Ruminant pestivirus infection in pigs

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Summary: Ruminant pestivirus infections of pigs have a worldwide distribution. The prevalence is varied and depends mainly on (i) contact with cattle, (ii) age of pigs and (iii) degree of homology of virus strains used for serology, with field strains of bovine virus diarrhoea virus (BVDV) infecting pigs. Emphasis should be laid on sources of BVDV other than cattle, e.g. contaminated vaccines and fetal calf serum.

The need for differentiation of pestiviruses (hog cholera, bovine virus diarrhoea and Border disease viruses) is highlighted by the fact that clinical disease syndromes, e.g. growth retardation and wasting, are reminiscent of hog cholera. Monoclonal antibodies are available which differentiate between hog cholera virus (HCV) and ruminant pestiviruses, presumably BVDV.

An up-to-date account of the antigenic relationship between pestiviruses is included in the review. Analysis of the in vitro host range of these viruses is considered to be important and may explain infections of pigs with pestiviruses other than HCV. Recent results have shown the existence of “specialists” amongst BVDV strains for bovine cells, and a few isolates also performed well in cultures of the PK15 cell line. In contrast, multipotent BVDV strains presumably have additional attachment sites for ovine and porcine cells. Identification of receptors on ovine and porcine cells could contribute to a clear distinction between BVDV and HCV infections of pigs.

Immediate control measures for BVDV infections of pigs are not required. However, such infections may interfere with serologic surveys and surveillance on a herd basis and, therefore, impair eradication programmes and efforts to maintain the status in countries declared free of hog cholera.


WORLDWIDE DISTRIBUTION

After the discovery of a close antigenic relationship between hog cholera virus (HCV) and bovine virus diarrhoea virus (BVDV) (8), susceptibility of pigs to BVDV was established by isolation of virus in culture following natural (11, 4, 40) or
experimental infection (32, 11, 4, 7). It was only recently that the transmissibility of pestiviruses of ovine origin to pigs — presumably Border disease virus — was reported (35). Like HCV, both ruminant pestiviruses also have the ability to cross the porcine placenta and are readily transmitted to pigs by contact with infected cattle (37). They induce neutralising antibodies in pigs, which cross-react with HCV (36, 17, 9, 37, 39, 38, 34, 13, 22, 7). By cross-neutralisation tests, using polyclonal antisera produced in rabbits (1) or monospecific porcine immune sera (29), considerable antigenic variation has been shown within the group of pestiviruses of ruminant or porcine origin.

Antibody titres of monospecific sera are highest against the homologous viruses and in most cases titres against heterologous strains of the same or another pestivirus species are considerably lower (20, 13, 22). In addition, antibodies against heterologous viruses, even within the same virus species, are almost invariably detected later in the course of infection than those against homologous strains (21, 29). The observed antigenic variation of pestiviruses has some practical implications concerning the detection of neutralising antibodies, in pig sera, against ruminant pestiviruses and their differentiation from antibodies induced by HCV (21, 38, 30). It stresses the necessity of using different antigenic variants of BVDV/Border disease virus in, e.g., neutralisation tests with porcine field sera.

Serologic surveys for detecting pig herds infected with HCV clearly revealed neutralising antibodies against various strains of ruminant pestiviruses in pigs with or without contact with cattle (37, 39, 38, 20, 13, 3, 7).

In order to establish the extent of worldwide distribution of ruminant pestivirus infections in pigs, serologic surveys of antibodies were carried out in various countries. The number/percentage of sera neutralising BVDV strains varied widely. In most cases the sera collections were not biased and blood samples were usually collected by random sampling from cases of suspected hog cholera or in surveys primarily aimed at the detection of antibody to HCV on a herd basis. The first of these surveys was apparently performed using a series of serum samples collected from pigs in abattoirs of three Australian states (38). In 115 pigs older than twelve months, about 50% had detectable antibodies to both viruses compared with only 2.8% in younger pigs.

A limited survey in the Federal Republic of Germany on 111 breeding pigs from 20 herds revealed a prevalence of 42% of sera with neutralising titres ranging from 1:5 to >1:640 against BVDV strain 1138/69 (20). This strain is known to be closely related to the Oregon strain C24V (22) which was used in the Australian survey (38). In the Republic of Ireland there is a high prevalence of BVDV infections in cattle, but only one outbreak of hog cholera has occurred during the past fifty years. Infections of pigs with ruminant pestiviruses varied according to the type of herd or contact with cattle: from 27.8% in cases of contact to about 4% of sows or pigs of bacon-weight without contact (19). Serologic investigations in the USA also confirmed that pestivirus-specific titres in pig sera were caused by natural infections with BVDV; titres against HCV were consistently lower than those against BVDV (12, 4). More recently the percentage of pigs with antibodies to BVDV in the Netherlands was reported to be in the range of 15 to 20% (42).

Summarising the above results of serological investigations, it is obvious that the prevalence of BVDV infection in pigs varies widely and depends mainly on contact with cattle, age of the pigs and the degree of homology between the strains of BVDV used for serology and the field isolates. All data available on the prevalence of
ruminant pestivirus infections indicate a more or less widespread distribution in the porcine population. However, it is questionable whether all cases are caused by natural infections. It is safe to assume that many pigs seroconverted after they received vaccines contaminated with BVDV, e.g. derived from fetal calf serum or produced from cell cultures fortuitously infected with BVDV (45, 43). More information on the serologic evidence of virus distribution is given in a recent review on the relationship between ruminant pestiviruses and HCV infections in pigs (14).

THE CLINICAL DISEASE SYNDROME

Isolation of ruminant pestiviruses from naturally infected pigs provided direct proof for the occurrence of this infection with or without clinical disease in this species (11, 42). Experimental infections of pigs with ruminant pestivirus left no doubt about their susceptibility. Infected animals developed a viraemia and antibodies, and recovered virus retained its virulence for calves (39, 11). Identification of the ruminant pestivirus was, however, not convincing until BVDV- and HCV-specific monoclonal antibodies (MAB's) were available. These enable a clear-cut differentiation between ruminant and porcine pestiviruses in sections of tissue (47) or after propagation of the isolate in suitable cell cultures (42, 45, 5).

The first report on the isolation of a non-cytopathogenic ruminant pestivirus from a sow and some of her hysterectomy-derived, colostrum-deprived pigs did not mention any clinical signs of disease. The brood sow was located on a farm where antibodies against BVDV had previously been detected in several sows and piglets (11).

The history of a pig herd in Western Iowa indicated that in a group of low-weight pigs (18 kg), clonic spasms and elevated rectal temperatures (40.0-40.6°C) might have been the result of infection with BVDV (40). This virus was isolated from one of the animals. Specimens of tissue from three other pigs were negative for BVDV and HCV when fluorescent antibodies were applied to sections of tonsils and when virus isolation using PK15 cell cultures was attempted. Pregnant gilts, at 38 days and 53-54 days of gestation, developed antibody titres when exposed oronasally to a high passage (143 passages) of the above isolate, but rectal temperatures remained normal. All live fetuses tested were negative for antibodies to BVDV and for virus isolation. Similar results which showed no clinical signs of disease, either in sows inoculated with BVDV or their offspring, were reported by other investigators even when isolates of low passage in cell culture were used (38; J. Dahle & B. Liess, unpublished data). In spite of this, viraemia was detected for a varying length of time following intravenous (38) or nasal inoculation (J. Dahle et al., unpublished data).

Viraemia is a prerequisite for transplacental transfer of BVDV and it was reported without signs of disease in the fetuses of pigs (41). It was therefore alarming when natural infections of pigs with bovine virus diarrhoea virus, associated with signs resembling hog cholera, were described (42). These virus isolates were typed with two MAB's, one of which recognises a conserved epitope present on all HCV strains. Isolates which were not recognised by this MAB were considered as being not HCV but ruminant pestivirus. Additional tests, e.g. comparative neutralisation performed on sera from the herds of origin, supported the differential diagnosis. Detailed inquiries on the pig breeding farms where thirteen incidents of BVDV infections were
detected between 1976 and 1985 resulted in a full list of clinical signs and post-mortem lesions resembling chronic and "late onset" hog cholera. It appears highly justified to stress the importance of these findings in view of international trade of pigs and pig products.

A case of growth retardation and wasting recently observed in farm pigs (J. Dahle et al., unpublished data) stressed once more the need for a clear differential diagnosis and differentiation of pestiviruses (42, 45). Virus was isolated in cultures of fetal calf kidney cells and identified as BVDV using a panel of MAB's against BVDV and HCV. Sera obtained from twelve sows of this farm had neutralising antibodies against BVDV (NADL strain) invariably at higher titres than against HCV (Alfort 187 strain). Sera obtained from eight piglets showed no antibodies to BVDV but low titres to HCV. When the sera were tested against the virus isolate (V725), obtained from the same herd, neutralising antibody titres in the same range or higher were detected in four of these piglets. This finding obviously leaves room for speculations but the following conclusion can be drawn: descriptions of disease in pigs caused by BVDV require a consideration of the aetiologic factors before a casual relationship can be proved.

**ANTIGENIC RELATIONSHIP AMONGST PESTIVIRUSES**

Although a fossil record is lacking, the close relationship between pestiviruses indicates that they have a common ancestor. Similarities in their respective host spectra are therefore not surprising. Detailed studies of their in vitro host range and their relationship at the molecular and antigenic level are now yielding an ever clearer concept of pestivirus evolution. In addition, an accurate picture is emerging which depicts common and differential features.

Investigations of the in vitro behaviour of pestiviruses might give some clues which help to explain infections of pigs with pestiviruses other than HCV. In the early stages of BVDV research it was shown that the virus replicates readily in bovine cells originating from various organs such as cornea, trachea, kidney, testis, embryo spleen and skin, fetal endometrium, bone marrow and leukocytes (16). The apparent ease with which pigs may be infected with ruminant pestiviruses suggests that it is important to analyse the in vitro host range of these viruses in cultured cells of heterologous species, e.g. pigs. It was reported that permanent and/or non-permanent cells of sheep, goats, primates (human), hamsters and pigs (PK15) can be successfully infected with BVDV. Virus adaptation to the latter cells was achieved by blind passages using the Oregon C24V strain of BVDV (10). The adapted virus was able to infect porcine and bovine cells equally well. It is tempting to speculate that, under appropriate natural conditions, this adaptation process is likely to occur in vivo. In this laboratory a total of 35 BVDV strains and isolates were grown in bovine (kidney), ovine (kidney) and porcine (PK 15) cells. None of the viruses had been passaged in porcine cells before. Four virus strains turned out to be "specialists" for bovine cells, whereas the others replicated with comparable efficiency in both bovine and ovine cells (27). Interestingly, two BVDV isolates also performed well on the PK15 cell line. This result indicated that the adaptation process described for cultured cells (10) seems to occur in vivo.

A closer look at the comparative in vitro experiment showed that viruses which produced insignificant or no measurable titres in heterologous cells only formed small
foci of infected cells when concentrated virus was used. The reasons for these differences in host preference are not yet understood. The restriction for the replication of most ruminant pestiviruses in pig cells could either be at the attachment or post-attachment level. In the first case the presence or absence of a specific receptor and its respective partner on the viral surface would determine the efficiency of replication in heterologous cells. Studies of host cell receptors for BVDV support this concept. Recently we described a MAB directed against a bovine cell surface protein which specifically mediated the infectivity of a number of BVDV strains for bovine cells (26). Infection of cells with several HCV and one BDV strain, as well as with parainfluenza 3 and infectious bovine rhinotracheitis viruses, was not impaired by the presence of the MAB. Although the protein specificity of the MAB has not yet been determined, the findings suggest that a specific cell surface receptor facilitates the entry of BVDV into bovine cells and that heterologous pestiviruses are unable to use that receptor unless they are adapted to bovine cells. However, when studied more closely, the inhibition of infection by the receptor-specific MAB was not always complete; a few foci of BVDV-infected cells were detectable in monolayers pretreated with the antibody. This indicates that a less efficient, receptor-independent mechanism of virus internalisation may be operative, as has been described for other viruses (25, 24). It has been shown that the receptor specificity of viruses can be altered upon passage in cultured cells (33). Provided that HCV and BDV viruses also use specific cell receptors for the infection of their respective host cells, the experimental evidence offers an explanation for the observations described in the first part of this paper. "Specialists" among BVDV isolates have attachment sites for bovine cells only, and multipotent viruses presumably have additional attachment sites for ovine and/or porcine cells. The identification of receptors on ovine and porcine cells would further clarify our present understanding of the biology of ruminant pestivirus infections in pigs.

The relatively high frequency of infections of pigs with ruminant pestiviruses raises the question whether there are criteria for laboratory diagnosis that provide a clear distinction for HCV. Early observations indicated that BVDV and HCV share a relatively close antigenic relationship (8). The exact nature of this is still not fully understood. However, recent results obtained with MAB and/or genetic analysis have provided fragmentary evidence that there are highly conserved and more variable viral proteins (6, 28). A large non-structural protein of 125 kDa is highly conserved among pestiviruses whereas the major envelope protein gp53 displays clear differences between HCV and ruminant pestiviruses, but the latter viruses cannot be differentiated from each other (44, 31, 15, 5). In terms of practical application for routine diagnosis, MAB's used either individually or in pools have proved to be reliable tools for antigen differentiation both in cell culture and on sections of tissue from infected animals (15, 47). HCV can be identified by MAB's directed against highly conserved epitopes on the major envelope glycoprotein of the virus. These epitopes are not shared by other pestiviruses (45, 46).

**CONTROL MEASURES**

The present state of ruminant pestivirus infections in pigs, despite a sometimes high prevalence, does not require any immediate control measures, e.g. eradication or vaccination strategies, since the direct economic loss associated with these infections
is still tolerable. However, the occurrence of seroconverted pigs interferes with the considerable efforts made to control hog cholera. Infections of pigs with pestiviruses other than HCV could lead to false interpretations of serological results (39). Serologic surveys and surveillance on a herd basis are considered necessary as long as the elimination of HCV from the pig population remains the backbone of any control policy (21). False-positive serologic results due to ruminant pestiviruses will therefore impair HCV eradication programmes as well as efforts to maintain the HCV-free status in countries declared free of hog cholera (2, 23). Consequently, a reduction in the incidence of ruminant pestiviruses in pigs is desirable.

Recommendations for an improvement in the hog cholera control programme with respect to BVDV infection of swine have been offered (4):

- Develop methods for an efficient differentiation of antibodies against HCV and BVDV
- Keep pigs segregated from cattle
- Avoid the use of BVDV vaccine in swine
- Regard attenuated BVDV vaccines as potential pathogens and use discretion in recommending their use in cattle
- Be alert to the possibility that BVDV may be detected in sections of tissue, with anti-HCV conjugates, or isolated in cell cultures and confirmed as HCV.

In addition, it must be mentioned that the use of any vaccine that may be contaminated with ruminant pestivirus and capable of inducing pestivirus-specific antibody, should be applied with care if at all. Future attempts to eliminate persistent BVDV infections in cattle would also reduce the risk of infections and therewith antibody production in pigs.

Fortunately most of the laboratory tools required are now available, including MAB’s, for the differentiation of HCV and ruminant pestivirus (31, 44, 42, 45, 5). The availability of immunoassays for the specific detection of antibodies induced by HCV in pig sera seems to be at hand (46, 18; Schagemann et al., unpublished data).

L’INFECTION DU PORC PAR UN PESTIVIRUS DES RUMINANTS. – B. Liess et V. Moennig.

Résumé : Les infections des porcs par un pestivirus des ruminants existent dans le monde entier. Leur prévalence est très variable, essentiellement en fonction (a) des contacts avec les bovins, (b) de l’âge des porcs et (c) du degré d’homologie entre les souches virales utilisées en sérologie et les souches sauvages du virus de la diarrhée virale (virus BVD) qui infectent les porcs. L’accent doit être mis sur les sources de virus BVD autres que les bovins, par exemple des vaccins ou sérums de fœtus bovin contaminés.

La différenciation entre les pestivirus (virus de la peste porcine classique (PPC), de la diarrhée virale bovine et de la «border disease») est d’autant plus nécessaire que des syndromes cliniques tels que le retard de croissance et l’amaigrissement font penser à la peste porcine classique. On dispose
actuallement d'anticorps monoclonaux permettant de différencier le virus PPC des pestiviruses des ruminants dont, vraisemblablement, le virus BVD.

L'article comporte une étude mise à jour des relations antigéniques entre les pestiviruses. L'analyse de l'éventail des hôtes in vitro de ces virus est jugée importante et peut expliquer les infections des porcs par des virus autres que le virus PPC. Des résultats récents ont montré l'existence, parmi les souches du virus BVD, de souches «spécialistes» des cellules bovines, et quelques isolats de ce virus se sont également bien multipliés dans des cultures de la lignée cellulaire PK15. En revanche, des souches de virus BVD à action multiple ont vraisemblablement des sites de fixation supplémentaires sur des cellules ovines et porcines. L'identification des récepteurs sur les cellules ovines et porcines pourrait faciliter une distinction nette entre les infections des porcs dues au virus BVD et celles dues au virus PPC.

Des mesures immédiates pour la prophylaxie des infections porcines par le virus BVD ne sont pas nécessaires. Toutefois, ces infections peuvent interférer avec les enquêtes sérologiques et la surveillance sérologique au niveau du troupeau ; par conséquent, elles peuvent compromettre les programmes d'éradication de la PPC ainsi que les efforts accomplis pour conserver leur statut aux pays déclarés indemnes de cette maladie.

MOTS-CLÉS : Anticorps monoclonaux - Incidence - Pestivirus - Porcs - Prophylaxie - Ruminants - Sérologie - Virus de la diarrhée virale bovine - Virus de la peste porcine classique.

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INFECCIÓN DE PORCINOS POR UN PESTIVIRUS DE LOS RUMIANTES. — B. Liess y V. Moennig.

Resumen: Existen infecciones de porcinos por un pestivirus de los rumiantes en todos los países. Su prevalencia es muy variable, esencialmente en función de (a) los contactos con los bovinos, (b) la edad de los cerdos y (c) el grado de homología entre las cepas virales usadas en serología y las cepas salvajes del virus de la diarrea viral bovina (virus BVD) que infectan a los cerdos. Se debe prestar particular atención a las fuentes del virus BVD distintas de los bovinos, por ejemplo las vacunas o sueros de feto bovino contaminados.

La diferenciación entre los pestivirus (virus de la peste porcina clásica (PPC), de la diarrea viral bovina y de la «border disease») es particularmente necesaria cuando síndromes clínicos tales como retardos en el crecimiento y adelgazamiento permiten pensar en la peste porcina clásica. Se cuenta actualmente con anticuerpos monoclonales que permiten diferenciar el virus PPC de los pestiviruses de los rumiantes entre los cuales, verosímilmente, el virus BVD.

El artículo incluye una actualización respecto de las relaciones antigénicas entre los pestiviruses. El análisis del conjunto de los huéspedes in vitro de estos virus se considera importante y puede explicar las infecciones de porcinos por virus distintos del virus PPC. Resultados de experiencias recientes han mostrado la existencia, entre las cepas del virus BVD, de cepas «especialistas» de las células bovina, y algunas cepas aisladas de este virus se multiplicaron bien, asimismo, en cultivos de la línea celular PK15. En cambio, probablemente cepas del virus BVD de acción múltiple tienen sitios de fijación suplementarios en células ovinas.
y porcinas. La identificación de los receptores en las células ovinas y porcinas podrían facilitar una distinción clara entre infecciones de porcinos por virus BVD e infecciones por virus PPC.

No se necesitan medidas inmediatas para la profilaxis de las infecciones de porcinos por virus BVD. Sin embargo, estas infecciones pueden interferir en las investigaciones serológicas y la vigilancia serológica al nivel de la piara, comprometiendo los programas de erradicación de la PPC así como los esfuerzos para conservar libres de la enfermedad a los países así declarados.

PALABRAS CLAVE: Anticuerpos monoclonales - Incidencia - Pestivirus - Porcinos - Profilaxis - Rumiantes - Serología - Virus de la diarrea viral bovina - Virus de la peste porcina clásica.

REFERENCES


