ARTICLE

Review Article

New Concepts in the Pathogenesis, Diagnosis and Control of Diseases Caused by the Bovine Viral Diarrhea Virus

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Abstract

The new information on the pathogenesis and epidemiology of mucosal disease of cattle is reviewed. It is now known that clinical mucosal disease occurs only in cattle which were infected with a pestivirus in early gestation and were born with persistent viral infection and specific immunotolerance. These animals may be clinically normal at birth but may develop fatal mucosal disease, perhaps following superinfection with another pestivirus, usually between 6 and 24 months of age. They may also remain clinically normal indefinitely and breed successfully. The progeny from persistently infected females will similarly be persistently viremic, and maternal families of such animals may be established.

Congenital defects may occur when infection of the fetus occurs in mid-gestation. Although fetuses may be infected *in utero* in late gestation, the infections do not persist, the fetuses develop antibodies, and they appear to suffer no ill-effects. Postnatal infection can result in subclinical disease (bovine viral diarrhea) with a normal immune response; the virus may also be responsible for enhanced susceptibility to other infections, diarrhea in newborn calves, and reproductive failure.

Prevention of the economically important diseases caused by the virus is dependent upon the identification and elimination of persistently viremic animals, which are reservoirs of infection, and the vaccination of immunocompetent females at least three weeks before breeding. However, because of serotypic differences between strains, there is some doubt whether vaccination will reliably provide protection against the transplacental fetal infections that are important in the pathogenesis of this disease. There is no substantial evidence to warrant the vaccination of feedlot cattle.

Résumé

Cet article fait la révision des plus récentes informations sur la pathogénèse et l'épidémiologie de la maladie des muqueuses chez les bovins. Il est bien connu que l'aspect clinique de la maladie des muqueuses survient seulement chez les sujets infectés par un pestivirus tôt dans la gestation et qui sont nés avec une infection virale persistante et une immunotolérance spécifique. Ces animaux peuvent être normaux au moment de la naissance mais peuvent aussi développer une forme mortelle de la maladie; ceci est possiblement dû à une surinfection avec un autre pestivirus survenant entre les 6^{e} et 24^{e} mois d'âge. Ils peuvent aussi demeurer cliniquement sains indéfiniment et se reproduire normalement. La progéniture de femelles souffrant d'infection persistante aura aussi une virémie persistante et des familles maternelles de tels sujets peuvent être établies.

Certaines anomalies congénitales peuvent survenir quand l'infection du foetus survient au milieu de la gestation. Quoique les foetus peuvent aussi être infectés *in utero* vers la fin de la gestation, l'infection ne persiste pas, les foetus développent des anticorps et les sujets ne semblent pas montrer de symptômes. Les infections post-natales peuvent entraîner une maladie subclinique (diarrhée virale des bovins) avec une réponse immunitaire normale; le virus peut aussi causer une augmentation de susceptibilité à d'autres infections, à la diarrhée chez les veaux nouveau-nés et aux problèmes de reproduction. La prévention des maladies virales ayant une importance économique dépend de l'identification et l'élimination d'animaux ayant une virémie persistante car ceux-ci servent de réservoirs pour l'infection. Elle dépend aussi de la vaccination des femelles immunocompétences au moins trois semaines avant l'accouplement. Cependant, à cause de la différence des sérotypes qu'il y a entre les souches, il existe certains doutes à savoir si la vaccination procure une protection adéquate contre les infections transplacentaires du foetus qui sont un facteur important dans la pathogénèse de cette maladie. Il n'y a donc aucune évidence certaine qui appuie la vaccination systématique des animaux de boucherie.

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The virus causing bovine viral diarrhea (BVD) and mucosal disease (MD) is distributed worldwide. It usually causes a benign infection resulting in minimal

clinical changes, sometimes recognized as BVD, but is also consistently associated with fatal MD. In keeping with common usage, the virus will be referred to as that of BVD (BVDV) although MD is the more important disease manifestation. The BVD virus may also cause congenital defects in calves, reproductive failure and, through an immunosuppressive effect, contribute to the severity of other infectious diseases or disease complexes.

The BVDV, strain Oregon C24V, is the type species of the genus Pestivirus within the family Togaviridae (1). It is closely related to the viruses causing hog cholera (swine fever) in pigs and "Border" disease in sheep.

This is a review and update of the literature of the BVD-MD complex with emphasis on new information about pathogenesis and epidemiology which has improved our understanding of the disease. It is intended to assist the veterinarian in making reliable recommendations for control of the disease.

Pritchard (2) published an excellent account of the BVD-MD complex in 1963 in which he reviewed the field and experimental observations which had been published since the first reports of the disease by Childs (3) and Olafson *et al* (4) in 1946. Duffell and Harkness (5) and Baker (6) have recently reviewed the current literature on the BVD-MD complex.

Much of the literature on the pathogenesis of the BVD-MD complex published prior to about 1980 may be of questionable validity. All of it should be read with caution, or reinterpreted, for two reasons. Firstly, it was incorrectly assumed that clinical MD was a direct consequence of a simple "virus + host = disease" relationship. Secondly, the frequency with which BVDV occurs as a covert contaminant of cell cultures or fetal calf serum, a common medium supplement, was not fully recognized. The risk of results being misinterpreted because of these mistakes cannot be overemphasized. The magnitude and complexity of problems resulting from the use of fetal calf serum in cell culture media and work on BVD/MD may be appreciated if it is recognized that noncytopathic pestivirus will be present in most commercial preparations, with a likely concurrent presence of, and effectively camouflaged by, homologous antibody from a fetal herdmate that was infected when more mature than the viremic fetus. This is both demonstrable (IRL, unpublished data) and predictable from epidemiological considerations. Diluted by the pooling of many samples, the antibody may not be detectable by conventional tests and so go unrecognized. Then, when the fetal serum is in use as a medium supplement, the period of contact with cells is prolonged and, as the neutralization is slowly reversible, infective virus may emerge, though still difficult to recognize until a change of medium results in total freedom from antibody. The ramifications of this hazard in pestivirus diagnosis and research are wide and the consequences have frequently been quite disastrous.

The current concepts of BVDV infection in cattle are as follows:

1. *Mucosal disease occurs only in animals which are virus carriers following intrauterine infection before about 125 days of gestation.* These carriers are persistently infected and viremic, specifically immunotolerant, and continuously infective by shedding virus in a wide variety of secretions and excretions. They may be clinically normal or unthrifty prior to overt disease developing.
2. *There is no evidence that postnatal pestivirus infection alone in an immunocompetent virus-negative animal causes typical MD.* In an immunocompetent animal, it may result in a mild transient illness known as BVD or increase susceptibility to other infections, but it is usually entirely subclinical and accompanied by a normal immune response. This accounts for the high percentage of seropositive animals in most populations of cattle.
3. *Carrier females that are clinically normal may breed successfully and their progeny will similarly be carriers.* In this way, maternal families of such carriers may be established.

A summary of the possible consequences of BVDV infection in cattle is given in Figure 1.

Terminology

The terminology used in the literature on BVD-MD has been confusing. Up to the early 1960's, BVD and MD were regarded as distinct entities with many clinical features in common, but with epidemiological differences. Bovine viral diarrhea was described as being of high morbidity (80 to 100%) and low case fatality (0 to 20%), whereas MD was of low morbidity (5 to 10%) and high case fatality (90 to 100%).

In the early 1960's, evidence was accumulating that both diseases were manifestations of infections caused by viruses which were antigenically related or identical (7). Then, BVD became the widely preferred term, especially in North America. Mucosal disease in both acute and chronic clinical forms, was often referred to as chronic BVD because the infection was persistent.

According to current concepts, BVD represents the inapparent or benign infection which occurs in cattle herds, sometimes with mild clinical signs from which most animals recover uneventfully in a few days. To avoid confusion, the term BVD should be limited to that sense and otherwise discarded in favor of MD.

The terms acute and chronic MD are used to describe the clinical forms of the fatal disease which may develop in carriers.

Diversity among the pestiviruses infecting cattle may be serotypic or biotypic. In neither category are types separated systematically into discrete identities but they cover a continuous range, within which individual isolates may have representative status for some purposes and be referred to, loosely, as serotypes or biotypes. For example, a "serotype" may be one chosen for use in a serological test, or one "biotype" may be recognized as a vaccine strain.

Serotypes are distinguished by their behavior in serological tests of identity. In practice, short of using monoclonal antibodies, serological techniques other than virus neutralization (VN) have little ability to distinguish among different pestivirus strains so that serotypic identification or differentiation of pestiviruses has generally been based on VN.

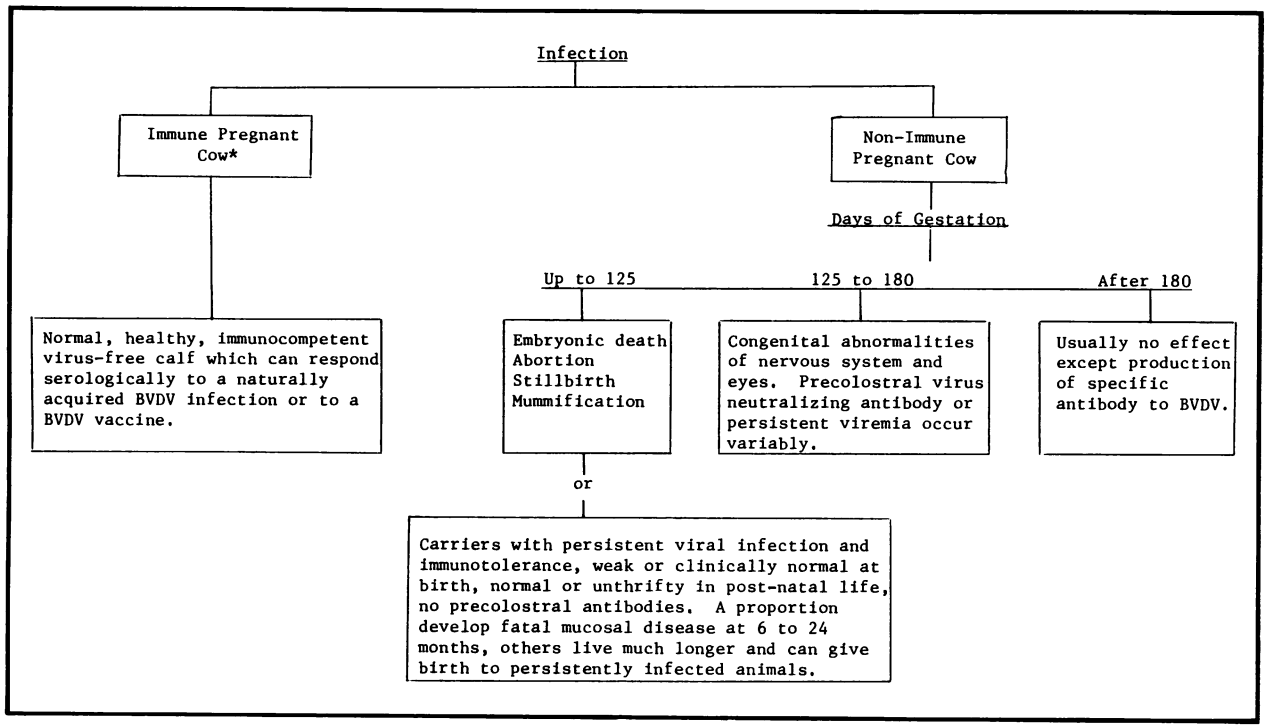

Figure 1. The possible consequences, to the bovine fetus and the calf after birth, of infection of pregnant cattle with bovine virus diarrhea virus (BVDV). *Immunity may be serotypically restricted.

Biotypes are described on the basis of biological behavior such as virulence or host range. Cytopathogenicity is one of the more important biotypic characters and the term cytopathic (or cytopathogenic) refers to the capacity of a strain of virus to produce visible cellular damage *in vitro*. Cytopathic isolates of the virus will destroy cells in culture. Noncytopathic isolates replicate in cell culture but do not destroy the cells and indirect methods for the detection of virus or associated antigens, such as fluorescent or enzyme-linked antibody staining, or immunodiffusion, must be used to detect their presence. As cytopathic effects may be evident only under defined conditions of cell culture, and the requirements may differ for different isolates, the distinction between cytopathic and noncytopathic viruses is not absolute.

Serotype and biotype vary independently, so that there are cytopathic and noncytopathic biotypes found which are serotypically indistinguishable. As described under "Pathogenesis", these may be concurrently present in naturally diseased animals and they provide for the only mechanism by which MD is really successfully induced experimentally. Some indication of the molecular basis of cytopathogenicity is provided by the observation that, when virus-specified proteins are compared, those in cells infected by cytopathic virus include one extra (~80 Kd) to those found in cells that are infected by noncytopathic virus (8,9). The full significance of this difference is not clear, and the extra protein is antigenically related to another (~115–120 Kd) which is found in cells infected by either biotype. Antisera are therefore not distinguishable according to the inducing biotype and it seems that any anti-BVDV serum may have antibody to all virus-specified proteins of either biotype (10). Monoclonal antibodies that do distinguish between cytopathic and noncytopathic viruses have been described (11) and may allow for the future serological distinction between the biotypes and their respective antisera, although it is not clear that the critical capacity to distinguish within a single serotype has been demonstrated.

Interestingly, it has been reported that, following experimental infection of pregnant cows with cytopathic viruses, only noncytopathic virus was recoverable from the fetus or calf (12). A similar result was found in sheep (13). The mechanism for this perplexing result is not at all clear but it could imply that the determinant of cytopathogenicity is separable from the virus identity, as it would normally be conceived.

Epidemiology

Geographical Distribution, Prevalence and Seasonal Incidence. Disease caused by the virus has been recorded in most cattle-raising countries of the world. Serological surveys indicated that 60 to 80% of cattle over one year of age may have VN antibodies to the virus (14). The prevalence of seropositive animals within affected herds may be much higher and, excluding carriers, may approach or equal 100% (15, IRL unpublished data). Note that these figures are derived from areas where vaccination is not practised. Because, vaccination apart, almost all reactors occur as a result of contact with a carrier (see under "Method of Transmission" heading) it can then be deduced that the same figures, of 60 to 80%, also give a rough indication of the minimum proportion of herds which contain one or more such carriers.

It is often stated in the veterinary literature that the BVDV is ubiquitous, which implies that the virus spreads easily between immunocompetent animals. However, it is unlikely that such secondary transmission

really occurs, at least not to any significant extent. If there are seropositive immunocompetent animals in a herd, it is likely that there is one or more carrier immunotolerant animals in the same herd excreting the virus and spreading it to the immunocompetent ones (primary transmission). Infection of immunocompetent animals (postnatally) will result in the production of antibodies with subsequent neutralization of the virus and only transient shedding, if any, of the virus.

In North America, MD occurs most frequently during the late fall and winter months but it can occur at any time of the year. A peak occurrence during the fall and winter months may be a reflection of mixing and crowding following weaning of beef calves at six to eight months of age.

Morbidity and Case Fatality Rates. Clinical MD is usually sporadic and only a small percentage of a herd of cattle, usually less than 5%, will be affected. Occasionally, outbreaks have been observed (OMR, unpublished clinical observations) in which up to 25% or more of the calves six to ten months of age in a herd are affected over a period of two to four weeks. To produce a prevalence of carrier calves, within a span of four months, of the order of 25% or more, it may be postulated that their dams, as a group of susceptible animals in early (up to 125 days) pregnancy, had contact with a carrier for an adequate period. The adequate period could be from as short as one day under conditions of close contact (barns, yards, trucks) up to a period of weeks under open grazing conditions, with other factors affecting the transmission rate, discussed below, being important.

The virus has been recovered from almost 1% of apparently normal cattle going to slaughter in Denmark (15). In Australian experience (IRL, unpublished observations), a prevalence of that order is commonly found when the endemically infected status of a herd is maintained by the presence of viremic cows and persistent infection is familial in its incidence. Higher prevalence rates are seen, such as 10% in the progeny of heifers (16), when they are first exposed to BVDV infection when in early pregnancy. This may occur regularly under some on-farm management systems but may also be an occasional event after such measures as enforced movement of cattle to another place for pasture or in emergencies caused by unpredictable events such as drought, flood, or fire. Even higher values, up to 27%, have been reported in individual herds (17). These latter may reflect exposure in circumstances similar to those responsible for the occasional high incidence of disease which has been mentioned. The prevalence of carriers in any population or group is primarily determined by the frequency with which dams of animals in that group were first infected during early pregnancy. When 15 individual heifers were *known* to have been naturally infected at the end of breeding, abortion or stillbirth resulted in six and *all* five live births were carriers (16).

Animals Affected. Mucosal disease occurs in all classes of cattle. Most cases occur between six and 24 months of age; rarely calves as young as four months of age, or cattle older than two years are affected. Limited serological surveys in sheep and goats revealed that 11% of sheep and 16% of goats in Quebec were serologically positive for the virus (18). Antibody to the virus has also been detected in captive exotic ruminants (19). Pestivirus of sheep and cattle will readily infect the alternate species, both naturally and experimentally, but the role that such cross-infections play in causing the respective diseases has not been determined.

Method of Transmission. Transmission is usually by direct contact with a carrier (20). The virus can be isolated from virtually any secretion or excretion including nasal discharge, saliva, semen, feces, urine, tears, and milk, each of which could allow wide dissemination of the virus.

Under grazing conditions (0.2 to 1 animal per acre) no spread from benign infections was recognized but, from newborn carrier calves to susceptible cows, new attack rates ranging from 0.006 to 0.04 per susceptible animal per day have been observed (16). In the same herd, a rate of 0.6 per susceptible animal overnight was noted when susceptible adults were yarded with an adult carrier. Undoubtedly, other factors, such as supplementary feeding, the nature of watering facilities, or the use of nose-grips for handling stock may dramatically influence transmission rates.

The fetus can be infected by transplacental transmission of the virus from the infected dam, whether the dam is transiently or persistently infected. In fact, this is probably the only circumstance in which transmission occurs efficiently from a transiently infected animal. Epidemics of abortion and congenital defects in calves have been attributed to the transplacental virus infection of the fetuses of cows in the first trimester following the introduction of carriers to previously virus-free herds (21). However, such cases are not adequately documented.

Carrier cows can remain clinically normally for years, during which time they may breed successfully. Their progeny may be apparently normal but are invariably also persistently viremic carriers (22,23,24). If female progeny breed, then a maternal viremic family, such as has been observed over several generations (25) is established and provides one of the major mechanisms for maintenance of the virus as endemic in a herd (Figure 2).

The infection has also been introduced into herds through the use of vaccines for other diseases, which were contaminated by the BVDV (26). Fetal calf serum used in certain high-technology procedures such as embryo transfer can also be a source of the virus. The virus probably does not infect the embryos directly but it is more likely that susceptible recipient cows would become infected and then back-transmit to the embryos after implantation. The significance of transmission by embryo transfer is that the procedure is usually used on purebred stock. If a carrier calf, produced as a result of transmission during embryo transfer, develops into a successful breeder, then a maternal viremic family may be established and the occurrence has virtually the same impact as introducing an undesirable gene into elite breeding stock.

A carrier bull can shed the virus in the semen for long periods (27), and introduction of such an animal

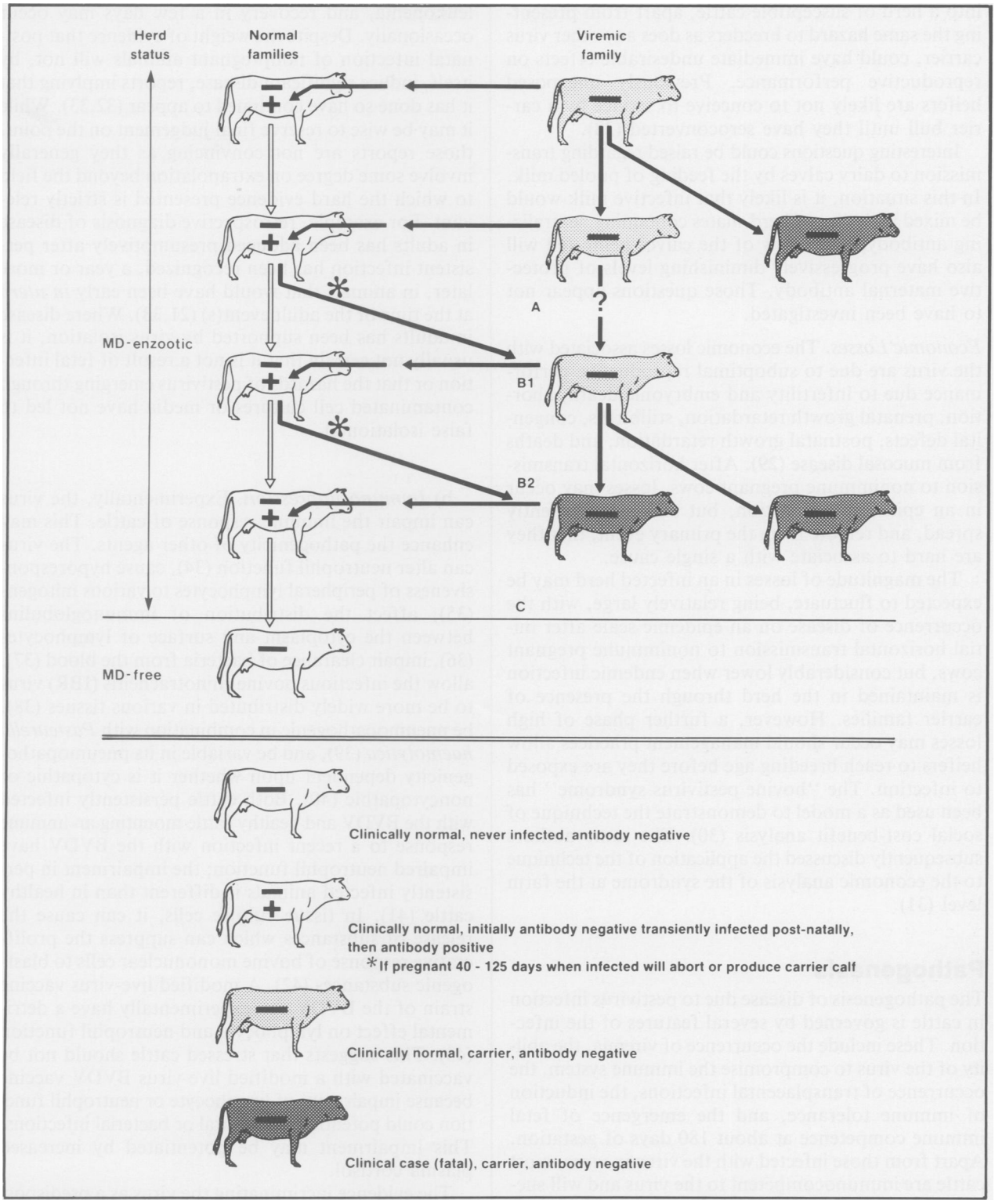

Figure 2. Structure of an MD-endemic herd showing alternative pathways of maintenance of the virus (after Littlejohns [24], modified). The persistently viremic maternal family, represented on the right hand side of the figure, may be important in maintaining true endemicity.

The open arrows represent reproduction in a normal family which is free of infection. Solid arrows represent virus transmission, either horizontally as postnatal infections or transplacentally through cows infected in early pregnancy or within carrier families. Note that,

A) Carrier cows are often poor breeders and maintenance of the virus over some generations may depend on horizontal and transplacental transmission in an early pregnant member of a normal family.

B) Carriers may, irrespective of their origin from normal or carrier families:
   1) Breed and establish continuous carrier families or
   2) Develop MD and die.

C) The line between the last two generations emphasizes that the endemic state tends to be terminating as viremic families die out. They are at a decided survival disadvantage compared to normal families in the herd.

into a herd of susceptible cattle, apart from presenting the same hazard to breeders as does any other virus carrier, could have immediate undesirable effects on reproductive performance. Previously unexposed heifers are likely not to conceive to service by a carrier bull until they have seroconverted (28).

Interesting questions could be raised regarding transmission to dairy calves by the feeding of pooled milk. In this situation, it is likely that infective milk would be mixed with that of herd-mates containing neutralizing antibody, and many of the calves being fed will also have progressively diminishing levels of protective maternal antibody. Those questions appear not to have been investigated.

Economic Losses. The economic losses associated with the virus are due to suboptimal reproductive performance due to infertility and embryonic death, abortion, prenatal growth retardation, stillbirths, congenital defects, postnatal growth retardation, and deaths from mucosal disease (29). After horizontal transmission to nonimmune pregnant cows, losses may occur in an epidemic proportion, but still be sufficiently spread, and remote from the primary event, that they are hard to associate with a single cause.

The magnitude of losses in an infected herd may be expected to fluctuate, being relatively large, with the occurrence of disease on an epidemic scale after initial horizontal transmission to nonimmune pregnant cows, but considerably lower when endemic infection is maintained in the herd through the presence of carrier families. However, a further phase of high losses may occur should management practices allow heifers to reach breeding age before they are exposed to infection. The "bovine pestivirus syndrome" has been used as a model to demonstrate the technique of social cost-benefit analysis (30). The same authors subsequently discussed the application of the technique to the economic analysis of the syndrome at the farm level (31).

Pathogenesis

The pathogenesis of disease due to pestivirus infection in cattle is governed by several features of the infection. These include the occurrence of viremia, the ability of the virus to compromise the immune system, the occurrence of transplacental infections, the induction of immune tolerance, and the emergence of fetal immune competence at about 180 days of gestation. Apart from those infected with the virus *in utero*, most cattle are immunocompetent to the virus and will successfully control a natural infection, develop antibodies, and eliminate the virus. Accordingly, infection may result in any of the following.

I. ***Postnatal Infections***

a) *Bovine Viral Diarrhea.* This is usually a clinically unrecognizable infection with the development of serum neutralizing antibodies and elimination of the virus from normal immunocompetent animals. This accounts for the high percentage of normal animals that are serologically positive (14). A mild transient clinical disease characterized by inappetence for a few days, depression, fever, mild diarrhea, transient leukopenia, and recovery in a few days may occur occasionally. Despite the weight of evidence that postnatal infection of nonpregnant animals will not, by itself, induce significant disease, reports implying that it has done so have continued to appear (32,33). While it may be wise to reserve final judgement on the point, those reports are not convincing as they generally involve some degree on extrapolation beyond the field to which the hard evidence presented is strictly relevant. For example, retrospective diagnosis of disease in adults has been adduced presumptively after persistent infection has been recognized, a year or more later, in animals that would have been early *in utero* at the time of the adult event(s) (21,33). Where disease in adults has been supported by virus isolation, it is usually not certain that it is not a result of fetal infection or that the hazards of pestivirus emerging through contaminated cell cultures or media have not led to false isolation(s).

b) *Immunosuppression.* Experimentally, the virus can impair the immune response of cattle. This may enhance the pathogenicity of other agents. The virus can alter neutrophil function (34), cause hyporesponsiveness of peripheral lymphocytes to various mitogens (35), affect the distribution of immunoglobulins between the cytoplasm and surface of lymphocytes (36), impair clearance of bacteria from the blood (37), allow the infectious bovine rhinotracheitis (IBR) virus to be more widely distributed in various tissues (38), be pneumopathogenic in combination with *Pasteurella haemolytica* (39), and be variable in its pneumopathogenicity dependent upon whether it is cytopathic or noncytopathic (40). Both cattle persistently infected with the BVDV and healthy cattle mounting an immune response to a recent infection with the BVDV have impaired neutrophil function; the impairment in persistently infected animals is different than in healthy cattle (41). In tissue culture cells, it can cause the release of substances which can suppress the proliferative response of bovine mononuclear cells to blastogenic substances (42). A modified live-virus vaccine strain of the BVDV can experimentally have a detrimental effect on lymphocyte and neutrophil function (43). This suggests that stressed cattle should not be vaccinated with a modified live-virus BVDV vaccine because impairment of lymphocyte or neutrophil function could potentiate other viral or bacterial infections. This impairment may be potentiated by increased plasma cortisol.

The evidence incriminating the virus as a predisposing pathogen in naturally occurring cases of bovine respiratory disease is largely circumstantial. The presence of the virus in the respiratory tract tissues of cattle affected with pneumonia is difficult to interpret. Several different viruses have been incriminated in the causation of acute bovine respiratory disease, but experimental evidence to support their involvement has centered on the IBR and parainfluenza-3 (PI-3) viruses (44). Bovine viral diarrhea virus may be present with other pathogens, such as those viruses or *Pasteurella* spp., and this may indicate that synergism occurs. However, it is also possible that the virus may be casually

present in some animals and have no significant adverse effect.

A recent report of an investigation of BVDV infection in a dairy herd of 200 milking cows indicated that 16% of the calves died of pneumonia in the year following the suspected introduction of the infection into the herd with purchased calves (33). A noncytopathic isolate of the virus was recovered from 55% of the calves which died from pneumonia and it was concluded that calves may have been carriers from early prenatal infection. Over a period of two years following the introduction of the infection, the herd also experienced an increased incidence of diarrhea in adult cattle, abortions, congenital defects and MD. A cytopathic isolate of the virus was recovered from the calves with MD.

c) *Diarrhea in Calves.* Calves born as carriers may have poor viability and suffer early disease and death with or without signs of diarrhea. To what extent these cases should be regarded as forms of either BVD or MD is arguable and the extent to which the manifest disease is due to other contributing pathogens has usually not been determined.

The virus has also been considered as a potential pathogen after postnatal infection of young calves. Following experimental infection with the BVDV, neonatal calves have been reported to develop enteric disease, occasionally fatal, with virus recoverable for up to 103 days (45,46). However, in the light of the new information, the relevance of these results to natural events may be difficult to assess, and reasonable caution could call for more evidence concerning the persistence of infection in these circumstances. On epidemiological grounds, it could be expected that calves born to susceptible (unexposed) cows would also be unlikely to be exposed to infection, whereas the dams of those born into an infective environment are likely to have also been exposed and so pass on maternal protective immunity. Perhaps, as noted elsewhere, the question of transmission via milk from a viremic cow is an important, but unresolved, question.

Whether or not the BVDV can cause a primary diarrhea, perhaps representing a juvenile form of BVD as a disease entity, it is suspected of complicity, with other agents, including rotavirus and coronavirus in producing disease (47). In older calves, it has also been shown to exacerbate the effects of infection by *Salmonella* spp. (*dublin* and *typhimurium*) (48). The novel possibility that bacterial infection may influence the extent of a viral disease seems not to have been investigated in regard to BVDV (49).

d) *Reproductive Failure.* Infection at the time of breeding may interfere with conception. Of five susceptible heifers mated to a carrier bull, all seroconverted within two weeks. Three did not hold to service until serum VN titers rose to 1:128, while a fourth aborted at six months (28). The alternatives of primary uterine infection by semen or systemic infection after nonsexual contact were not distinguished. Experimentally, the intrauterine infusion of virus into cattle at the time of insemination has prevented conception and has been attributed to prevention of fertilization (50) or simply recognized as an empty uterus at five weeks after breeding (51). It seems that intrauterine infection at the time of breeding may have some effects on the very early stages of reproduction in addition to those that could be attributed to infection by other contact routes (52).

Numerous reports describe abortion as a result of infection of susceptible cows in early pregnancy. In one study, experimental infection of pregnant cows with the BVDV during the first 100 days of gestation caused abortion or mummification, but inoculation of fetuses or pregnant cows in the second and third trimester failed to cause abortion, although fetuses were infected and developed VN antibody (53). Much of the information in this area, and also on the closely related subject of fetal infections, dealt with in the next section, is derived from experimentation, often using very artificial conditions, such as the direct inoculation of fetuses *in utero* with large doses of virus. Results obtained, and their likely consonance with natural events, should be seen in this context.

II. *Fetal Infections*

Following the infection of a nonimmune pregnant animal, the virus is capable of crossing the placental barrier and invading the fetus. The congenital infection can result in a wide spectrum of abnormalities from death of the fetus to congenital defects, to the persistent lifelong infection of a carrier, perhaps without clinical signs. The results are mainly dependent on the stage of fetal development at which infection takes place (Figure 1) (53,54). In general, the risk for the fetus is highest during early pregnancy.

The bovine fetus gains immune competence to the BVDV around day 180 of gestation (55). However, it can produce immunoglobulins without detectable specificity in response to a BVDV infection before attaining immune competence to the virus (56). No fetal disease is recognized to result if infection occurs after full immune competence has been acquired. Antibody to the virus is then demonstrable at birth, before colostral intake has occurred.

a) *Persistent Viremia.* If the fetus is infected with a noncytopathic isolate of the virus before about 125 days of gestation, it will not develop serum VN antibodies and may be carried normally to term and be born with a persistent infection with the virus. From birth these animals are specifically immunotolerant and persistently viremic, and may appear either clinically normal or unthrifty. They continuously shed virus in secretions, even while carrying maternal antibody. Although immunotolerant to the homologous noncytopathic strain of BVDV, carriers are immunocompetent to other antigens since they develop neutralizing serotiters to the IBR and PI-3 viruses and agglutinating titers to *P. haemolytica* (57). They may also produce VN antibody, following administration of a commercial live BVDV vaccine of different serotype against the vaccine virus as well as other laboratory strains (58). Furthermore, in spite of this antibody formation, the original virus may persist.

b) *Mucosal Disease.* Mucosal disease will develop in a proportion of these, and only in these, carriers. During the postnatal period, superinfection with a

cytopathic isolate of the virus may precipitate fatal clinical MD in these animals (59,60,61). Death from acute MD usually occurs within two weeks of the onset of clinical signs, and both cytopathic and noncytopathic isolates of the virus have been recovered from the tissues of affected cattle (62). Because maternal colostral antibodies to the BVDV may persist for about six to eight months (63,64), it is interesting to speculate if those antibodies protect the carrier calf from clinical MD. This is not known. Such calves do subsequently remain seronegative to, and do not respond to experimental infection with, the homologous noncytopathic virus (57). A different result was obtained in sheep, when disease, but not antibody, was induced in carrier lambs after they were challenged with the cytopathic form of the homologous virus, which had been used originally to infect the dams of the trial lambs while the latter were *in utero* (13,65).

In spite of the new information on the pathogenesis of mucosal disease, some important unresolved questions remain. What is the source of the cytopathic isolate of the virus? The clinically normal carrier state for cytopathic virus has not been recognized. Brownlie *et al* (66,67) suggest that a mutation of the noncytopathic virus within the animal is a possibility and more likely than the introduction of the cytopathic virus by way of an infected animal introduced into the herd. Also, although MD as a consequence of superinfection by a cytopathic pestivirus has been convincingly demonstrated experimentally and some diagnostic results indicate that, in the field, it is one of the triggers to manifest MD, it seems not to be the complete answer to the questions of etiology and pathogenesis. Disease is not always induced (68,69), or not by all cytopathic strains (70). On the other hand, serotypic identity between original and superinfecting viruses, presumably a condition for persistence of infection by the latter, has not always been necessary (59). This raises the possibility that the superinfecting virus contributes only the essential determinant of cytopathogenicity (see "Terminology"), perhaps as a subviral agent, to the infection and an alternative hypothesis suggested that a defective infectious agent, which is incapable of independent replication but uses pestivirus as helper, may contribute, in some cases, to both the cytopathogenicity in cell culture and the pathogenicity for the animal (25). A close serological relationship between cytopathic and noncytopathic viruses from individual diseased animals has been demonstrated (71). However, this result was largely predictable and it may be wise to recognize that there is, conceptually, a subtle, but crucial, distinction to be drawn between the cytopathic virus that initiates the superinfection and the cytopathic virus that is recoverable.

Recent work has shown that typical MD occurs within two to three weeks of superinfection of persistently viremic calves with the serologically homologous cytopathic virus (66,67), to which they do not respond serologically. Superinfection with a serologically different cytopathic virus did not result in MD within two to three weeks, but such infected animals could develop a nonfatal form of MD several months later, or not at all, and respond serologically to the heterologous cytopathic virus (66,67).

It has also been noted that there are a number of pathological facets to MD and that it may be necessary to consider different pathogenetic mechanisms for those different facets (25). Some lesions may be caused by direct cytolytic effects of the virus, especially when cytopathic strains are involved, but glomerulonephritis (72,73), and other evidence of immune complex disease (16,74,75), present something of a paradox in a disease which is generally dominated by immune tolerance. The presence of these lesions suggests that at least this aspect of MD, like many other late pathological consequences of various virus infections recognized these days, is due to an inappropriate immune response rather than to any direct effect of the virus. Although the viral proteins responsible for serotypic character have not been identified, remarkable heterogeneity among the field viral isolates of BVD has been noted (9). A superinfecting virus which is serologically similar to the persistent virus, and hence is also able to persist, may differ from the original virus in regard to other antigens, i.e. antigens that are not involved in neutralization or the determination of serotype. This could conceivably provide a basis for immune complex disease, as the host's tolerance is strictly limited to the antigens of the original virus strain. Hypothetically, this should not require that the superinfecting virus necessarily be cytopathic.

c) *Congenital Disease.* Congenital defects of newborn calves can result from infection of the fetus with the field strain of the virus between approximately 125 and 180 days of gestation (76). Cerebellar hypoplasia occurs (77); ocular abnormalities consist of retinal atrophy, optic neuritis, cataract, and microphthalmia with retinal dysplasia (78,79). A modified live BVDV vaccine given to seronegative pregnant cows between 90 and 118 days of gestation can result in congenital cerebellar hypoplasia and hydranencephaly in the calves (80). The teratogenic effect of that vaccine was restricted to the period between 90–118 days.

Calves with cerebellar hypoplasia are unable to stand and walk normally immediately after birth. Defects of the eyes result in varying degrees of blindness; the cataracts are obvious when they occur.

Congenital morphological defects follow infections which occur somewhat later in gestation than do infections which result in persistent viremia and may be due, in part, to the emerging immunological capability. The presence of either persistent infection or antibody is variable.

Border disease of sheep is caused by an *in utero* infection with a related pestivirus which cross-reacts with the BVD virus (13,65,81,82,83). Ewes are clinically normal, but affected newborn lambs have a hairy fleece, clonic rhythmic tremors, and are unthrifty. The lesions consist of hypomyelination and abnormal cells in the central nervous system. The hairy birthcoats have been attributed to hypertrophy of primary follicles and medullation of wool fibers (81). Surviving lambs are also infected carriers of the virus (82). The virus can be isolated in cell culture and detected by immunofluorescent staining of the peripheral leukocytes, cellular debris in urine, and cerebrospinal fluid

in lambs up to one year of age. Affected lambs, like calves, have no detectable serum neutralizing antibody. Sheep, after recovery from postnatal infection by the virus, have no detectable virus in the leukocytes and have VN antibodies (84).

Clinical Findings

Inapparent or Subclinical Infection (BVD). The most frequent form of BVDV infection in cattle is nonclinical or a mild disease of high morbidity and low case fatality characterized by a mild fever, leukopenia, inappetence, and mild diarrhea followed by rapid recovery in a few days and the production of VN antibodies. This form occurs in cattle which are infected after birth and presumably accounts for the high proportion of adult animals which possess serum neutralizing antibodies to the virus (14). The literature commonly refers to this subclinical infection as BVD. Similar infection, with no long-term consequences other than the development of antibody, occurs in fetuses over about 150–180 days of gestation.

Acute Mucosal Disease. This form is characterized by the sudden onset of clinical disease in animals from 6 to 24 months of age which were infected during early fetal life. The morbidity is low but the case fatality rate is usually 100%. Within herds, from 5 to 25% of animals in this age group may develop the disease over a period of several days, or sporadic cases may occur over several weeks or months. Well-nourished, clinically normal animals can be affected.

Affected animals are depressed, anorexic and slobber saliva, wetting hair around the mouth. Their body temperatures are elevated to 40°–41°C, and tachycardia and polypnea are common. Ruminal movements are usually absent and a profuse and watery diarrhea occurs two to four days after the onset of clinical illness. Their feces are foul-smelling and may contain mucus and variable quantities of blood. Occasionally, small fibrinous intestinal casts are present. Straining at defecation is common and the perineum is usually stained and smeared with feces.

The lesions of the buccal mucosa consist of discrete, shallow erosions which become confluent, resulting in large areas of necrotic epithelium becoming separated from the mucosa. These erosions occur inside the lips, on the gums and dental pad, on the posterior part of the hard palate, at the commissures of the mouth, and on the tongue. The entire oral cavity may have a cooked appearance with the gray necrotic epithelium covering the deep-pink raw base. Similar lesions occur on the muzzle and may become confluent and covered with scabs and debris. Although the oral lesions are highly significant in the identification of the disease, they may be absent or difficult to appreciate visually in up to 20% of the affected animals, particularly in the latter part of an outbreak.

There is usually a mucopurulent nasal discharge associated with some minor erosions on the external nares and similar lesions in the pharynx. Lacrimation and corneal edema are sometimes observed. Lameness occurs in some animals and appears to be due to laminitis, coronitis, and erosive lesions of the skin of the interdigital cleft, which commonly affect all four feet.

Usually dehydration and weakness are progressive and death occurs five to seven days after the onset of signs. Occasionally, in peracute cases, which die within a few days after the onset of illness, the diarrhea is not evident even though the intestines are distended with excessive fluid. Presumably, there is paralytic ileus and fluid is not being moved down the intestinal tract.

Chronic Mucosal Disease. Some acute cases of MD will not die within the expected time of several days and become chronic cases. There may be intermittent bouts of diarrhea, inappetence, progressive emaciation, rough dry hair coat, chronic bloat, hoof deformities, and chronic erosions in the oral cavity and on the skin. Shallow erosive lesions covered with scabs can be found on the perineum, around the scrotum, preputial orifice and vulva, between the legs and at the skin-horn junction around the dew-claws, in the interdigital cleft and at the heels, and there may be extensive scurfiness of the skin. The failure of these skin lesions to heal is an important clinical finding suggesting chronic mucosal disease. Chronic cases will sometimes survive for several weeks or months during which time they are unthrifty and ultimately die from chronic inanition.

The chronic clinical form of the disease described above must be distinguished from the unthrifty carrier described next.

Unthrifty Persistently Viremic Calves. Calves which are born persistently viremic carriers may be smaller than their contemporaries and may fail to grow normally. They may survive and appear unthrifty for several months or more until they develop fatal MD or some other infectious disease such as pneumonia (12,54). These calves do not have detectable clinical evidence of MD and they are seronegative to the BVDV (29).

Laboratory Diagnosis

The diagnosis of MD is usually made on the basis of the presence of characteristic clinical and pathological findings. A severe leukopenia is frequently observed in acute MD. The decrease is commonly to below 50% of normal, and total leukocyte counts of 1.0–3.0 $\times$ 10^9/L are common and may persist for weeks.

The definitive etiological diagnosis of MD by virus isolation, can be time-consuming, expensive, and elusive, however there is continuing progress being made in resolving these difficulties. It can be attempted by inoculation of nasopharyngeal swabs, ocular swabs, intestinal tissues, spleen, or most other tissues, or any fraction of blood into cell cultures. Recovery of virus from feces is generally difficult. Isolation of virus, from any source, in cell culture may require more than one passage before the virus is detectable. It is then recognized by cytopathic effects or, in the case of noncytopathic strains, either interference with a cytopathic virus or various serological methods may be used to demonstrate the presence of virus or virus-associated antigens. Both cytopathic and noncytopathic pesti-

viruses have been isolated from spleen (61) or blood (60) of individual cattle with MD and it has been suggested that both should be present in cases of mucosal disease (85). The serological methods used to detect noncytopathic virus or antigen in cell culture or tissues, such as intestine, kidney or spleen from affected animals or aborted fetal tissue, include direct or indirect immunofluorescent antibody staining, immunoperoxidase staining, (15,86,87,88), and gel diffusion (GDP) techniques (89,90). Nasal epithelial cells collected on cotton swabs were stained by fluorescent antibody for the diagnosis of field cases of BVD in calves (91) and, using a similar technique, the detection of virus antigen in cells, obtained from the nasopharynx using Belmont brush swabs, was shown to be a rapid and efficient method for identifying carriers, agreeing perfectly with virus isolation from leukocytes and clotted blood (20).

Serological techniques are also used to detect antibody. The various tests available differ somewhat in the range of viruses to which they detect antibody. Neutralization tests best detect antibody to strains of virus that are identical, or closely related, to the laboratory strain that is used in the test and so are, to some extent at least, serotype-specific. Guanosine 5′-diphosphate (GDP) tests can be based on one of several virus structural or virus-associated precipitable proteins (92). One test detects antibody to a single virus-associated molecular antigen that appears to be common to all pestiviruses, including those of other host species, and so is generally held to be group-specific. Other methods, including complement fixation (CF), immunofluorescent (IF) staining, or enzyme-linked immunosorbent assays (ELISA) also tend to detect antibody to all virus strains although it could be anticipated that the exact breadth of the specificity of any of these tests may vary with the way in which the antigen is prepared. Tests of wider or narrower specificity will have advantages for different purposes and, if available, should be selected accordingly. A number of recently described ELISA tests (93,94,95,96) are of high serological sensitivity and appear likely to be group-specific.

In the past, the VN test has sometimes been used to determine the occurrence of a rising titer between acute and convalescent sera. It is now apparent that this is only a valid procedure in the case where clinical BVD is under consideration. It is not valid for the diagnosis of MD because the specific immune tolerance precludes the development of VN antibody. In the specific case in which immune complex disease is a component of the pathology, weak antibody may be demonstrable by a GDP test. Development of this reaction in a persistently viremic animal would presage clinical deterioration.

Precolostral sera from calves infected *in utero* as immunocompentent fetuses may have virus-specific neutralizing antibodies (57) and their demonstration is meaningful for the diagnosis of late *in utero* infection.

Despite the limitations to the use of serology in the initial diagnosis of MD, it could be argued that diagnosis is of little use in itself, but must be followed by investigation of the herd in some depth to guide further action. Tests for antibody provide the main basis for action in this phase and their use is described under "Control and Prevention".

The pathological criteria for the diagnosis of BVDV as a cause of abortion have not been established (97). Finding antibody in a fetus, as in an unsuckled neonate, indicates that intrauterine infection had occurred but its diagnostic significance in regard to the abortion is not clear. In the absence of congenital malformations, the diagnostic significance is not likely to be great since the fetus has recovered from the infection. The recovery of virus from, or the serological identification of virus or viral antigen in, fetal tissues is more suggestive of a diagnosis of pestiviral abortion but is not conclusive. If something like 1% of the adult population are carriers, than presumably virus should be detectable in at least that proportion of either normal fetuses or those aborted for any reason other than the infection by the BVDV. Experimentally, it has been found that viral antigen was demonstrable by immunocytochemical methods in secretions of several fetal organs, primarily lymphoid tissues, even though virus was not recoverable (98). These observations were made on viable fetuses, recovered surgically, three weeks after direct fetal inoculation with a large dose of virus, so the relevance of the finding to the diagnosis of natural fatal fetal infection is not certain.

While each of the various methods for virus isolation, antigen identification or detection of antibody has its own particular advantages, all laboratory units cannot be practiced in the execution of all procedures. Accordingly, the better diagnostic strategies will be those which are designed around the tests that are readily available in the cooperating laboratory.

Control and Prevention

The successful control and prevention of the BVD-MD complex in a herd will depend on the identification and eradication of carriers and immunization of breeding animals before their first breeding.

1. ***Detection and Elimination of Persistently Viremic Carriers.***

With the knowledge presently available concerning the epidemiology of pestivirus infections, certain recommendations can be made. However, it should be anticipated that there is yet much to learn, particularly in regard to anomalies not yet recognized, which may alter these recommendations. Also, it is important that the laboratory tests available and their best use should be determined in consultation with the laboratory providing the diagnostic service.

It could be argued that vaccination prior to breeding should be sufficient to achieve control. Even the untoward sequelae that may follow vaccination with modified-live BVDV probably only affect carriers, which are doomed and should be eliminated from the herd anyway. However, a single vaccine may not be fully effective against all serotypes so it is recommended that other steps to eliminate infection and to prevent its reintroduction should be taken.

Serological testing of the whole herd will allow the search for virus carriers to be limited to seronegative

animals. Even when it is not practicable to attempt virus isolation from individual animals on the scale necessary to identify carriers directly, much useful information may be obtained from a careful analysis of the serological results in the light of the herd structure and management. In the description that follows, it is important that the serological technique employed should have, in relation to all strains of virus likely to be encountered, very high sensitivity and specificity, with the latter being the more important. Note that the titers achieved in a test, reflecting what might be regarded as serological sensitivity, have very little to do with the sensitivity for epidemiological purposes, as this is only measured as the proportion, often expressed as a percentage, of previously infected animals that are detected, regardless of titer or reaction strength. However, it is essential that the specificity of the test should be effectively equal to 100% (i.e. no false positives). Achieving this may incur some compromise in sensitivity, but it is imperative that false positives, which could allow a carrier to be cleared of suspicion, be avoided. The performance of the test or tests available, and how the results are best interpreted for specific purposes, must be determined in consultation with the laboratory providing the service.

With an understanding of the epidemiology of BVDV infection, the best use of diagnostic tests, and the management and background of the particular herd, the strategy is then best designed individually for that herd. The following provides a general guide.

The first step is to take blood samples, which are allowed to clot, from all animals over six months of age. Sera are removed from the samples and tested for antibody, preferably using a group-specific test so that all serotypes are equally represented. The clot residues should be retained frozen for later attempts at virus isolation should this be necessary. The overall prevalence of antibody, and its distribution within recognizable subgroups within the herd, for example by age, management or origin, should be carefully considered before further action is taken.

If there are few negatives, of the order of 1%, then, in the absence of any explanation for their negative status, they should be regarded as likely carriers and culled immediately. They may be examined for virus but, since this diagnostic procedure may be less than 100% efficient, they will never be free of suspicion and so it is probably a waste of time and effort. However, in other circumstances, for example, if a controlled exposure program is to be contemplated, it may be useful, or even necessary, to confirm the viremic status of these animals.

If there are marginally more negatives, of the order of 5–10%, too many to willingly cull, then either they should all be examined for virus or, if this is not feasible, the serological results should be analyzed in an attempt to deduce the status of the negatives, i.e. whether they are carriers or unexposed. Subgroups within the herd, defined by age, origin or location, should be considered separately. Small numbers of negative animals within subgroups that are otherwise positive come under suspicion and should be eliminated. In some cases, the proper interpretation of serological results may be clarified, and carriers identified, by further testing after some tactic to promote seroconversions. Suitable tactics for this purpose, provided that the animals are not pregnant, might include vaccination or simply intensive management of the group to maximize intimate contact with an unrecognized carrier that may be present. Although it is obviously impossible to forecast precise transmission rates for all situations, interpretation of data for single groups should be made in the knowledge that transmission rates are likely to be relatively low under extensive grazing conditions but greatly increased by any handling or intensive husbandry. Progeny of suspect virus carriers will also initially be under suspicion, although the finding of antibody in healthy progeny would initially absolve the claim of suspicion.

As the endemic presence of the virus appears to depend almost entirely on the presence of one or more carriers, and these animals are at a survival disadvantage compared to normal animals, it can be expected that the infection will be naturally eliminated from some herds. Then, barring reintroduction of infection, reactors will be confined to those animals which were present in the herd prior to the elimination of the infection. If a substantial part of a herd, which has been of stable composition for some time, is serologically negative, and reactors can be recognized to be confined to groups defined by either age or origin, this may indicate that the virus has been active in those groups in the past but is no longer active in the herd. In these cases, no further investigation is necessary before vaccination and precautions against the reintroduction of infection are employed.

A final scenario which should be considered is the herd which has already been comprehensively vaccinated. As in a herd with a very high natural antibody prevalence, the carriers might be recognized as those that are still lacking antibody. However, in the case of the vaccinated herd there is a further complication, in that viremic animals may have responded to the vaccine strain, if it differs serotypically from the persistent virus, and have produced antibody that will be recognized in a VN test, provided that there is a serotypic relationship between the strains used in the vaccine and the test. A group-specific test may be more informative than the VN test because the group antigen, shared by all serotypes, is included within the scope of the carrier's immune tolerance and so it is more likely to remain seronegative in that respect. On theoretical grounds, a group GDP test could be preferred, as it involves only a single common antigen, whereas those antigens which are specific to the serotype may also contribute to reactivity in other tests, even though those tests may normally be dominated by group-specific reactions.

Apart from its use in identifying carrier animals, the detailed analysis of the distribution of serological reactions in the herd may also indicate when transmission usually occurs and, hence, the pattern of spread that has allowed endemicity to be maintained and disease to occur. For example, in a dairy operation, calves fed from the milk pool may be effectively vaccinated while a carrier cow is in production. However, the same cow may represent a health hazard if it subsequently goes into a dry lot and contacts pregnant

heifers who were raised as calves out of phase with the virus in the milk pool. If management factors involved can then be corrected or avoided a basic improvement in disease control is achieved.

Calves present a special case and testing should be deferred until they are over six months of age and are likely to have lost maternal antibody. It has been reported that colostral antibodies, presumably detected by VN, may be demonstrable for a lesser period of time in the persistently viremic calf compared to the normal calf (6). However, a different result was observed in regard to precipitating antibody in six carrier calves which were born to immunocompetent cows which had been infected in early pregnancy (IRL and TM Jessep, unpublished data). They carried antibody detectable by GDP test significantly longer than did their 27 normal herd-mates (140 v 70 days, $t = 7.0$, $p < 0.001$), presumably because of their prenatal hyperimmunizing effect on their dams who were then able to pass on unusually high levels of colostral antibody. To allow a margin of safety, final clearance tests might best be made after one year of age, particularly if a group test of high sensitivity, for example ELISA, is used. Calves at foot should be treated as animals of unknown status and particular care should be taken to avoid new contacts between them and likely susceptible breeders in early pregnancy.

After virus carriers have been eliminated, the new virus-free status of the herd has to be maintained by the careful selection and/or testing of all introductions. This is a wise precaution whether the herd is vaccinated or not because of uncertainty whether the protection offered by vaccination will be absolute in the most crucial requirement, that of preventing transplacental infection in the pregnant animal (99). In many cases safety can be assured, as far as possible, if introductions either have convincing titers of serum antibody, due to either vaccination or natural exposure, or are negative and are derived from a totally negative herd or subherd. What are significant titers are best advised by, or decided in consultation with, the laboratory conducting the tests. In practice, it may be difficult to establish that the herd is "totally negative" and free of infection unless it has been closed to introductions for a lengthy period, at least a year. If any doubt exists in this direction, antibody-negative introductions should be examined for virus and/or held for a period of on-farm quarantine in close contact with a few seronegative nonviremic test animals, which are subsequently examined for antibody. Bulls which are destined for artifical breeding units should be tested serologically and their blood examined for the presence of virus. Persistently viremic bulls should be disqualified from entry into artificial breeding units.

2. *Vaccination*

a) *General Considerations.* The efficacy of the currently available vaccines is a major question. Both modified live virus and inactivated virus vaccines are available. Modified live BVDV vaccines have been available for the past 20 years and have been used with various degrees of apparent success. The modified live-virus vaccines are potentially fetopathogenic and should not be used in pregnant cows. Some other modified live-virus vaccines (not specifically BVDV vaccines) have been contaminated with BVDV and their use has been followed by serious economic losses (26). A temperature-sensitive vaccine will cause seroconversion, produces no clinical signs of disease or leukopenia and, when used experimentally in pregnant cows, does not result in fetal infection as evidenced by lack of virus isolation and absence of precolostral antibodies in the calves which are born healthy (100). The inactivated virus vaccines are safe but must be given twice, ten days to two weeks apart (101). More research and field experience are necessary to more accurately assess the relative value of each vaccine (102).

Historically, the vaccines have been tested in young cattle using a titer response as the major criterion of efficacy. It is now clear that testing the efficacy of BVDV vaccines by vaccinating calves at four to six months of age followed by experimental challenge a few weeks after vaccination is not an adequate method of determining the efficacy of a BVDV vaccine (102). Immunocompetent calves whether vaccinated or not, will not develop MD following experimental or natural infection. There are few, if any, reports of controlled trials to examine the efficacy of the vaccines under field conditions.

It is important to emphasize that vaccination be done at least three weeks before breeding so that breeding females become seropositive to the virus before conception. This is necessary regardless of the type of vaccine used

b) *Vaccination of Calves.* The prevention of MD in young cattle from 6 to 24 months of age has been misguided until recently, because the pathogenesis was not clearly understood. Conventional wisdom suggested that calves should be vaccinated at or around weaning time when the level of colostral antibody had declined to a level where it did not interfere with the vaccine. When outbreaks of MD occurred, vaccination of the in-contact animals to prevent further occurrence of the disease was commonly practiced. However, the results were difficult to evaluate. In some herds no further cases occurred and success was attributed to the vaccine. In other herds, outbreaks of clinical disease occurred about 10 to 14 days following vaccination (103). The possible causes postulated for these so-called "*vaccination breaks*" included the following: the vaccine virus may not have been sufficiently attenuated and actually caused the disease; the calves may have been incubating the disease when vaccinated; and, some calves were immunotolerant because of infection during fetal life thus allowing the vaccine virus to cause the disease. These vaccination breaks gave the vaccines a poor reputation and as a result they have not been used on a regular basis. Also, veterinarians began to make regular reports that the vaccine was ineffective against mucosal disease, but the reasons were unknown (104).

The new information on the pathogenesis of mucosal disease explains why vaccination of calves at about six months of age may not provide protection against their developing MD. Those calves that are already persistently infected at the time of vaccination will not be cured of that original infection and so may eventually develop MD whether they respond to the vaccine or not (70). It can also now be seen the so-called "untoward sequelae", when, infrequently, new cases of MD may occur in 5–10% of calves within a few weeks of live-virus vaccination, may not really be a debit against vaccination. It has been postulated that these cases of disease are likely to be due to the vaccine fulfilling the role of a superinfecting virus and precipitating clinical disease in carriers. If this is so, then those animals were likely to eventually develop MD anyway, or were best culled for disease control purposes.

There is some experimental and circumstantial evidence that the virus may predispose cattle to pneumonia. If the virus predisposes young immune competent cattle to respiratory tract disease, then vaccination prior to the expected occurrence of respiratory disease must be examined using well-designed field trials. However, at the present time there is no substantial evidence to warrant the vaccination of feedlot cattle. In the Bruce County beef cattle project, the use of modified BVDV live-virus vaccines in feedlot calves on arrival in the lot after transportation of 3200 km increased the risk of mortality (105). The risk declined following decreased use of the vaccine (106). This observation may support the experimental work that field isolates of the virus and live-virus vaccines may both cause immunosuppression.

c) *Vaccination of Breeding Females.* The key to successful control of pestivirus infection of the fetus and the consequences thereof is vaccination of the breeding female several weeks before breeding (107,108,109). Experimental exposure of pubertal heifers to the virus six weeks before breeding stimulated the production of serum neutralizing antibodies which protected against transplacental infection of the fetuses when the pregnant dams were challenged with homologous virus at 100 days of gestation. A high incidence of fetal death and intrauterine growth retardation occurred in the nonimmune dams. Thus, the presence of maternal immunity protected the fetus from homologous infection (107). These observations provide justification for the use of BVDV vaccines in females before breeding in an attempt to stimulate maternal immunity to provide protection of the fetus. However, to date there is little published information on the efficacy of the vaccines for the protection of the fetus. Immunization, in terms of protecting the fetus, may not be effective against serotypes which are different from that contained in the vaccine. One report claimed that the use of an inactivated BVDV vaccine will provide protection of the fetus even though, depending on the heterogeneity of the infecting strains, the protection may be incomplete (110). However, an inactivated quadrivalent vaccine, which induced a serological response in cows before insemination, failed to protect approximately one-third of the fetuses against transplacental infection from a multiple heterologous strain challenge (111). This indicates a need for additional understanding of the antigenic relationships among the many isolates of the virus and to examine the spectrum of strains of the virus which must be included in a vaccine.

Vaccination of pregnant cows cannot be recommended at this time in spite of published advice (112). Vaccination of pregnant cattle with modified live BVD virus can have the same age-determined effects on the fetus as does natural BVDV infection (113,114). This may not be true of a temperature-sensitive strain which may be safe in this regard (100), as also are inactivated vaccines. However, it is important to emphasize that vaccination be done at least three weeks before breeding so that breeding females become seropositive to the virus before conception. This is necessary regardless of the type of vaccine used. The suppliers of inactivated virus vaccines may promote their vaccines on the basis that they can be given safely to pregnant cows. While it is true that the inactivated virus vaccines are not fetopathogenic, only successful vaccination before conception will protect the fetus from natural infection for the entire gestational period.

With the present state of knowledge, a rational vaccination program, for both beef breeding herds and dairy herds, would consist of vaccinating all of the cows and heifer replacements at least three weeks before breeding. Each year thereafter, all new heifer replacements are vaccinated. Colostral immunity is present for up to six months of age in calves born from immune cows. Calves with low residual titers of colostral antibody may have an active response to vaccination (34), but it is questionable whether this serves any useful purpose. If vaccination of the dam before conception is the vital part of the program, the vaccination of calves may be unnecessary until they approach breeding age. There is no evidence that postnatal primary infection with BVDV will cause MD in immunocompetent calves.

A final precaution is to prevent cows or heifers from making new contacts shortly before or during the first half of pregnancy. It should be emphasized that control of the infection, and of MD, depends entirely on control among the breeding stock. Infection among nonbreeders is of no long-term consequence except in so far as they may be a source of infection to breeders and compromise the continuing freedom from infection of that group. CVJ

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