise. Ce test est par ailleurs beaucoup plus rapide et moins onéreux que l'isolement du virus. L'auteur met l'accent sur les procédures qui permettent d'attester avec certitude que les animaux destinés à l'exportation ou à faire l'objet de prélèvements de semence ou d'embryons sont exempts d'infection à pestivirus.

MOTS-CLÉS : Diagnostic - ELISA à capture d'antigènes - Pestivirus - Reproduction artificielle.

INFECCIÓN DE RUMIANTES POR PESTIVIRUS EN AUSTRALIA. – P.D. Kirkland.

Resumen: El diagnóstico de infecciones por pestivirus en el ganado es muy corriente, pero es más bien raro en otras especies de rumiantes en Australia. El aislamiento del virus es una técnica muy segura para detectar los pestivirus en muestras, sobre todo cuando se utilizan anticuerpos monoclonales específicos de grupo con coloración por inmunoperoxiðase. Debe tenerse cuidado en prevenir la contaminación accidental del suero o las células utilizadas para los cultivos. Recientemente, se ha desarrollado una técnica ELISA de captura de antígenos, que, tras haberse sometido a numerosas pruebas, se ha encontrado de suma precisión. Además, esta prueba es mucho más rápida y menos costosa que el aislamiento del virus. El autor describe los procedimientos para certificar con toda seguridad que los animales están libres de infección por pestivirus para ser exportados o servir de donantes de semen o embriones.

PALABRAS CLAVE: Cría artificial - Diagnóstico - ELISA de captura de antígeno - Pestivirus.

REFERENCES

