Differential Diagnosis of Unusual Viral Diseases

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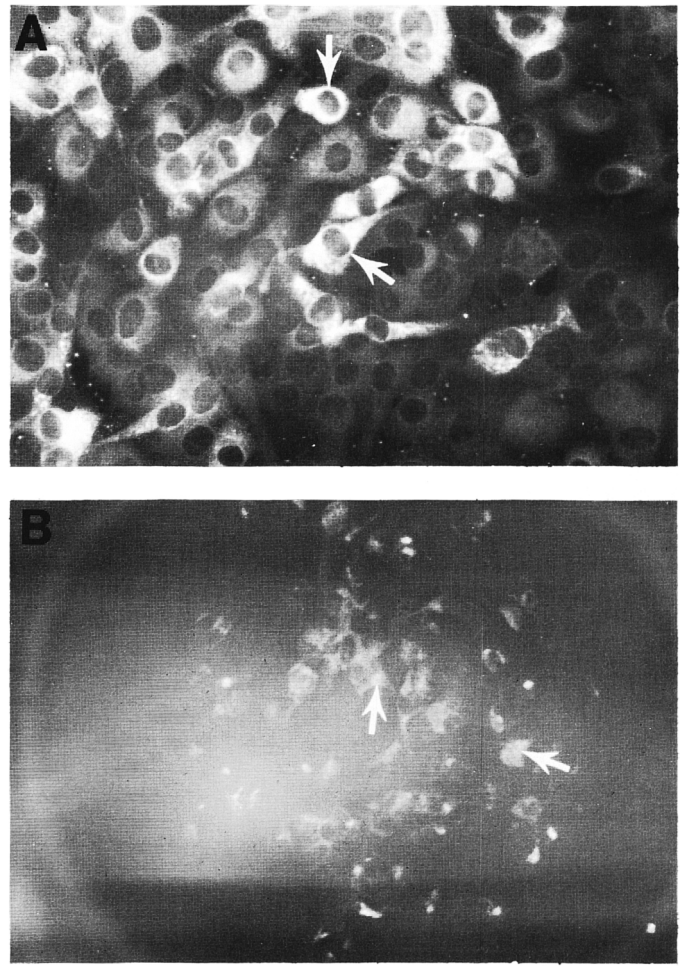
Most viral diseases have certain characteristic features that enable veterinary practitioners to establish a preliminary diagnosis at the clinical level (1,2). To the veterinarian, the most important features of an animal virus are its virulence and pathogenicity (3). In many instances, it is essential that a viral diagnosis be established by the isolation and characterization of the putative virus. By serologic techniques, a viral agent can be implicated as the cause of a disease if paired sera collected 10 to 21 days apart demonstrate a rise in titer to the test virus (4,5,6,7). Occasionally, certain viral agents can be diagnosed serologically by the use of a single serum sample (e.g., bovine leukemia, bluetongue) because these viruses usually persist in the host and the concomitant presence of antibody is usually indicative of infection (8). Sometimes the serologic result indicates a previous exposure to virus or vaccination and clinically may not reflect the current disease status of the host and thus does not implicate the virus as the cause of disease (4).

Because the methods for the isolation and identification of viruses usually require from several days to weeks, a laboratory diagnosis for a specific virus may not influence the immediate course of treatment in an individual animal (4,8). Certain viral diseases, however, can be diagnosed within 24 to 48 hours by use of fluorescent antibody stain on nasal scrapings, conjunctival smears, blood smears or on frozen tissue sections (3,4). This fluorescent antibody procedure can also be done on viral-infected cell cultures for the identification of a virus (Figure 1). The immunofluorescent procedure is rapid and specific, as in the diagnosis of rabies by the detection of viral antigens in affected brain tissue. However, the drawbacks to immunofluorescence as a diagnostic tool are the plethora of viruses and the availability of fluorescein-conjugated antibodies specific for each viral-induced disease. Another rapid diagnostic procedure is the enzyme-linked immunosorbent assay for the detection of antigens of rotavirus in feces from calves (9,10); however, even with these rapid procedures, most viral diagnoses are in retrospect (4). Furthermore, most viral diagnostic results arrive to the veterinarian usually after the animal has died or recovered (4,8).

Therefore, why identify viral agents? Virus identification should be considered in the establishment of the etiology of an unknown disease, in herd health programs, in public health problems, and in the determination of serologic types in viral-caused epidemics (4,11,12). In addition, in situations where serology fails to identify a viral agent due to cross reactions, other diagnostic methods must be employed to determine the identity of the virus.

Because groups of viruses have certain common morphologic characteristics (Figure 2), the visualization by electron microscopy (EM) of a viral particle in animal

Figure 1. Specific immunofluorescence (arrows) in cell cultures for viral antigens. (A) Cytoplasmic fluorescence in fetal bovine lung cells produced by BVD virus; (B) Nuclear and cytoplasmic fluorescence in fetal bovine lung cells produced by IBR virus.



Paper presented at the 16th Annual AABP Convention, Oklahoma City, on November 30, 1983.

Figure 2. Morphology and size of certain viruses (arrows) which affect the bovine. (A) Rotavirus (65 nm); (B) Bluetongue (Orbivirus) (70 nm); (C) Herpesvirus of MCF (100-240 nm); (D) Bovine parvovirus (20-22 nm).

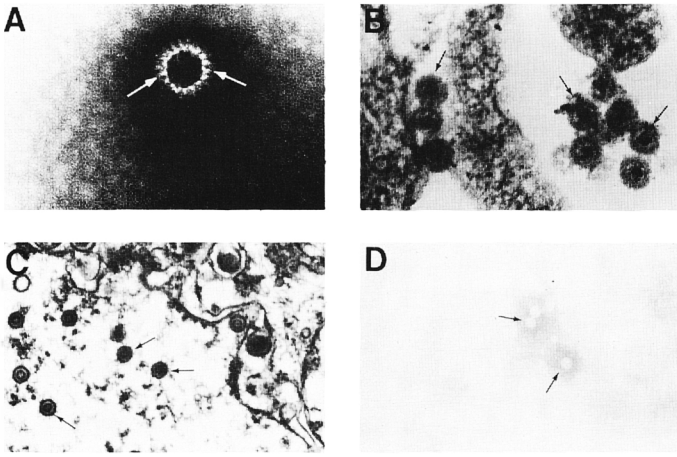
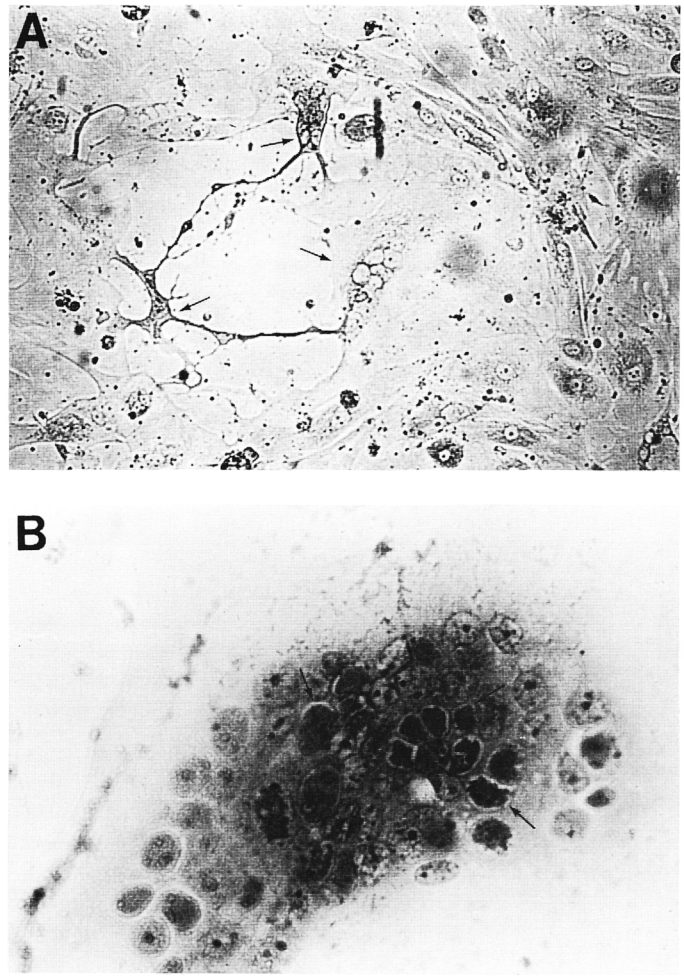


Figure 3. Cytopathology in fetal bovine kidney cells produced by MCF virus. (A) Syncytia (arrows) and refractile cells; (B) Intranuclear Cowdry type "A" inclusions (arrows).



tissues, secretions, excretions, or in cell cultures has become a routine diagnostic procedure (5,13,14). The determination of the morphologic structure of a virion by EM is an aid in the establishment of a differential diagnosis (3,6,13,14). However, EM has certain limits for detection of viruses since the minimum number of particles which can be observed by EM is 10^5 particles/ml with an increased rate of detection at virus concentrations of 10^6 to 10^8 particles/ml (15). In most viral diseases, concentrations of virus of 10^5 particles/ml are not usually present; however, virus detection by EM is enhanced by the technique of immune EM or the addition of specific antibody to a virus preparation (5). The immune EM technique has been used in the identification of the surface antigen of hepatitis A virus in humans (6).

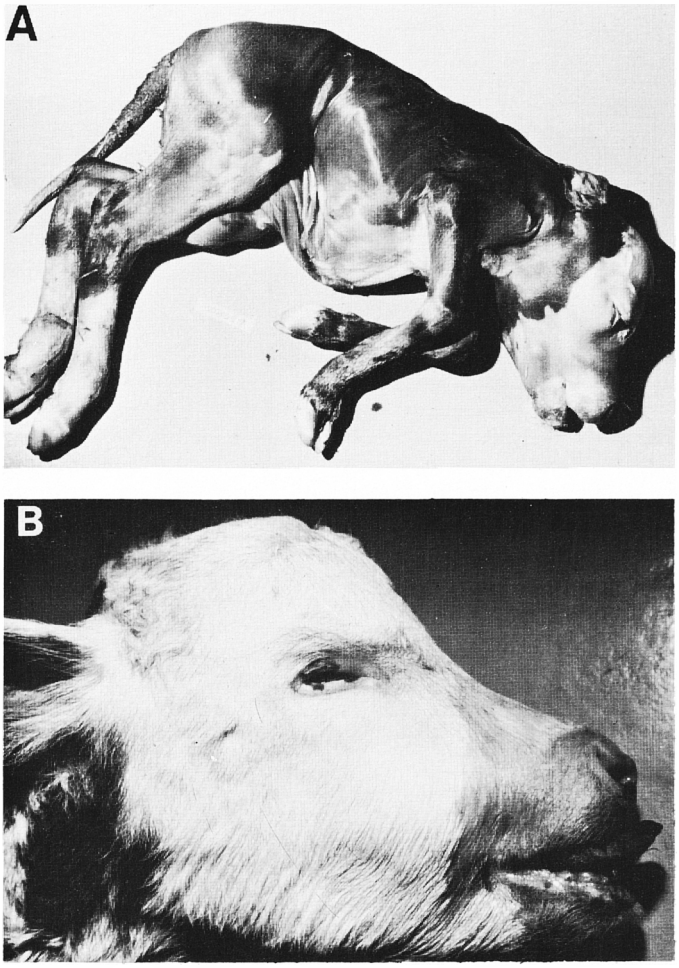
Other microscopic techniques can be used in the identification of viruses which include the visualization of the cytopathic effects (CPE) of virus in susceptible cell cultures (3,6,14). Also, certain groups of viruses have characteristic CPE (Figure 3A) which aids in their identification. Cytopathic changes like inclusions in cells (Figure 3B) are a key in the diagnosis of several viral-induced diseases (e.g., rabies, poxvirus, herpesvirus). However, virus-produced inclusions are usually transient and require experience in recognition in both tissues and cell cultures (4).

Viruses can multiply only in living cells and disease arises from cell damage associated with the intracellular replication of the virus. Hence, the cellular changes produced by a virus dictate the methodology used in diagnostic laboratories for virus isolation (3,6). Virus isolation is necessary in undescribed disease, in acute disease with death, in situations where viruses of multiple antigenic type are present, and in exotic or foreign animal diseases (3). The isolation of a virus does not in itself constitute a diagnosis but must be related to the clinical signs of disease (1,3).

Embryonated chicken eggs, living cell cultures, and animal inoculations provide the known vehicles for the isolation of viruses. The verification of a virus isolate as the etiology of a specific animal disease usually requires experimental reproduction of disease in a susceptible host and reisolation and serologic identification of virus, thus fulfilling Koch's postulates. Many clinical diseases such as fetal developmental anomalies of calves require that a multifactorial virus etiology be given consideration (e.g., bluetongue, bovine viral diarrhea and Akabane viruses). Certain reported lesions (16,17) in the bovine fetus can be used in the diagnosis of a bluetongue-caused infection (Figure 4A, B). Nevertheless, final confirmation of the presence of bluetongue virus or other viruses in the tissues of the fetus requires virus isolation and identification (18).

To make a virus diagnosis requires an understanding of the pathogenesis of disease and the associated clinical, epidemiologic and laboratory procedures to confirm a diagnosis (1,3,7). Diagnosis of a virus infection requires a

Figure 4. Developmental anomalies in a bovine fetus produced by bluetongue virus. (A) Stillborn fetus with arthrogyprosis (contracture of limbs); (B) Calf with domed cranium and prognathism.



complete history with information pertaining to age, breed, vaccination of the animal, clinical signs, type of lesions observed on gross necropsy, and the number of deaths in the herd. Futhermore, a list of the clinical specimens collected and appropriately labeled (Figure 5) should be included with each submission. The early collection of clinical specimens is vital in the isolation of viruses. Since many viral agents are susceptible to changes in temperatures, packing with wet ice is recommended when sending a specimen to the laboratory (3,4). A telephone call will assist in alerting the diagnostic laboratory in the preparation of appropriate procedures for the identification and isolation of virus from the submitted tissues or intact animal.

Because of the different procedures used for the isolation and identification of viruses, the approach used for the diagnosis of the virus of malignant catarrhal fever will be presented as a model for handling an unusual viral disease.

Diagnosis of Malignant Catarrhal Fever: isolation and identification of virus

Malignant catarrhal fever (MCF) is a viral disease of domesticated and exotic ruminant species. This viral disease has been characterized as sheep-associated MCF and wildebeest-associated MCF (19). The diagnosis of MCF of cases described in the United States had been done by clinical signs and histopathic lesions in tissues (19,20). The clinical signs reported (21,22) include a generalized lymphadenopathy, leukopenia, necrosis of the oral mucosa (Figure 6) and nasal cavity, high fever, a profuse nasal discharge, ophthalmia and corneal opacity (Figure 7B).

The etiologic agent of MCF was isolated in Africa from a blue wildebeest (Alcelaphine species) by Plowright et al. (23). Although a similar disease entity has been described in cattle in Europe and the United States as sheep-associated MCF, a virus has not been isolated from any clinical case (20,21,22).

In 1981, the first isolation and identification of MCF virus in the United States was made from a clinically ill gaur *Bos gaurus,* an exotic bovine housed within the Oklahoma City Zoo (24, 25).

Figure 5. A typical packet for the submission of clinical specimens to the diagnostic laboratory for virus isolation.

Virus isolation was made from cultures of buffy coat cells separated from heparinized blood collected from the gaur at the peak (40.5° to 41.1°C) of the febrile period (25). Malignant catarrhal fever was suspected in the gaur herd because another gaur had previously died and MCF was diagnosed by the histopathic lesions (vasculitis) seen in the tissue of the gaur. The cocultivation of buffy coat cells from the gaur with either bovine fetal kidney or thyroid cells produced CPE typical for the herpesvirus of MCF. The CPE of MCF virus was characterized by the formation of syncytia or multinucleated giant cells and production of Cowdry type A intranuclear inclusions (Figures 3A, B). Although the CPE in cell cultures suggested a herpesvirus (26), other possible herpesviruses of the bovine that were considered included: bovine cytomegalovirus; DN599, a respiratory virus of cattle; and infectious bovine rhinotracheitis virus.

The process in the diagnosis of MCF virus included the

preparation of virus-infected cells for observation by EM. Thus, by a determination of the morphology and size of the virus isolate and the formation of virus in the cell nucleus, a herpesvirus was confirmed (Figure 2C). Further identification of the isolate as MCF virus required the serologic identification of the virus by antiserum against a reference strain (WC 11) of MCF virus. By indirect immunofluorescence using the reference antiserum to the WC 11 strain of MCF virus, the virus isolated from the gaur was found to be serologically related to wildebeest-associated MCF virus (24, 25).

Because viruses by serial *in vitro* passages in cell cultures usually lose their virulence for their animal hosts, certain passages of the MCF virus isolated in bovine cell cultures were inoculated intravenously into susceptible ruminant hosts to reproduce clinical MCF (24,25,27). Since MCF virus was a potential danger for the cattle population in Oklahoma, all animal transmission studies were done in an animal facility classified as a P3 isolation unit.[a] Because the mode of transmission for MCF virus was also unknown, additional precautions were instituted to restrict access of personnel to the isolation building. Furthermore, fecal and urine waste from each inoculated animal was collected and disinfected by autoclaving. Disposable garments were also used by all personnel handling MCF-inoculated animals. These precautions should be routinely instituted in transmission studies with virulent viruses and in situations where susceptible domestic species have never been previously exposed to exotic viruses.

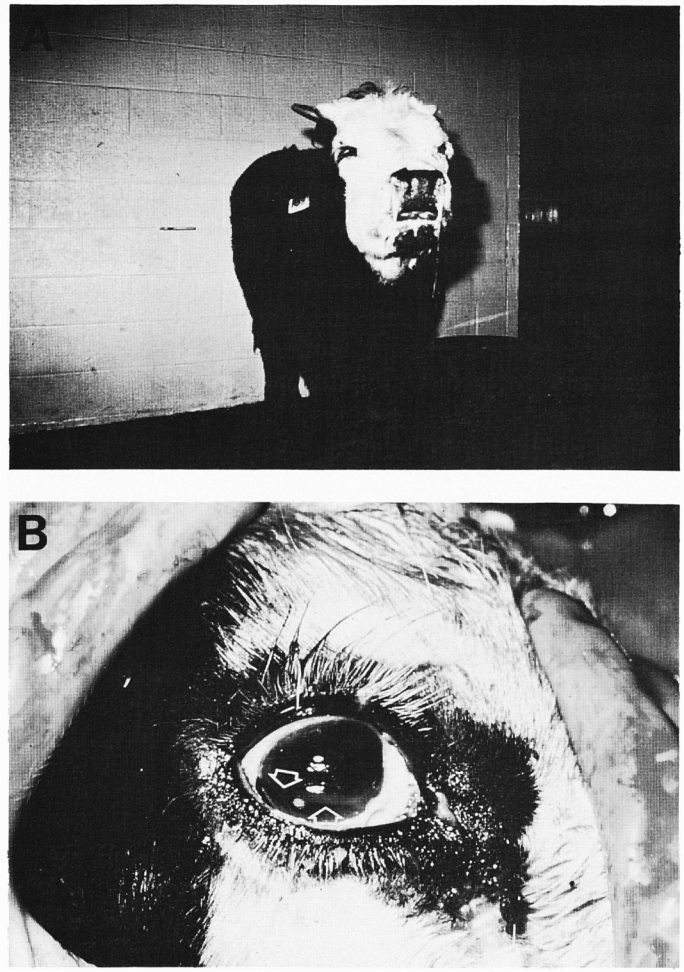
By these transmission studies (24,25), we found that the inoculated animals became ill at 16 to 18 days with clinical signs typical for MCF (Figure 7A) and the lesions seen on gross necropsy were also typical for MCF (Figures 6,7B). The tissues from the experimentally inoculated animals, when examined by histopathology, contained a vasculitis which was compatible with the lesion reported for an MCF virus infection (20,21). The virus isolated from an experimentally inoculated heifer was characterized as MCF virus by procedures which fulfilled Koch's postulates (25).

The methodology described for the isolation and identification of MCF virus was time-consuming and required elaborate laboratory procedures for isolation, transmission, and the elimination of other adventitious viruses. The identification procedures in these studies on MCF necessitated that other known viruses present in the experimentally inoculated hosts be ruled out by either serology or cell culture isolation.

For the veterinary practitioner, the diagnosis of similar diseases will usually not aid in the treatment of the affected individual animal. However, the establishment of a diagnosis of an unusual viral agent in a herd will enable the development of an effective program of prevention and

Figure 6. Erosions on hard palate of a bovine inoculated with MCF virus.

Figure 7. Malignant catarrhal fever in a heifer. (A) Nasal mucopurulent catarrhal and depression in experimentally infected animal; (B) Corneal opacity (arrows) produced following infection by MCF virus.



[a] *Richardson, H. J., Barkley, W. E., 1983, Proposed Biosafety Guidelines for Microbiological and Biomedical Laboratories. Manual of the U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia.*

control. Furthermore, the knowledge established in the confirmation of a diagnosis of an unusual virus provides for approaches to develop diagnostic reagents for future outbreaks of a similar nature.

The purpose of this presentation was to provide an insight into a possible approach in the diagnosis of an unusual viral disease and to alert veterinarians of the virologic facilities available in many diagnostic laboratories. The cooperation engendered between each veterinarian and personnel in a diagnostic laboratory should provide a confirmation of a viral diagnosis and also offer approaches in the prevention and control of unusual viral problems which can affect our domestic cattle.

Acknowledgements

The author thanks the staffs of the Oklahoma Animal Disease Diagnostic Laboratory and the Oklahoma City Zoo for their support and assistance in these studies and Dr. Robert W. Fulton of the Department of Microbiology, Parasitology, and Public Health, College of Veterinary Medicine, Oklahoma State University, for the electron photomicrograph of rotavirus.

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