CASE REPORT

Concurrent Bovine Virus Diarrhea and Bovine Papular Stomatitis Infection in a Calf

J. G. BOHAC AND W. D. G. YATES*

Summary

A case of concurrent infection with the viruses of bovine virus diarrhea and papular stomatitis in a calf is reported. The difficulties posed by such situations are described and the criteria used for diagnosis outlined. The two diseases are reviewed briefly and the possible mechanisms whereby bovine virus diarrhea virus is suspected of facilitating infection by other agents are discussed.

Résumé

Rapport de la présence simultanée de la diarrhée à virus et de la stomatite papuleuse, chez un veau

Les auteurs rapportent qu'un veau souffrait à la fois de diarrhée à virus bovine et de stomatite papuleuse. Il décrivent les difficultés que présente une telle situation et énumèrent les critères qu'ils utilisèrent pour poser leur diagnostic. Ils présentent aussi une brève revue des deux maladies et commentent les mécanismes plausibles grâce auxquels le virus de la diarrhée à virus bovine faciliterait l'infection par d'autres agents infectieux.

Introduction

Bovine virus diarrhea (BVD) is described as the most widespread and among the most economically significant bovine diseases characterized either primarily or secondarily by enteritis (21). It is caused by strains of an RNA virus of the non-arbo togavirus group. Cattle and deer are infected naturally, whereas goats and sheep can be infected experimentally. Bovine virus diarrhea has been reported from Canada, the United States, Europe, Africa, India, Australia and New Zealand (3). There are five forms of the disease (20): (a) the acute form characterized clinically by fever, leukopenia, anorexia, diarrhea, skin lesions, and oral erosions or ulcers, and pathologically by erosions or ulcerations throughout the alimentary tract; (b) subclinical BVD, with serum neutralizing antibodies but no disease; (c) chronic infection with or without serum antibody and characterized by interdigital hyperkeratosis, oral ulceration, diarrhea, and progressive emaciation; (d) an intrauterine form which, depending on stage of gestation, may result in fetal deformities such as cerebellar hypoplasia and (e) immune tolerance with persistent infection, where the animals have no serum antibody and shed virus in their semen and nasolacrimal secretions. Virus can also be isolated from leukocytes in these animals.

Bovine papular stomatitis (BPS), also an infectious disease, does not usually cause systemic effects in the host. It is not thought to be of economic importance and concern only occurs when it causes difficulties in diagnosing other more serious or exotic diseases such as rinderpest, foot and mouth disease, vesicular stomatitis, etc. (9). There has been considerable confusion between BPs and reports of ulcerative stomatitis, proliferative stomatitis, erosive stomatitis and muzzle disease, but there is increasing agreement that these conditions are probably clinical variations of BPS (4, 28, 33).

The etiological agent of BPS is a DNA virus classified as a parapoxvirus (formerly paravaccinia virus). As such it is included as one species within the genus Parapoxvirus, the others being pseudocowpox (milker's nodules), contagious ecthyma (orf), and seal pox viruses (4, 23, 24, 31). It is considered possible that the etiological agents of BPS, pseudocowpox, and contagious ecthyma are merely strains of one virus (24).

Bovine papular stomatitis occurs in Canada, U.S.A., Mexico, U.K., Europe, Africa, Australia and Asia (1, 4, 17). It is a disease of cattle, although incidental transmission to humans has been reported (1, 5). In cattle, BPS is often subclinical but may be associated with mild fever. Characteristic raised lesions (papules) are found on the muzzle, buccal mucosa and external nares (2) and can also be seen either grossly or histologically in the tongue, esophagus, reticulum, rumen, omasum and skin (11).

Fraser and Savan (9) stated that uncomplicated cases of BPS should be readily diagnosed, but other concurrent disease problems could pose diagnostic difficulties. This report describes a case of concurrent BPS/BVD virus infection.

Case History

During the summer of 1979, regulatory veterinarians were asked to examine a calf that was showing clinical signs suggestive of foot and mouth disease. The animal, a Hereford crossbred steer weighing approximately 200 kg, had been purchased three months earlier at an auction market and it had never been considered healthy enough to be pastured with the other 90 steers on the farm. It was confined to a two-acre pasture shared with two healthy horses. The other steers on the farm remained healthy except for one which died suddenly of suspected bloat.

Clinical examination revealed a moribund, emaciated calf with normal temperature, pulse and respiration. Corneal ulcers and keratitis were present. Lymph nodes were normal on palpation. There were extensive raised, hypertrophic lesions of the oral mucosa, muzzle, and skin of the coronary band, scrotum and prepuce. The calf had suffered from profuse diarrhea for the preceding ten days. Treatment was not attempted and the animal was euthanized.

Necropsy Findings

A postmortem examination was

*Agriculture Canada, Animal Diseases Research Institute, P.O. Box 640, Lethbridge, Alberta, Canada T1J 3Z4.

performed in the field and tissues were submitted to this laboratory. The entire muzzle was covered by a thick, dry crust of exudate and necrotic debris containing fly larvae. Lesions similar in texture to these but roughly spherical and about 2 cm in diameter were present on the scrotum. Skin proximal to the coronary bands was affected similarly. Individual round, raised (papular) lesions up to 3 cm in diameter were present on the lips, gums and hard and soft palates. Involvement of the tongue included a single papule on the dorsal aspect and ulcers at the tip and lateral surfaces. There was a severe catarrhal rhinitis. The esophagus contained two types of lesions: some were linear erosions while others were proliferative and resembled those in the mouth. Similar proliferative lesions were present on the mucosal surface of the rumen (Figure 1.) Incidental findings included hepatic abscessation and pulmonary congestion.

Laboratory Examinations

Histological sections, prepared by conventional techniques and stained with hematoxylin and eosin, revealed no abnormalities in brain, kidney, liver, popliteal and bronchial lymph nodes, adrenal gland or urinary bladder. The lungs were somewhat congested. Sections of heart and tongue contained numerous cysts presumed to be *Sarcocystis* spp. There was no lymphoid necrosis or vasculitis in any of the tissues.

The proliferative lesions seen on skin surfaces (scrotum, teat areas, coronary bands and muzzle), tongue, esophagus and rumen were similar in character but different in degree. In general, there was increased thickness of the stratum spinosum and stratum granulosum, with ballooning degeneration of the composite epidermal cells. Large intracytoplasmic inclusion bodies, particularly numerous and prominent in ruminal lesions (Figure 2), were also present in the teat area, tongue and skin lesions at the coronary band. Mixed inflammatory cells, some of them degenerate, were usually present in affected layers, especially if the overlying stratum corneum had been damaged. Ulcerative lesions in the esophagus and tissues from the digestive tract distal to the rumen were not available for histological study.

Tissue cultures of secondary bovine fetal skin cells were used to detect the presence of viruses in the lesions. Cytopathic effects (CPE) were present on day 7 after infection with an inoculum prepared by pooling lesions of the scrotal skin, coronary band, muzzle, tongue and palate. Cytopathic effects occured on day 8 after infection with a pool of tissue lesions from the mucosal surfaces of the esophagus and rumen. Electron microscopic examination, by negative staining with 2% phosphotungstic acid, was conducted on infected tissue culture material following ultracentrifugation. This revealed a mixed population of virions, including large particles approximately 140 x 280 nm, having the spiral "ball of wool" appearance typical of parapoxviruses (3) (Figure 3), and smaller, spherical particles (Figure 4) resembling the *Togaviridae*, of which BVD virus is a member (12, 13, 34). The togaviruses were of two sizes: complete virions, approximately 50 nm in diameter; and core particles, 35 nm in diameter.

Sonication and density gradient centrifugation studies of the cellular fraction of the harvested tissue cul-

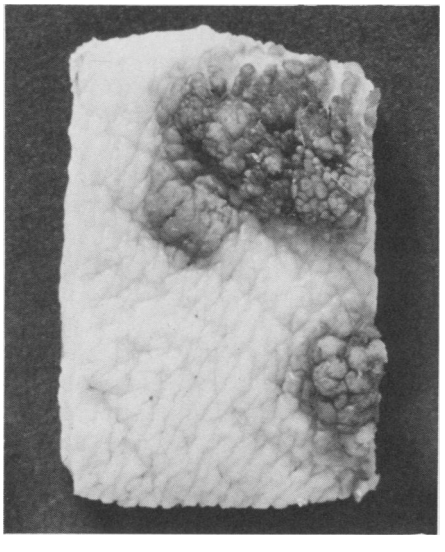

FIGURE 1. Focal, raised lesions on the mucosal surface of the rumen. By definition, the smaller lesion is a papule whereas the larger one is a plaque or nodule.

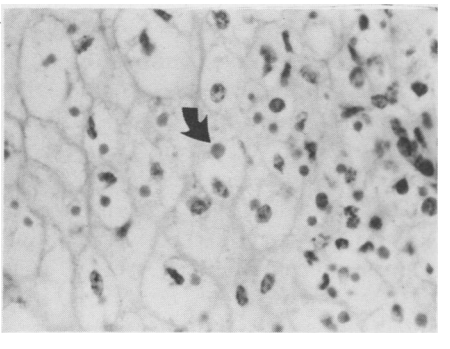

FIGURE 2. Ballooning degeneration and eosinophilic, intracytoplasmic inclusion bodies (arrow) in a papular lesion of the rumen. H. & E. X400.

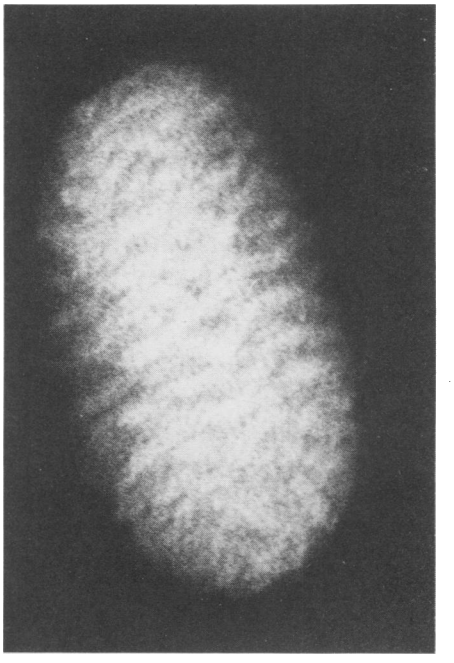

FIGURE 3. Typical "ball of wool" morphology of the parapoxvirus isolate, BPS virus, prepared from third tissue culture passage. Negative stain with 2% phosphotungstic acid (PTA). X250 000.

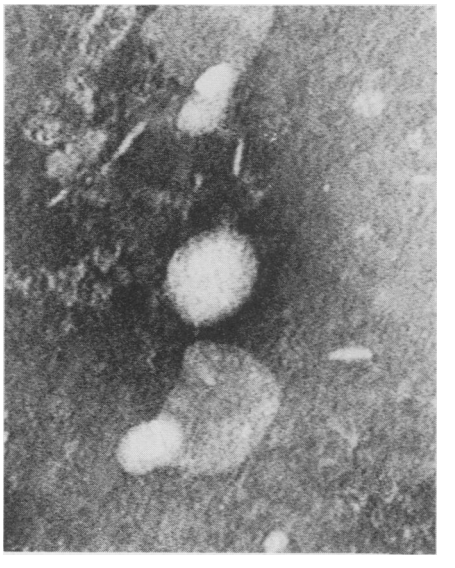

FIGURE 4. Approximately 60 nm diameter viral particle, suggestive of BVD, prepared from third tissue culture passage. Negative stain with 2% PTA. X200 000.

tures resulted in defined bands at 1.15 g/mL and 1.25 g/ mL. These correspond to published values for BVD virus and poxviruses respectively (8, 25).

Homogenized material from lesions was injected intradermally into guinea pig foot pads. This resulted in redness and heat but not in lesions suggestive of foot and mouth disease. After 11 days, the guinea pig serum showed two distinct precipitin lines when tested against a reference strain of BVD virus[1] and the parapoxvirus antigen obtained from the 1.25 g/mL density band described above. This indicated that foot pad injections of the crude homogenate had resulted in an immunological response by the guinea pigs to BVD virus and to the parapoxvirus present in the inoculum from the calf's pooled lesions. The precipitating antibody test was used since detectable neutralizing antibodies are not formed after parapoxvirus infection (31).

Serological tests for BVD and bluetongue antibodies in the calf's serum were negative.

Discussion

Evidence has been presented that this calf suffered from simultaneous infection with the viruses of BPS and BVD. The facts supporting a diagnosis of BPS included: (a) clinically evident proliferative and papular lesions of the muzzle, upper digestive tract and various areas of skin, (b) intracytoplasmic inclusion bodies in epithelial lesions of the rumen, tongue and other sites, (c) ultrastructural demonstration of typical parapoxvirus particles and (d) a buoyant density of 1.25 g/mL which is again typical of the poxvirus group. Findings supporting the presence of BVD virus were: (a) clinical signs of diarrhea, keratitis, and erosions of the tongue, (b) linear erosive lesions in the esophagus, (c) demonstration of particles suggestive of *Togaviridae* by electron microscopy and (d) a buoyant density of 1.15 g/mL, typical of BVD virus.

There are several diseases of cattle which are manifested by oral lesions, and this may make differential diagnosis somewhat difficult (2, 15, 29). In this case, consideration was given to a range of diseases, including BVD, BPS, malignant catarrhal fever, bluetongue, foot and mouth disease, vesicular stomatitis, and rinderpest, although the history and morbidity allowed provisional elimination of most of the exotic diseases. An assumption that a single etiological agent was involved would have led to an impossible conflict of clinical signs, lesions, and laboratory findings in attempting to arrive at a diagnosis. This case serves as a reminder of the possible occurrence of combined infections and encourages clinicians and pathologists to be cautious in situations where disease does not follow typical patterns. Bovine papular stomatitis can be especially confusing, because both erosive and ulcerative as well as papular lesions can occur in pure BPS infections (10, 30). Furthermore, new lesions can occur throughout the prolonged course of the disease (10, 17, 36).

Bovine virus diarrhea has been suggested to interact with other conditions, such as neonatal colibacillosis (21) and acute helminthiasis (42), and there is a commonly held view that it may play a role in predisposing to feedlot respiratory disease.

Fraser and Savan (9) commented that diagnosis of BPS in uncomplicated cases does not pose a problem but difficulties could arise if enteritis was concurrent. Plowright *et al* (32) have reported and quoted other authors on the interactions of BPS with rinderpest, cutaneous streptothricosis, bluetongue and other diseases.

It is a relatively common occurrence for more than one pathogen to infect a herd of animals at one time, but clinical manifestation of two simultaneous viral infections in a calf is reported rarely. Dual infections of tissue culture cells can occur under laboratory conditions, although one virus usually suppresses the other (7).

Many virus infections of animals and man can affect lymphocyte reactivity (14, 26) and a great deal has been said and written in recent years about the putative immunosuppressive role of BVD virus. Recovery from virus infections is thought to depend on the cell mediated immune system (26) and it has been stated that chronic BVD virus infection always causes depression of host cell mediated immunity (CMI) (26). Animals for which BVD infection is fatal are those which, in addition to having depressed CMI, cannot mount a humoral response due to decreased B cell function (19, 26, 27). The animal under discussion was shown to have no serum antibody to BVD and yet to be infected with the virus. Thus, the calf probably fitted into either the chronic infection or immune tolerance categories mentioned previously. It has been stated that secondary infections with bacteria or other viral agents may be the consequence of such BVD infections (22). Another factor of possible relevance may be that BVD infection of tissue cultures has been shown to suppress interferon production (22). It is interesting that the humoral immune failure of some BVD-infected animals can be limited to that virus, since some such animals have been found with titers to other agents, such as infectious bovine rhinotracheitis virus (32). It is known, however, that animals capable of mounting humoral defences against BVD are protected from infection (16, 35) probably for life (35) and that the antibodies usually appear within 21 to 28 days postinfection (19). It is still a source of debate, however, whether BVD-related immunosuppression is strictly the effect of infection with the virus or whether immunosuppression by another factor permits secondary BVD infection (27). Coria and McClurkin (6) discuss possible mechanisms that might explain an animal's failure to produce antibodies, such as tolerance, immune paralysis, and unresponsiveness.

In the case described, it is possible that the calf was chronically infected with BVD and that this contributed to severe and prolonged infection by BPS virus. The observation that no problem was noted in the other animals on the farm suggests that this calf was individually incapable of an appropriate protective immune response to the infecting agents.

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[1]NADL strain, National Animal Disease Laboratory, Ames, Iowa.

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