Comparison of serologic techniques for the detection of antibodies to malignant catarrhal fever virus

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Malignant catarrhal fever (MCF) is an acute viral disease of domestic and exotic ruminants. It has a worldwide distribution and is generally a sporadic disease with low morbidity and high mortality (Plowright, 1968). Historically, MCF has been separated into two epidemiological forms, a sheep-goat-associated form and a wildebeest-derived form based on reservoir host and ease of experimental transmission (Daubney and Hudson, 1936). On a clinicopathologic basis the two forms are indistinguishable (Goetze and Liess, 1930, cited by Daubney and Hudson, 1936). The etiological agent for the sheep-goat-associated form has not been determined. Based on serological evidence, Rossiter (1983) hypothesized the causative agent to be a cell-associated herpesvirus antigenically related to the cell-associated herpesvirus, alcelaphine herpesvirus-1 (AHV-1), which is the etiological agent for the wildebeest-derived form of MCF (Plowright et al., 1960).

Malignant catarrhal fever is classified as an exotic disease even though both forms of the disease have been diagnosed in the United States. Accurate diagnosis of exotic diseases is critical to the protection of our domestic livestock. Diagnosis on a clinical basis is not reliable, as MCF resembles other viral diseases including infectious bovine rhinotracheitis, bovine virus diarrhea-mucosal disease, bluetongue, vesicular stomatitis, and rinderpest (Heuschele and Castro, 1984). Gross pathologiclesions are not distinctive (Jubb et al., 1985). Histopathological diagnosis of MCF is considered reliable due to the presence of characteristic lesions consisting of a disseminated fibrinoid

vasculitis with adventitial accumulation of primarily mononuclear cells in virtually all cases of the disease (Jubb et al., 1985). Standard virus isolation techniques are not practical due to the cell-associated nature of the virus. Viral infectivity depends on the maintenance of tissue cell viability, which is destroyed by freezing and post mortem autolysis (Piercy, 1953). Low tissue viral antigen levels (Rossiter, 1980a; Rossiter, 1980b; Patel and Edington, 1980) makes diagnosis of MCF using electron microscopy for detection of virions or immunofluorescence on frozen sections of tissue unreliable.

In order to provide laboratory diagnostic assistance for MCF diagnosis, studies were initiated to compare serologic assays for detection of antibodies to MCF virus. Three serologic assays, the indirect immunofluorescence assay (IFA), the virus neutralization assay (VN), and the enzyme-linked immunosorbent assay (ELISA) were compared.

LITERATURE REVIEW

History

In the Masai Reserve of Kenya and Tanzania, the Masai were the first to recognize a connection between wildebeest and a disease in cattle, "snotsiekte", which occurs annually during the wildebeest calving season. The Masai believe cattle become infected from grazing pastures contaminated by wildebeest after-birth or hair shed by young wildebeest calves (Daubney and Hudson, 1936).

In South Africa, Mettam, in 1923, published the first description of "snotsiekte" which he believed was a separate clinical entity from the disease in European cattle known as malignant catarrh (cited by Daubney and Hudson, 1936). "Snotsiekte" occurred in cattle grazing common pastures with wildebeest which he hypothesized served as reservoir hosts for the etiological agent. Transmission of "snotsiekte" from a black wildebeest (Connochaetes gnou) to a calf by injection of whole blood substantiated this hypothesis. Mettam, 1923, was the first to transmit "snotsiekte" between cattle by inoculation of large volumes of fresh blood. He suggested that blood-sucking vectors might play a role in the natural transmission of the disease, but was unsuccessful in his attempts to transmit the disease using cattle lice or wildebeest lice. Contact spread between cattle did not occur (cited by Daubney and Hudson, 1936).

In Hannover, Germany, research on malignant catarrh, a clinical entity recognized in Europe for over one hundred years, was stimulated by Dobberstein's findings in 1925 of characteristic infiltrative lesions in the brain of cattle dying from the disease (cited by Daubney and Hudson,

1936). Glamser, 1926, confirmed Dobberstein's observation. Goetze and Liess, in 1929, successfully transmitted malignant catarrh between cattle by inoculation of fresh blood, but failed to transmit the disease with brain, aqueous humor, or lymphatic tissue. Natural transmission was suggested to occur via blood-sucking vectors, but attempts to transmit the disease with cattle lice by Goetze and Liess, 1929, and sheep keds by Goetze, 1930, were unsuccessful (cited by Daubney and Hudson, 1936). Goetze and Liess, 1929, could not demonstrate direct contact spread between cattle (cited by Daubney and Hudson, 1936). Transmission of the disease in cattle by blood inoculation was successful 40% of the time with incubation periods of 16 days to 10 months. The disease was not transmitted by filtered blood, centrifuged blood or blood collected more than a few hours before animal inoculation. Goetze and Liess, in 1929, failed to transmit the disease to mice, rats, guinea pigs, cats, dogs,and pigs (cited by Daubney and Hudson, 1936). Based on epidemiological evidence, Goetze and Liess, 1929, proposed sheep to be the reservoir host for malignant catarrh. Goetze, in 1930, suggested transmission to cattle in contact with placental or uterine discharge from lambing ewes (cited Daubney and Hudson, 1936).

Quinlan, who was in Hannover, Germany, during the transmission studies of Goetze and Liess drew their attention to the similarities between malignant catarrh and Mettam's findings on "snotsiekte" (cited by Daubney and Hudson, 1936). After reviewing Mettam's publication, Goetze and Liess, 1929, concluded that "snotsiekte" and malignant catarrh were a single clinical entity (cited by Daubney and Hudson, 1936). This view was

supported by Daubney and Hudson (1936) with the results they were obtaining from experimental transmission with both forms of the disease. Researchers have continued to separate the disease, now referred to as malignant catarrhal fever (MCF), into two epidemiological forms, the sheep-goat-associated form and the wildebeest-derived form based on reservoir host and ease of experimental transmission. Experimental transmission of MCF has been reported by many investigators (Appendix I).

Etiology

The etiological agent for the sheep-goat-associated form has not been identified. It is hypothesized, based on serological evidence, to be a cell-associated herpesvirus antigenically related to the AHV-1 viruses (Rossiter, 1983). A cell-associated herpesvirus was isolated from a clinical case of sheep-goat-associated MCF (Hamdy et al., 1978), but the isolate failed to induce clinical disease characteristic of MCF in cattle. A cytotoxic lymphoblastoid cell line (MF120) has been established from mesenteric lymph node cells of a rabbit (57th serial passage) with experimental sheep-goat-associated MCF (Reid et al., 1983). The MF120 cells have the morphology and functional characteristics of large granular lymphocytes (LGL). Intravenous inoculation of rabbits with as few as 10^2 of these cells induced MCF-like clinical disease (Reid et al., 1983). Viability of the LGL cells was critical to successful disease transmision suggesting a cell-associated etiological agent. No etiological agent has been identified in these cells using indirect immunofluorescence or electron microscopy (Reid et al., 1983). Other LGL cell lines have been isolated from tissues of cattle and deer with sheep-goat-associated MCF,

and from rats experimentally infected with wildebeest-derived MCF (Reid and Buxton, 1985). None of the other LGL cell lines have induced clinical disease in cattle, deer or rabbits. The use of molecular virologic techniques may be necessary to identify the causative agent (Heuschele et al., 1984a; Harkness, 1985; Plowright, 1986).

Development of primary bovine thyroid (BTh) cell cultures by Plowright and Ferris (cited by Plowright et al., 1960) made isolation of the etiological agent for the wildebeest-derived form possible. Prior to this, cattle inoculation was the only means for identification and maintenance of MCF isolates. Bovine thyroid cutures have equal sensitivity to cattle inoculation for MCF isolation (Plowright, 1968).

The etiological agent for the wildebeest-derived form was first isolated in BTh cultures from heparinized whole blood of blue wildebeest (Plowright et al., 1960). The agent was described as a filterable virus which would pass through a 300nm filter, but not a 275nm filter. The isolate was ether and chloroform senstive. Infectivity of the virus was inhibited by 5-iodo-2'-deoxyuridine. Electron microscopy revealed the virus to be a herpesvirus with 100nm nucleocapsids, and 140 to 240nm enveloped virions. Viral cytopathic effects (cpe) were characterized by the formation of small multinucleated, often vacuolated, syncytia. The isolate induced clinical disease characteristic of MCF in cattle with a mean incubation period of 20 days. Reisolation of the virus from infected cattle fulfilled Koch's postulates for identification of etiological agents (Plowright et al., 1963; Plowright, 1964). This established wildebeest as reservoir hosts for MCF.

Studies were initiated to determine the means of virus maintenance in wildebeest, and the natural modes of transmission from wildebeest to cattle (Plowright, 1965a; Plowright, 1965b). Plowright (1965a) detected viremia in 31% of wildebeest calves less than three months of age. Isolation of virus from a wildebeest fetal spleen, and from wildebeest calves less than one week of age suggested congenital transmission as a mode of virus maintenance in the wildebeest (Plowright, 1965a; Plowright, 1965b). Horizontal spread between wildebeest calves, and between wildebeest calves and cattle occurred with exposure to wildebeest calves less than three months of age which had persistent high level viremia (Plowright, 1965a; Plowright, 1965b). Contact spread rarely occurred with exposure to wildebeest calves over three months of age (Mushi et al., 1981c). Rweyemamu et al., in 1974, were the first to isolate MCF virus from the nasal secretions of wildebeest (cited by Mushi et al., 1980b). Mushi et al. (1980b) demonstrated that wildebeest calves less than three months of age shed cell-free virus in their nasal and ocular secretions. Viral shedding appeared to cease at approximately three months of age with the development of neutralizing antibodies in the nasal secretions (Mushi et al., 1980b). Mushi et al. (1981c) hypothesized inhalation or ingestion of cell-free virus-laden droplets produced by the snorting of young wildebeest calves as the mode of natural transmission to wildebeest calves and cattle. Virus shed in the nasal and ocular secretions of infected cattle was cell-associated (Mushi and Rurangirwa, 1981c) which would account for the lack of contact spread between cattle.

Reid and Rowe (1973) isolated a MCF-like virus (K/30) from a Cokes hartebeest (Alcelaphus buselaphus cokei). This isolate induced clinical disease characteristic of MCF in cattle. A cell-associated MCF-like virus was isolated from topi (Damaliscus lunatus korrigum) using topi cell cultures (Mushi et al., 1981a). This isolate failed to induce clinical disease in cattle or rabbits.

The terminology alcelaphine herpesvirus (AHV) was proposed by Reid et al. (1975) based on the classification of reservoir host species (wildebeest, topi, and hartebeest) as members of the subfamily Alcelaphinae. The designation of AHV-1 for wildebeest isolates, and AHV-2 for topi and hartebeest isolates has recently been proposed (cited by Heuschele and Castro, 1984). The AHV viruses have been further classified as members of the subfamily Gammaherpesvirinae based on the lymphotropism of the virus, latency, abscence of in vivo inclusion bodies, but development of Cowdry type A inclusion bodies and syncytia in cell cultures, and the highly variable incubation period (Heuschele, 1984).

Restriction endonuclease profiles of AHV-1 isolates demonstrated this group of viruses to be distinct from other bovine herpesviruses (Osorio et al., 1985).

Pathogenesis

The exact pathogenesis for MCF has not been determined. Plowright (1968) suggested an immune mediated hypersensitivity to the AHV-1 virus.

Rweyemamu et al. (1976) described MCF as an immune complex disease with circulating immune complexes comprised of AHV-1 infected lymphocytes or lymphocytes expressing virus induced plasma membrane antigens, specific

anti-AHV-1 immunoglobulins, and complement. Deposition of circulating immune complexes in blood vessel walls was hypothesized to cause Arthus type III vasculitis typical of immune complex diseases (Rweyemamu et al., 1976). Arthus type III vasculitis was also proposed as the basis for the pathogenesis of the sheep-goat-associated MCF (Selman et al., 1974). Many researchers have attempted to demonstrate immune complexes in MCF lesions in both cattle and rabbits with little success (Liggitt and DeMartini, 1980; Mushi and Rurangirwa, 1981b; Patel and Edington, 1982b). Patel and Edington (1982a) used anti-AHV-1 IgG ($F(ab)_2$) fragments to demonstrate virus induced antigens on the surface of lymphocytes expressing intracellular viral antigens by indirect immunofluorescence. They were unable to determine when in the virus replication cycle the surface antigens were produced or if latently infected cells expressed surface antigens. Patel and Edington (1982a) detected antibodies against the surface antigens in terminal sera of cattle and rabbits infected with AHV-1 virus. Attachment of these antibodies to the lymphocyte surface antigens did not result in immune cytolysis. When bovine, human, or guinea pig complement was added to the resulting antigen-antibody complex, immune cytolysis occurred. Rabbit complement did not cause immune cytolysis. Patel and Edington (1982a) from this work hypothesized that complement played an important role in MCF pathogenesis. Using indirect immunofluorescence, Patel and Edington (1982b) were able to demonstrate deposits of IgG-$C(_{3b})$-conglutinin (Cg) in renal arteries and glomeruli of AHV-1 infected cattle, but the complexes were not always associated with histologic lesions. Patel and Edington (1982b) were unable to demonstrate

viral antigens or AHV-1 specific immunoglobulins in any of the deposits. Only rarely could AHV-1 infected lymphocytes be detected in vascular lesions by indirect immunofluorescence. Clinically affected cattle were shown to have decreased conglutinin and complement (C_3) levels in the terminal stages of disease (Patel and Edington, 1982b). Patel and Edington (1982b) were unable to demonstrate virus induced transformation of lymphoid cells. Mushi and Rurangirwa (1981b) also found a 25-35% decrease in serum C_3 levels of cattle with terminal stage MCF. No change was detected in serum levels of hemolytic complement, IgM, IgG_1, or IgG_2. Mushi and Rurangirwa (1981b) demonstrated C_3 glomerular deposits by indirect immunofluorescence, but couldn't demonstrate AHV-1 specific immune complexes in vascular lesions or in circulation. Rabbits hyperimmunized with a virulent AHV-1 developed high titers of neutralizing antibodies, but were still susceptible to virulent AHV-1 challenge suggesting that serum antibodies do not play an important role in pathogenesis of the disease (Rurangirwa and Mushi, 1984). Based on these findings, Mushi and Rurangirwa (1981b) hypothesized that MCF was not a immune complex mediated disease, but had a cell-mediated immunopathologic (CMI) basis.

Further evidence for CMI pathogenesis was demonstrated by Mushi and Rurangirwa (1981d) with their work on delayed hypersensitivity (DTH) skin testing in AHV-1 experimentally infected rabbits. Intradermal injection of homologous virus antigen produced DTH nodules by 24 hours post-inoculation. Maximal DTH response occurred after 48 hours in rabbits injected during the seventh day of AHV-1 infection. Delayed

hypersensitivity did not occur in rabbits injected after the onset of pyrexia. Mushi and Rurangirwa (1981d) hypothesized that this failure of DTH resulted from AHV induced T-lymphocyte dysfunction or suppression of the cell-mediated immune system. Rurangirwa and Mushi (1982a) found decreased antibody response to sheep red blood cells (SRBC) in AHV-1 infected rabbits when they were inoculated with SRBC during the third to tenth day post AHV-1 infection. Parallel decreases in AHV-1 specific antibody was not seen. Since antibody production to SRBC requires B-lymphocytes, T-lymphocytes, and macrophage interaction, they hypothesized that the decreased antibody formation was related to T-lymphocyte dysfunction occurring between the second and fourth day of AHV-1 infection. Normal antibody production to the AHV-1 virus suggests that humoral initiation occurs before the T-lymphocyte dysfunction. Rurangirwa and Mushi (1984) treated rabbits with cyclophosphamide, which has a mitostatic effect on B-lymphocytes, before and during AHV-1 infection. The treated rabbits had milder lymphadenopathy, slower viremia development, delayed virus neutralization (VN) antibody response, and prolonged clinical disease with intermittent pyrexia. The mean incubation period of treated rabbits was not significantly different from the mean of control rabbits. This was taken as further evidence for a CMI pathogenesis.

Liggitt and DeMartini (1980) suggested a CMI reaction of the vascular endothelium similar to graft rejection or direct viral induced cytolysis for the pathogenesis of MCF. Viral cpe could not be demonstrated in tissues of animals infected with AHV-1 (Plowright et al., 1960; Straver

and Van Bekkum, 1979; Rossiter, 1980a). Buxton et al. (1984) could not demonstrate viral cpe or antigens in tissues of rabbits experimentally infected with sheep-goat-associated MCF.

Hunt and Billups (1979) described AHV-1 as a oncogenic herpesvirus. They hypothesized that the viral DNA was incorporated into the genome of infected lymphocytes or existed as episomal DNA causing lymphocyte transformation. This hypothesis was based on several factors, including lack of viral cpe in lesions, rare detection of viral antigen in tissues, the cell-associated nature of the virus, failure of contact spread between cattle, and the presence of Cowdry type A inclusions and viral cpe in cell culture systems similar to that seen with H. saimiri, H. ateles, Marek's disease virus, and Epstein-Barr virus. Virus induced transformation of lymphoid cells causing dysfunction of T-suppressor cells with subsequent T-lymphocyte proliferation and B-lymphocyte cytolysis was also hypothesized by other investigators (Edington et al., 1979, cited by Buxton et al., 1984; Denholm and Westbury, 1982).

Reid and Buxton, in 1981, suggested a pathogenesis based on virus induced dysfunction of the immunoregulatory system from their results with serial tranmission studies of sheep-goat-associated MCF in rabbits (cited by Buxton et al., 1984). Tissues from a red deer with sheep-goat-associated MCF were inoculated into rabbits inducing clinical disease suggestive of MCF (Buxton and Reid, 1980). The isolate was maintained by serial passages in rabbits using intravenous injection of washed lymph node cells (Buxton et al., 1984). Progression of lesions was studied. Lymphoid cell proliferation in the T-dependent areas of lymphoid

tissues and interstitial areas of non-lymphoid organs occurred from day three to day 13 post-infection, and was comprised of primarily T-lymphocytes (Buxton et al., 1984). Buxton et al. (1984) described the proliferation as hyperplasia since the structural architecture of the involved tissues remained unchanged. Cyclosporin A (20mg Kg^{-1}), a potent T-lymphocyte suppressor which prevents initiation of T-lymphocyte mediated immune reactions, was used in rabbits to demonstrate that suppression of the viral induced T-lymphocyte proliferation did not affect the incubation period, disease course, or development of tissue necrosis (Buxton et al., 1984). Based on this evidence, they hypothesized MCF pathogenesis to involve virus induced dysfunction of a subpopulation of T-lymphocytes termed large granular lymphocytes (LGL). Mesenteric lymph node cells from a rabbit with sheep-goat-associated MCF (57th serial passage) were treated with polyethylene glycol to fuse them with fetal ovine kidney (FOK) cells (Reid et al., 1983). The resulting cell monolayers degenerated leaving single and small clumps of lymphoblastoid cells (MF120) in the supernatant fluid. These cells had morphological and functional characteristics of LGL (rabbit karyotype) cells (Reid et al., 1983). The MF120 cells appeared very similar to lymphocytes generated from marmosets infected with H. ateles or H. saimiri suggesting a similar pathogenesis. Large granular lymphocytes could not be identified in MCF lesions (Reid et al., 1983). High levels of natural killer cell (NKC) activity were detected in experimentally infected rabbits during clinical disease, but not during the incubation period (Reid and Buxton, 1985). Rabbits, cattle, and deer with sheep-goat-associated MCF, and rats with wildebeest-derived MCF had

detectable levels of NKC activity (Reid and Buxton, 1985). Reid and Buxton (1985) hypothesized the LGL cells to be targeted by the MCF virus with integration of the viral DNA into the cell genome or existing as episomal DNA. They hypothesized that virus induced dysfunction of the LGL cells with subsequent production of interleukin-2 or other lymphokines was responsible for the benign polyclonal T-lymphocyte hyperplasia. Viral induced dysfunction of the NKC activity of the LGL cells with indiscriminant extension of NKC cytotoxicity to normal cells was hypothesized to be responsible for development of necrotic lesions, onset of clinical disease, and ultimately death (Buxton et al., 1984). This hypothesis for the pathogenesis of MCF could account for the long and variable incubation period, and the variable distribution and severity of histopathologic lesions seen with both epidemiological forms the disease (Reid and Buxton, 1985).

Clinical Disease

Goetze, in 1930, arbitrarily divided the sheep-goat-associated form into four clinical forms, namely, the peracute, the intestinal, the head and eye, and the mild form (cited by Daubney and Hudson, 1936). Considerable overlap occurs between the clinical forms. Daubney and Hudson (1936) observed that the wildebeest-derived form of MCF in cattle could be divided into the same clinical forms with the head and eye form being the most prevalent.

Clinical signs in the peracute form range from acute death without clinical disease to a brief clinical illness of one to three days

characterized by hyperthermia, mucosal hyperemia, and hemorrhagic gastroenteritis (Heuschele and Castro, 1984; Jubb et al., 1985).

The intestinal form is characterized by pyrexia, mucosal hyperemia, lacrimation, lymphadenopathy, and diarrhea generally ending in death within four to nine days (Heuschele and Castro, 1984; Jubb et al., 1985).

The head and eye form is the typical clinical syndrome with a clinical course of seven to 18 days. Mortality is generally greater than 95%. Pyrexia (104-107^{0}F) with progressive serous, mucoid to purulent nasal and ocular discharges are classical signs of this form. Muzzle and nasal encrustation, often with secondary excoriation, leads to blockage of the nasal passages, dyspnea and open mouth breathing. Hyperemia of the oral mucosa with focal to diffuse necrosis involving the lips, gums, hard and soft palate causes increased salivation and drooling. Sloughing of the tips of the buccal papillae is frequently seen. Ophthalmia with lacrimation, photophobia, conjunctivitis, and centripetally progressive corneal opacity results in partial to complete blindness. Corneal opacity is generally bilateral, but can be unilateral. Hypopyon may be present. Enlarged superficial lymph nodes, especially of the head and neck, are present in most cases. Anorexia with constipation is generally present late in the clinical disease, but diarrhea has been observed during terminal stages. Central nervous signs can include muscle tremors, incoordination, and terminal nystagmus. Occasionally a generalized exanthema or laminitis has been noted (Heuschele and Castro, 1984).

The mild form is generally a nonfatal clinical episode characterized by mild to moderate pyrexia, lacrimation, muzzle encrustation, mucosal

hyperemia, and enlarged superficial lymph nodes. Clinical regression generally occurs over a three to nine day period (Kalunda, 1975).

Clinical signs in exotic ruminants, with the exception of members of the subfamily Bovinae, tend to be more subtle. Pyrexia, conjunctivitis, photophobia, moderate corneal opacity, serous nasal discharge, diarrhea, depression, and a variable lymphadenopathy are seen. Oral and nasal cavity inflammation is generally less severe than that seen in cattle (Heuschele and Castro, 1984). The peracute form was most commonly seen in deer (Reid et al., 1979; Denholm and Westbury, 1982).

Clinical signs in experimentally infected rabbits include mucosal hyperemia, keratitis, catarrhal nasal and ocular discharges, depression, anorexia, incoordination, and frequently hind leg paralysis with loss of the ability to adduct the hind limbs (Daubney and Hudson, 1936).

Malignant catarrhal fever can rarely be diagnosed on a clinical basis. Differential diagnoses would include bovine virus diarrhea-mucosal disease, bluetongue, rinderpest, vesicular stomatitis, foot and mouth disease, and ingestion of caustic chemicals, poisonous plants or mycotoxins (Heuschele and Castro, 1984). Clinical cases of MCF have been reported in a wide range of domestic and exotic ruminants (Appendix II).

Gross Pathology

The extent and severity of the gross lesions seen with MCF in cattle varies depending on the severity and clinical course of the disease (Jubb et al., 1985).

Peracute cases have few lesions with the most consistent being a hemorrhagic gastroenteritis. In more prolonged forms, a variety of gross

lesions are seen. Condition of the carcass varies from normal to extreme dehydration and emaciation. A mucopurulent conjunctivitis with unilateral or bilateral corneal opacity is frequently noted. Encrustation of the muzzle and nares with secondary excoriation is often seen. Skin hyperemia especially in the unpigmented regions is common. Occasionally a generalized exanthema is observed.

Respiratory tract lesions range from nasal mucosal hyperemia with mild serous discharge to severe inflammation and congestion of the mucosa of the nasal passages, turbinates, pharynx, and larynx with focal erosions and grayish yellow pseudomembranes. The tracheobronchial mucosa is generally hyperemic with petechiation or erosions. Lungs might appear normal or be edematous and emphysematous. Bronchopneumonia may complicate prolonged cases.

Gastrointestinal tract lesions range from no visible lesions to severe hemorrhagic gastroenteritis. The most commonly seen lesions included mucosal inflammation with or without erosions in the oral cavity especially on the dental pad, around the base of the incisors, on the palate and the tongue. Erosion of the tips of the buccal papillae is a common finding. Petechial hemorrhages and focal erosions of the esophageal mucosa are occasionally observed. Hyperemia and congestion of the abomasal mucosa with scattered petechial hemorrhages and occasional erosions is commonly seen. Petechial hemorrhages and congestion of the mucosa with occasional erosions occurs in both the small and large intestines. Button ulcers in the jejunum and ileum of cattle with chronic cases of MCF have been described (Kalunda, 1975; Rweyemamu et al., 1976).

Lesions of the urinary system include hyperemia and congestion of the mucosa of the urinary bladder with petechial and ecchymotic hemorrhages. Occasionally more severe hemorrhage is associated with erosions of the epithelium of the urinary bladder. Kidneys may be normal in appearance or have small raised greyish-white foci in the capsular area of the renal cortex. Petechial and ecchymotic hemorhages are frequently present in the renal pelvis and ureteral mucosa.

The liver is generally enlarged. A diffuse mottling with small white foci is especially prominent in the areas of the portal triads. The gall bladder wall is often thickened due to edema, and the mucosa frequently has petechial hemorrhages and erosions.

Enlargement of lymph nodes and hemolymph nodes, especially of the head and neck, are characteristic lesions of MCF. Lymphocytic hyperplasia accounts for most of the increase in lymph node size. The spleen is generally enlarged with prominent Malpighian corpuscles (Jubb et al., 1985).

Petechial hemorrhages on the epicardium of the heart have been noted. Blood vessels, especially small arterioles, are prominent due to thickening of the vessel walls secondary to the vasculitis and infiltration of mononuclear cells (Heuschele and Castro, 1984).

Gross lesions in rabbits experimentally infected with MCF are virtually identical to those in cattle with the exception of a much less marked lymphadenopathy (Heuschele and Castro, 1984).

Exotic ruminants which die from MCF have gross lesions similar to cattle. The nasal and ocular discharges are generally less prominent and

remain serous in nature. Fewer erosive lesions of the nasal and oral cavities are generally seen (Heuschele and Castro, 1984).

The marked variability of gross lesions makes diagnosis of MCF on a gross pathological basis unreliable. Histopathology is generally required to confirm a clinical diagnosis of MCF.

Histopathology

Diagnosis on a histopathological basis is considered reliable due to characteristic histologic changes present in virtually all cases of the disease. Vascular lesions consisting of a disseminated fibrinoid necrotizing vasculitis with mononuclear cell infiltration of the adventitia are present in small and medium sized arteries and arterioles (Jubb et al., 1985). The severity of the vasculitis and subsequent tissue necrosis increases with progression of the disease. The best tissues to examine for vascular lesions are the carotid rete, kidney, liver, adrenal capsule, brain, and leptomeninges (Jubb et al., 1985).

All lymphoid tissues demonstrate follicular necrosis with loss of small mature lymphocytes and a concomitant increase in macrophages and proliferation of immature lymphoblasts. Infiltration and proliferation of mononuclear cells generally associated with blood vessels can be profuse forming raised white foci in many organs including the capsular area of the renal cortex and portal triad regions of the liver.

A nonsuppurative meningoencephalitis is frequently seen secondary to vascular lesions.

Hyperemia, petechial hemorrhages, erosions and edema of the alimentary and respiratory tract mucosa occur secondary to the vascular lesions.

Ophthalmitis involving all portions of the globe is frequently seen with MCF. Corneal edema and vascularization occur secondary to vasculitis of the limbic vessels. Vasculitis of the retina frequently occurs with occasional hemorrhage and subsequent retinal detachment.

Virus Isolation

Permissive cell cultures for the isolation of the etiological agent of the sheep-goat-associated form have not been found. Efforts to isolate and characterize the causative agent continue.

Virus isolation of AHV-1 and AHV-2 viruses from several different species has been accomplished (Appendix III). Permissive cell cultures include, primary and secondary bovine thyroid cultures, embryonic bovine kidney cultures, bovine testes, fetal aoudad kidney, and mouflon kidney cultures (Plowright et al., 1960; Castro et al., 1981; Plowright, 1986; Heuschele, 1982). Two cell-associated virus isolation techniques have been described. The first being cocultivation of infected tissue or buffy coat leukocytes with permissive cell cultures (Plowright et al., 1960), and the second a fusion technique using polyethylene glycol to fuse blood leukocytes with permissive cells (Castro et al., 1983). Tissues of choice for AHV virus isolation included whole uncoagulated blood (EDTA, heparin, or ACD solution), spleen, lung, lymph nodes, adrenals, tonsils, thyroids, liver, and kidney (Plowright et al., 1960; Kalunda et al., 1981a; Mebus et al., 1979). Tissues for virus isolation need to be collected within hours

of death, chilled to 4C, and processed as soon as possible to achieve virus isolation (Plowright, 1964). Viral infectivity in blood and tissues is completely cell-associated (Plowright, 1968). Freezing, lyophilization, or desiccation of infected blood and tissues destroys viral infectivity (Piercy, 1953). Early isolation attempts used a 37C incubation temperature. Under these conditions, typical viral cpe was observed in three to 21 days (Plowright et al., 1963). Early viral passages were acomplished using cell-associated techniques. Plowright et al., 1965, noted a gradual change of viral cpe from syncytial formation to foci of rounded refractile cells with change of the virus from cell-associated to cell-free with serial passage in cell cultures (cited by Harkness and Jessett, 1981).

Harkness and Jessett (1981) demonstrated a marked effect of incubation temperature on the growth characteristics of AHV-1 virus in bovine thyroid cultures. Incubation of infected cultures at 37C produced cell-associated virus with nonprogressive syncytial cpe which reached a maximal virus titer at nine days post-infection. While incubation of infected cultures at 32-34C produced virus which formed foci of rounded refractile cells two to three days later, and reached a maximal virus titer at 12 days post-infection. The 32-34C virus had measurable quantities of cell-free virus which accounts for the more progressive nature of the viral cpe. Reciprocal temperature shift experiments demonstrated that the type of viral cpe was directly related to incubation temperature. Harkness and Jessett (1981) hypothesized that incubation temperature influenced the accumulation of virus coded material at the

surface of the cells. Due to the more progressive nature of the cpe, and the enhanced production of cell-free virus, Harkness and Jessett (1981) suggested that all primary isolations should be done at a 32-34C incubation temperature.

Viral isolates are identified on the basis of viral cpe (Plowright et al., 1960), direct or indirect immunofluorescence (Ferris et al., 1976), virus neutralization (Plowright, 1967), and electron microscopy (Castro and Daley, 1982; Castro et al., 1985).

Heuschele and Fletcher (1984) described a direct immunofluorescence technique for detection of viral antigens in blood leukocytes from reservoir hosts and clinically affected animals. Whole blood or leukocyte smears were air dried, fixed in acetone, and stained using a direct anti-AHV-1 conjugate (Heuschele et al., 1984b) produced from a seropositive whitebearded wildebeest antisera (VN 1:256). Positive smears demonstrated specific fluorescence in both the nucleus and cytoplasm of infected leukocytes.

Serology

History

Cell-associated WC11 virus was isolated from a female blue wildebeest calf less than one week of age in primary BTh cultures (Plowright, 1965b). Serial passage by cell-associated techniques in calf kidney line cultures (BK/165) resulted in the liberation of measurable amounts of cell-free WC11 virus by the forty-ninth passage. The cell-free virus could subsequently be passaged by cell-free technique in primary BTh cultures (Plowright, 1968). Early passages of the WC11 cell-free virus

($10^{3.8}$-$10^{5.8}$ $TCID_{50}$/ml) could be stored at 4C up to seven weeks without loss of viral infectivity (Plowright, 1967; Plowright, 1968). The advent of cell-free WC11 virus made development of serologic techniques for detection of antibody to wildebeest-derived MCF possible.

Virus neutralization assay

The first serologic technique developed was a constant virus tube neutralization (VN) assay (Plowright, 1967). Serum which inhibited viral cpe from developing in three or more tube cultures was considered positive. Antibody titers were expressed as the reciprocal of the serum dilution which protected 50% of the BTh cultures ($log_{10}SN_{50}$). Plowright (1967) used the VN technique to survey East African blue wildebeest for the presence of neutralizing antibodies. Of 181 animals sampled, nine were negative, 26 had titers ranging from $10^{0.1}$ to $10^{0.8}$, 92 titers $10^{0.9}$ to $10^{1.8}$, and 54 had titers greater than $10^{2.0}$. The mean titer of animals three years or older was $10^{1.7}$. Six of the VN negative wildebeest were less than four weeks of age and were also negative for rinderpest antibodies which seemed to indicate lack of colostral antibody transfer, as the dams were all WC11 VN positive. Passive antibody titers in young wildebeest were generally equivalent to, or higher than their dam's antibody titer. The mean antibody titer was generally high ($10^{1.9}$) the first two months of life due to passively acquired antibodies, then declined from the second to fourth month of life to a low of $10^{1.19}$, and then increased to a mean titer of $10^{2.29}$ at 13 to 18 months of age. Plowright (1967) demonstrated that wildebeest did not pass through a seronegative phase between loss of passive antibodies and development of

active immunity. Eleven of 12 viremic wildebeest calves one to 20 weeks of age were VN positive. Failure of passive antibody transfer, and lack of adequate time for development of active immunity was used to explain the seronegative calf. All viremic wildebeest greater than six weeks of age were VN positive. Plowright (1967) concluded that passive antibodies did not interfere with AHV-1 infection or subsequent development of active immunity. The presence of neutralizing antibodies in viremic wildebeest whole blood didn't prevent transfer of infection to cattle. Immune tolerance could not be demonstrated (Plowright, 1967). Plowright (1967) hypothesized that the cell-associated nature of the AHV-1 virus might be responsible for both the lack of passive antibody interference and failure of immune tolerance to develop. The presence of virus induced surface antigens on lymphocytes expressing intracellular virus was suggested to explain the development of neutralizing antibodies to a cell-associated virus (Plowright, 1964).

Plowright (1968) used the VN to follow development of MCF neutralizing antibodies in cattle. Neutralizing antibodies were rarely detected in animals during clinical disease. Low level neutralizing antibody (1:6) were first detected approximately eight weeks after the onset of viremia in cattle which survived (Plowright, 1968). Cattle which recovered were shown to be solidly immune to challenge with virulent AHV-1 (Plowright, 1968). Seropositive cattle challenged with virulent AHV-1 virus demonstrated an anamnestic humoral response with development of high neutralizing antibody titers (1:60) (Plowright, 1968). Cattle hyperimmunized with avirulent virus developed high neutralizing antibody

titers, but were susceptible to challenge with virulent AHV-1 (Plowright et al., 1975). Neutralizing antibodies in cattle were primarily IgG_1 based on their half life of seven to nine days (Plowright et al., 1972). Colostral antibodies were primarily IgG_1 (Plowright et al., 1972).

Kalunda (1975) used the tube VN to follow development of neutralizing antibodies in 50 cattle experimentally infected with AHV-1 virus. He was unable to detect neutralizing antibodies in cattle with acute clinical disease. Thirteen cattle which had prolonged clinical disease or had recovered were seropositive with titers ranging from 1:8 to 1:128. Kalunda used the WC11 VN to investigate antigenic cross reactivity between AHV-1 and other herpesviruses. Antisera to infectious bovine rhinotracheitis virus, dermopathic herpesvirus, Marek's disease virus, duck plaque virus, and cytomegalovirus did not neutralize the WC11 virus. Antisera to the WC11 virus didn't significantly neutralize infectious bovine rhinotracheitis virus or dermopathic bovine herpesvirus. Kalunda (1975) tested 36 sheep sera from an Australian flock with a high incidence of MCF in associated cattle (Dr. Snowden, Australia), sera from 16 cattle with sheep-goat-associated MCF and 22 contact cattle (Dr.Storz, Colorado), and six bovine which had recovered from MCF-like disease (Kahrs, USA and Snowden, Australia). He found five sheep to be seropositive with titers of 1:4 to 1:16, and three of the recovered cattle to be seropositive with titers of 1:4 to 1:16.

A survey of East African game animals using the tube WC11 VN was completed by Reid et al. (1975). Neutralizing antibodies were detected in sera from three species of the subfamily Alcelaphinae (topi, hartebeest,

and blue wildebeest), and one species of the subfamily Hippotraginae (fringe-eared oryx). In topi, passive neutralizing antibodies were detectable in calves up to two months of age. The majority of topi calves then passed through a seronegative phase before developing active immunity at around seven months of age. A seronegative phase was not detected in the hartebeest or wildebeest. Seropositive topi and hartebeest had titers of 0.095 $\log_{10}SN_{50}$ or less. Antibody titers in two oryx were $10^{0.8}$ and $10^{1.4}$. The mean WC11 VN titer for wildebeest was $10^{1.93\pm0.11}$. This difference in antibody titer suggested that neutralizing antibodies detected in oryx, topi, and hartebeest were in response to infection with viruses antigenically related, but not identical to the AHV-1 virus (Reid et al., 1975).

Adaptation of the VN technique to a microtiter plate system was achieved by Mushi and Plowright (1979). The microtiter VN was demonstrated to be highly reproducible with comparable sensitivity to the tube VN. Advantages of the microtiter system included use of smaller quantities of reagents, elimination of dispensing the serum virus mixtures, and shorter assay time. The microtiter VN demonstrated a strong correlation between serum antibody titer and virus dose (correlation coefficient r=0.96). A standard virus dose of 50 to 200 $TCID_{50}$ was recommended (Mushi and Plowright, 1979). The microtiter VN was used to follow development of antibodies in rabbits and cattle experimentally infected with AHV-1 (Rossiter et al., 1977). They were unable to detect neutralizing antibodies in cattle. In rabbits, neutralizing antibodies were detected as early as one day before the onset of pyrexia, but not all

of the rabbits developed neutralizing antibodies. A mean titer of $10^{0.85}$ was present on day three of clinical disease. The highest antibody titer detected in rabbits was $10^{1.7}$. In a second study, Rossiter (1982b) failed to detect neutralizing antibodies in five experimentally infected rabbits. Rurangirwa and Mushi (1984) demonstrated that hyperimmunized rabbits developed good neutralizing antibody titers.

The addition of complement to the serum virus mixture prior to neutralization was investigated to determine if complement would enhance virus neutralization (Rossiter et al., 1977). Addition of guinea pig complement did not appear to enhance neutralization of the WC11 virus regardless of disease stage when the serum was drawn.

Sera from 13 Kenyan cattle with wildebeest-derived MCF were tested by the microtiter VN (Rossiter et al., 1980). Six of the cattle were seropositive and one was questionable. Serum from normal cattle were all negative. The VN technique was determined to have good specificity, but low sensitivity for detection of neutralizing antibodies in clinically affected cattle.

Mushi and Karstad (1981) found neutralizing antibodies in 50 fringe-eared oryx (Oryx gazella callotis) without wildebeest exposure. The mean titer in adults was $10^{1.23}$. A seronegative phase between loss of passive antibody and development of active immunity (two to nine months of age) was observed in oryx calves. Natural transmission between oryx and cattle has not been documented.

Rossiter (1981a) used the VN to test sera from 167 sheep, many of which had been associated with MCF outbreaks, for neutralizing antibodies.

All were seronegative. Virus neutralization assays completed by Harkness (1985) on sheep, and cattle with sheep-goat-associated MCF in England found 86 of 508 sheep to be seropositive with titers from 1:2 to 1:32, and two of 15 cattle with confirmed cases of MCF to be seropositive. Harkness (1985) detected neutralizing antibodies in one of 21 clinically affected cattle, seven of 28 contact sheep, and one of 12 contact cattle from clinical MCF outbreaks in Shrewsbury and Northhampton. Rossiter (1983) used the VN to test sera from seven deer and 48 cattle with sheep-goat-associated MCF. All of the sera were negative. Heuschele and Castro (1984) also reported that clinically affected cattle, and domestic sheep and goats were rarely VN positive. Wan and Castro (1986) detected neutralizing antibodies in four of 98 cattle with natural and experimental cases of sheep-goat-associated MCF. All of these serologic findings lent support to Rossiter's hypothesis of antigenic relationship between the etiological agent of sheep-goat-associated form, and the AHV-1 virus group. A standardized microtiter WC11 VN technique has been described (Heuschele et al., 1985c).

Antigenic relationships, as detected by the serum virus neutralization assay, were not demonstrated between alcelaphine herpesvirus-1 and bovine herpesviruses (BHV) 1-3 (Heuschele, 1982; Hamblin and Hedger, 1984).

Serologic surveys for the detection of neutralizing antibodies to MCF virus in exotic ruminants were completed by Heuschele et al. (1984a), Hamblin and Hedger (1984), and Barnard (1985). A high prevalence of neutralizing antibodies were demonstrated in whitetailed wildebeest,

whitebearded wildebeest, brindled wildebeest, topi, hartebeest, addax, scimitar-horned oryx, fringe-eared oryx, and gemsbok (Heuschele et al., 1984a). Species tested positive for neutralizing antibodies to alcelaphine herpesvirus-1 are presented in Appendix IV.

Indirect immunofluorescence assay

Indirect immunofluorescence assays (IFA) have been shown to be easier, faster, and more sensitive than VN assays for many herpesviruses. The IFA was adapted for use with the cell-free WC11 virus using embryonic bovine kidney (BEK) cultures (Rossiter et al., 1977). Positive fluorescence patterns included both particulate and diffuse intranuclear and cytoplasmic fluorescence often with an intensely staining paranuclear body. Specificity of the fluorescence was demonstrated using blocking and absorption techniques. The IFA titer was the reciprocal of the highest dilution demonstrating specific fluorescence. Low level antigenic cross reactivity with other herpesviruses, especially BHV-1 and BHV-3, has been reported by several investigators (Appendix V). A single serotype for the AHV-1 group of viruses was proposed based on IFA serological findings (Rossiter et al., 1977).

The IFA was used to follow antibody development in rabbits, and cattle experimentally infected with AHV-1 virus (Rossiter et al., 1977). Antibodies could be detected in cattle as early as six to seven days before the onset of pyrexia (IFA titer of 1:8). Antibody titers continued to rise through the incubation period to a mean titer of 1:768 on the first day of clinical disease, and remained high through the course of the disease (mean titer > 1:512). In rabbits, IFA antibodies were detected

four to six days post inoculation (IFA titer of 1:8). Antibody titers rose through the incubation period to a mean titer of 1:200 on day one of the clinical disease, and continued to rise through the course of the disease (mean titer of 1:1024). Antibodies against the AHV-1 virus, in both cattle and rabbits, could be detected earlier and with higher titers by the IFA than by the VN (Rossiter et al., 1977). They hypothesized that antibodies detected by the IFA were not directed against the same surface viral antigens as neutralizing antibodies, but were directed against viral antigens expressed earlier in the infectious process.

Rossiter (1981a) used the IFA technique to test sheep sera for antibodies directed against the AHV-1 virus. He found 162 of 167 sheep seropositive (mean titer 4.4 $\log_2$), including gnotobiotic lambs which he took as evidence of congenital transmission of the etiological agent of sheep-goat-associated MCF.

Rossiter (1983) used the IFA technique to demonstrate antibodies to the AHV-1 virus in 38 of 48 cattle with histopathologically confirmed cases of sheep-goat-associated MCF. The pattern of IFA fluoresence was the same with positive sera from both forms of the disease. Rossiter (1983) further hypothesized that the etiological agent of the sheep-goat-associated form is antigenically related, but not identical to the AHV-1 virus since few IFA positive sera from sheep or cattle with sheep-goat-associated MCF were VN positive. Harkness (1985) used IFA to examine sera from 21 cattle with clinical sheep-goat-associated MCF, 50 normal cattle, and 51 cattle presented for other clinical diseases. Sixty-two percent of the cattle with clinical MCF, six percent of the

normal cattle, and two percent of cattle presented for other diseases were IFA positive. Harkness (1985) then used the IFA on cattle and sheep sera from MCF clinical outbreaks in Shrewsbury and Northhampton. In the Shrewbury outbreak, three of four clinically affected cattle, none of the contact cattle, and three of 19 contact sheep were IFA positive. Whereas, two of 17 clinically affected cattle, and none of the contact sheep or cattle were IFA positive in the Northhampton outbreak. The low percentage of IFA positives in the Northhampton outbreak Harkness felt could be explained in one of three ways, either not all clinically affected animals were infected with MCF virus, a percentage of the infected animals fail to develop detectable levels of antibodies or sheep-goat-associated MCF has multiple etiological agents, some of which may not be antigenically related to the AHV-1 virus group. Indirect immunofluorescence antibodies were also detected in cattle with clinical sheep-goat-associated MCF by Heuschele et al. (1984a).

To study antibody development to early antigens of the AHV-1 virus, cytosine arabinoside (25 μg/ml), a DNA inhibitor, was used to prevent the production of WC11 late antigens in BEK monolayers used for cover slip preparation (Rossiter et al., 1978). With this technique, termed IFA(E), two early WC11 antigens, a diffuse whole cell antigen (DEA) and a particulate intranuclear antigen (PEA) were identified. The DEA antigen was present in the cytoplasm of WC11 infected BEK cells at 12 hours and in the nucleus at 24 hours post-inoculation. Whereas, the PEA antigen took 24 to 36 hours to develop. Antibodies to the PEA antigen were detected in acute and hyperimmune cattle and rabbit antisera. Antibodies to the DEA

antigen was detected only in hyperimmune antisera. Rabbits produced few antibodies to early antigens during clinical disease. In cattle, antibodies to early antigens developed later and had lower titers (four to eight-fold) than antibodies directed against late antigens (IFA(L)). Adsorption and blocking techniques demonstrated the fluorescence to be specific. The IFA(E) assay was used to test sera from 13 Kenyan cattle with acute wildebeest-derived MCF (Rossiter et al., 1980). Positive fluorescence (PEA antigen) was detected in all 13 cattle with a mean titer of 1:26 (range 1:8 to 1:64). Fluorescence was rarely detected in sera from normal cattle. Rossiter (1981a) used the IFA(E) technique to test antisera from 13 clinically normal sheep. All 13 sheep were IFA(E) positive with a mean titer of 1:22 as compared to the mean IFA(L) titer of 1:90 for the same sheep. The pattern of IFA(E) fluorescence noted with the positive sheep sera was very similar to that seen with positive cattle or wildebeest sera (Rossiter, 1981a). Rossiter (1983) used the IFA(E) assay to test sera from cattle with sheep-goat-associated MCF. Seven of the 12 cattle tested were positive with a mean titer of 1:32 as compared to a mean titer of 1:282 for the IFA(L) assay. Detection of antibodies to WC11 early antigens in sheep and clinically affected cattle with sheep-goat-associated MCF supported Rossiter's hypothesis of antigenic relationship between the AHV-1 virus and the etiological agent of sheep-goat-associated form of MCF.

Species of animals in which IFA antibodies against AHV-1 virus have been detected are presented in Appendix VI.

Plaque reduction assay

A plaque reduction assay using cell-free WC11 virus was described by Russell (1979). Plaque reduction titers were reported as the reciprocal of the last dilution of antiserum reducing plaques by 90%. Bovine testes cultures were highly variable in growth and virus sensitivity making reproducibility of this assay difficult. An enhanced plaque reduction phenomenon with the use of hypotonic media (50-fold dilution with distilled water) was described by Russell (1979). Using this assay, he was able to demonstrate enhanced plaque reduction (six to 13-fold) of a single WC11 hyperimmune bovine serum. The virus infectivity appeared unchanged. The increased titer was hypothesized to be due to enhanced antigen-antibody binding (Russell, 1979).

A rabbit kidney cell line (RK_{13}) was used in a separate plaque reduction assay described by Hazlett (1980). The plaque reduction titer was calculated by linear regression and represented the dilution reducing 50% of the virus control PFU (ND_{50}). Hazlett (1980) used the assay to detect neutralizing antibodies in the sera of three blue wildebeest (1:120 to 1:480) and nasal secretions of one wildebeest. He felt the RK_{13} line had a sensitivity comparable to primary thyroid cultures.

Complement fixation assay

Kalunda (1975) described a tube complement fixation assay (CF) for MCF based on the method of Casey. Serum dilutions inhibiting more than 70% hemolysis were considered positive. Kalunda (1975) adapted the assay to a microtiter system. The assay was performed on serial serum samples from 50 cattle experimentally infected with AHV-1. Antibody titers

ranging from 1:16 to 1:128 were detected in eight cattle (16%) which had recovered or had prolonged chronic cases of MCF. Complement fixing antibodies were never detected before virus neutralizing antibodies, and the CF titers were generally equivalent to, or lower than their respective VN titer. The CF assay could not detect viral antigens in tissues of infected cattle.

A modified microtiter CF assay using normal bovine serum in the complement dilution was described by Rossiter et al. (1980). This assay detected significant levels of background antibodies (mean titer 1:8) in normal cattle. Anticomplementary activity of the bovine sera or cross reactivity with other bovine herpesviruses were suggested as two possible explanations for the high background (Rossiter et al., 1980). Thirteen cattle with wildebeest-derived MCF had a mean CF titer of 1:32. Detection of tissue viral antigens was not possible with this assay.

Hamdy et al. (1980) used two methods of complement fixation, serum dilution (Laboratory Branch CF Method, 1965) and complement dilution (modified Nakamura method, 1958), for detection of CF antibodies in 44 cattle experimentally infected with AHV-1 virus. Seventeen of 44 (39%) were positive with titers ranging from 1:8 to 1:256 by the serum dilution technique beginning at 28 days post-inoculation. Antibodies were detected in 32 of 44 (72%) cattle by the complement dilution assay beginning at 21 days post-infection. The complement dilution technique could be used to test sera with anticomplementary activity. Cross reactivity with antisera against foot and mouth disease virus, rinderpest virus, bovine virus diarrhea virus, infectious bovine rhinotracheitis virus, Ibaraki virus,

and bovine herpes mammillitis virus were not demonstrated. All CF positive sera were also VN positive (Kalunda and Dardiri unpublished, cited by Hamdy et al., 1980).

Immunodiffusion assay

Kalunda (1975) described an immunodiffusion assay (ID) to detect MCF precipitating antibodies. Using this technique with cattle experimentally infected with AHV-1 virus, he demonstrated precipitating antibodies only in animals which had recovered or had prolonged chronic cases. The ID assay was less sensitive than either the CF assay or the VN assay.

An improved ID technique was described by Rossiter (1980b). This assay was used to detect AHV-1 precipitating antibodies in three of nine experimentally infected rabbits, zero of 14 experimentally infected cattle, 62 of 176 wildebeest, and three of 20 hartebeest. Precipitating antibodies were detected in one of 13 cattle with acute wildebeest-derived MCF. This was the first report of precipitating antibodies in cattle with clinical disease. Precipitating antibodies were not detected in the sera of 19 topi, 20 sheep, or 144 normal cattle. Antigenic cross reactivity between the positive ID antigen and hyperimmune sera against BHV-1, BHV-2, rinderpest virus, and BVD virus were not detected. The ID assay was less sensitive than the VN, the CF, and the IFA assays. The ID assay had a relative sensitivity compared to IFA (positive threshold 1:32) of 10% for cattle sera, 36% for wildebeest sera and 38% for rabbit sera. Rossiter (1980b) used the ID assay to demonstrate antigenic relationship between the WC11 (AHV-1) and K/30 (AHV-2) isolates. The ID assay failed to detect

viral antigens in tissues of cattle with acute MCF (Kalunda, 1975; Rossiter, 1980b).

Indirect immunoperoxidase technique

Rossiter (1981b) examined the indirect immunoperoxidase (IIP) technique for detection of MCF antibodies in experimentally AHV-1 infected cattle. Staining of particulate intranuclear early and late antigens, and particulate and diffuse cytoplasmic antigens by acute phase sera of cattle were observed. Nonspecific staining of rounded cells was also observed. The IIP titer was reported as the reciprocal of the highest serum dilution giving positive staining of nonrounded cells. A positive threshold of 1:32 was selected. Antibody titers greater than 1:32 were not detected in the sera of normal cattle. Antibody titers greater than 1:32 were detected in 23 of 23 cattle with wildebeest-derived MCF (mean titer 1:320). Rossiter (1981b) detected IIP antibodies in 14 cattle experimentally infected with AHV-1 one to two days prior to detection of IFA antibodies. In cattle, IIP titers were up to eight-fold higher than IFA titers during clinical disease.

The IIP technique was used by Rossiter (1982b) to follow development of MCF antibody in experimentally AHV-1 infected rabbits. Rabbits developed both IgM and IgG IIP antibody as early as six days before the onset of pyrexia. All rabbits were IIP positive by two days before the onset of pyrexia (mean titer IgM 1:32, mean titer IgG 1:22). The mean IgM titer increased less than two-fold over the course of the clinical disease to a mean titer of 1:83. The IgG mean titer rose exponentially during clinical disease to a mean titer of 1:218.

Counterimmunoelectrophoresis assay

A counterimmunoelectrophoresis (CIEP) assay for detection of MCF precipitating antibodies was described by Rossiter (1980b). MCF hyperimmune rabbit and cattle sera formed two to three precipitin lines against the positive (WC11) antigen. Precipitin lines against the negative (BEK) antigen were not detected with normal or hyperimmune rabbit sera, but were present in 54% of the normal bovine sera, and more than 33% of the alcelaphinae sera. Possible causes for the nonspecific reaction were not determined. Precipitating antibodies against the WC11 antigen were detected in three of nine experimentally AHV-1 infected rabbits (all ID positive), 133 of 176 wildebeest, four of 19 topi, and two of 20 hartebeest. The CIEP assay was two to four times more sensitive than the ID assay, but less sensitive than the IFA assay. The high levels of nonspecific reaction with bovine sera prevented the use of this assay to detect precipitating antibodies in cattle. Cross reactivity did not occur between the WC11 antigen and hyperimmune sera produced against BHV-1, BHV-2, rinderpest virus, or BVD virus. The CIEP assay failed to detect viral antigens in infected tissue.

Enzyme-linked immunosorbent assay

Wan and Castro (1986) described a verification enzyme-linked immunosorbent (ELISA) assay using positive (greater kudu isolate, Castro et al., 1981) and negative (BEK) sonicated nuclear homogenate antigens. The nuclear extracted whole virus antigens were used because they were hypothesized to result in lower background absorbance values than semipurified cell-free whole virus antigen. The higher background absorban

absorbance with cell-free virus was suggested to be related to the incorporation of host cell proteins into the viral envelope during budding through cellular membranes. The mean absorbance for 88 seronegative cattle was 0.085 $\pm$ 0.067. Mean absorbance of the positive reference antiserum was 1.125 $\pm$ 0.140. Positive threshold was set at 0.150. Antigenic cross reactivity was not demonstrated between the positive ELISA antigen and hyperimmune antisera produced in cattle to BHV-1, BHV-2, and BHV-3. Serial dilutions of antisera from three WC11 VN postive ruminants demonstrated decreasing linear absorbance values with increasing dilutions. A 1:20 serum dilution was selected to screen antisera. Antisera from 42 exotic ruminant species (216 sera) were tested by the ELISA using anti-bovine ELISA conjugate (Wan et al., 1988). Levels of positive ELISA reaction were affected by the binding affinity of the anti-bovine ELISA conjugate to each exotic species IgG. Many of the ELISA positive sera were from clinically normal exotic ruminants (wildebeest, hartebeest, topi, oryx, addax, and exotic sheep and goats). The ELISA assay detected antibodies to AHV-1 in nine exotic ruminants with clinical MCF. Virus was subsequently isolated from eight of these animals. The ELISA results were compared to VN results (VN results supplied by Heuschele, San Diego Zoo, and Metz, National Veterinary Services Laboratories) for sensitivity and specificity. The results were in agreement for 73 negative and 110 positive sera. Fourteen sera were positive by the ELISA, and negative by the VN. Nineteen sera were VN positive, and ELISA negative. The VN titers for positive sera ranged from 1:4 to 1:516, and the ELISA absorbance values ranged from 0.284-1.534.

The assay had a significant correlation as calculated by linear regression ($r=0.564$, $P<0.001$). The sensitivity of the ELISA compared to the VN was 85.3%, and the specificity of the ELISA was 83.9%.

Antisera from cattle with clinical cases of sheep-goat-associated MCF, both natural and experimental, were tested by the ELISA and the VN (Wan and Castro, 1986). Thirty of 128 sera from cattle associated with MCF outbreaks in Colorado and California were ELISA positive. Neutralizing antibodies to AHV-1 were not detected in any of these sera. Sera from six of 24 cattle with natural cases of MCF in Colorado were ELISA positive. Three of the six cattle were VN positive. Preinoculation and post-inoculation sera from 24 cattle used in experimental transmission studies were tested by the ELISA and VN. One of 24 preinoculation sera was ELISA positive. Examination of paired serologic results demonstrated three seroconversions by the ELISA, and one seroconversion by the VN assay. The finding of ELISA seropositive cattle with the sheep-goat-associated form of MCF lends support to Rossiter's hypothesis of antigenic relationship between AHV-1 and the etiological agent of the sheep-goat-associated form. Wan and Castro (1986) concluded that the ELISA was a rapid, specific serologic technique which could be used with both forms of the disease for diagnostic purposes, and for screening large numbers of domestic and exotic ruminants for the presence of antibodies against MCF virus.

Western blot

Reid and Buxton (1985) used AHV-1 antigen in a western blot technique. Several major bands were detected using antisera from positive

wildebeest. A portion of these bands also reacted with positive sheep antisera. This was further evidence for partial antigenic relationship between AHV-1 and the etiological agent of the sheep-goat-associated form of MCF.

Disease Control

An efficacious vaccine for the AHV-1 virus is not currently available (Russell, 1980; Rossiter, 1982a; Plowright, 1986). Current method of disease control is separation of susceptible species from potential reservoir hosts, which makes identification of potential reservoir hosts critical. Serologic techniques can readily be used to assist in this identification process.

MATERIALS AND METHODS

Cell Cultures

Vero-Maru (VM) cell cultures (green monkey kidney cells, Panama) were split 1:5 once per week using minimum essential medium (F-15) and 7.5% fetal bovine serum (FBS). These cultures were used for propagation of the Indian gaur AHV-1 isolate (Castro et al., 1981), production of complement fixation antigens, and as the indicator cell system in the AHV-1 VN assays. Bovine turbinate (BT) cell cultures (McClurkin, National Animal Disease Center (NADC), Ames, Iowa) were split 1:3 once per week using F-15 medium and 10% FBS. These cultures were used for propagation of the WC11 AHV-1 isolate (Plowright, 1965b), preparation of viral cover slips, production of complement fixation antigens, as the indicator cell system in the bovine and porcine herpesviruses VN assays, and for preparation of WC11 and normal host cell (NHC) enzyme-linked immunosorbent antigens. Cell cultures were periodically tested for the presence of mycoplasma and bovine virus diarrhea virus (BVD) with negative results.

Viruses

Two AHV-1 isolates were used in this study. The WC11 isolate, considered to be the prototypic virus of the AHV-1 group, was isolated by cocultivation of the leukocyte fraction from 10 milliliters (ml) of uncoagulated (EDTA) blood from a female blue wildebeest calf less than one week of age with primary bovine thyroid (BTh) cultures (Plowright, 1965b), and characterized as a highly cell-associated herpesvirus. Serial passage of this isolate by cell-associated techniques in calf kidney (BK/165) line cultures resulted in the liberation of measurable amounts of cell-free virus by the forty-ninth passage (Plowright, 1967). The cell-free virus

was subsequently passaged in Bth cultures. Cell-free WC11 virus was provided by the Director, East African Veterinary Research Organization, Kikuyu, Kenya, to the Foreign Animal Disease Diagnostic Laboratory (FADDL), Greenport, New York, where it was maintained by tissue culture passage in primary or secondary BTh cultures. The cell-free WC11 virus was subsequently lyophilized after sixty passages in BTh cultures. The lyophilized cell-free strain of WC11 virus was received by the Diagnostic Virology Laboratory, National Veterinary Services Laboratories (NVSL), Ames, Iowa, from FADDL. The lyophilized WC11 virus was propagated in VM cultures. Five blind passages in VM cultures using cell-associated passage techniques where completed before viral cytopathic effects (cpe) were detected. The WC11 virus grown in VM cultures had reverted to cell-associated virus. Serial passage of the virus in BT cultures with selection for cell-free virus by filtration of virus inoculum through a 0.45μm millipore filter prior to inoculation of culture flasks, and incubation at 34C allowed for the development of cell-free viral stocks which could be harvested, and stored at -70C.

The Indian gaur isolate (IG30359) was isolated in embryonic bovine kidney (BEK) cultures (Castro et al., 1981) from buffy coat cells collected from an Indian gaur with clinical disease suggestive of MCF at the Oklahoma City Zoo, Oklahoma City, Oklahoma. The isolate was characterized as a cell-associated herpesvirus of the AHV-1 group by indirect immunofluorescence using a WC11 reference antiserum provided by FADDL (Ferris et al., 1979). The isolate also neutralized the reference WC11 antiserum. Low passage virulent IG30359 virus was provided to NVSL by Dr. A. E. Castro, Oklahoma State University, Stillwater, Oklahoma. The

isolate was maintained for over three years by serial passage every seven to 10 days in VM cultures using cell-associated passage techniques. Serial passage of this isolate in VM cultures with selection for cell-free virus by filtration of virus inoculum through a 0.45μm millipore filter prior to inoculation of flask cultures, and incubation at 34C resulted in the liberation of cell-free virus stock which could be harvested, and stored at -70C.

The bovine and porcine herpesviruses used in the antigenic relationship studies were propagated in BT cultures from reference NVSL viruses. They included bovine herpesvirus-1 (infectious bovine rhinotracheitis virus, Colorado strain), bovine herpesvirus-2 (bovine herpes mammillitis virus), bovine herpesvirus-3 (DN599 virus), and porcine herpesvirus-1 (pseudorabies virus, Shope strain).

All viral stocks were titered, examined by electron microscopy, streaked on blood agar plates for detection of bacterial comtaminants, and passaged through three blind passages to detect the presence of BVD virus or other adventitious viruses. All viral stocks used in this study were free of adventitious agents.

Antisera

Control negative and positive WC11 antisera were obtained from conventional calf 9471. Preinoculation serum was screened at a 1:20 dilution by indirect immunofluorescence for the presence of antibodies against pseudorabies virus (PHV-1), infectious bovine rhinotracheitis virus (BHV-1), bovine herpes mammillitis virus (BHV-2), DN599 virus (BHV-3), WC11 virus, IG30359 virus, and BVD virus with negative results. The calf was inoculated intravenously with 10 ml of cell-free WC11 virus,

and bled daily for 21 days. On day 23, the calf was given a second 10 ml dose of cell-free WC11 virus by the intravenous route. Serum was drawn on day 30, and the antibody titers determined by the IFA and VN assays. The calf was exsanguinated on day 34 post-inoculation. All sera were tested for the presence of antibodies against the WC11 virus by the IFA, the VN, and the ELISA. A positive control serum against the WC11 virus was also produced in a gnotobiotic calf (GC #16). Weekly serum samples were tested for antibodies against the WC11 virus by the IFA, the VN, and the ELISA.

Control positive (OSU715, OSU713) and negative (OSU696) sheep antisera were provided by Dr. S. K. Wan, Oklahoma State University, Stillwater, Oklahoma. The positive antisera were produced against the greater kudu AHV-1 isolate (Castro et al., 1981).

Field sera from goats tested by the IFA and the VN and found to be negative (Windy, Lolli) and weakly positive (0717-55, VN 1:4) were used as negative and positive control sera.

Field sera from 63 different animal species submitted to NVSL for MCF serology (1982 to 1986), 20 sera from three exotic species (two Formosan sika deer, 16 wildebeest, and two scimitar-horned oryx) provided by Dr. W. P. Heuschele, San Diego Zoo, San Diego, California, and six sera from three exotic species (one Indian gaur, one cape hartebeest, and four wildebeest) provided by Dr. S. K. Wan were tested by the IFA, the VN, and the ELISA for the presence of antibodies against the WC11 virus.

Animal species were classified and grouped for data presentation according to the International Species Inventory System (1974), with the exception of the subfamily Hippotraginae which was separated into two subfamilies, the subfamily Alcelaphinae which included the Alcelaphus,

Connochaetes, and Damaliscus genera, and the subfamily Hippotraginae which included the Addax, Hippotragus, and Oryx genera.

Complement Fixation Assay

Antigen preparation

Positive and negative complement fixation antigens were prepared for both viral isolates using a modification of the procedure described by Kalunda (1975). Bovine turbinate cultures were used for the WC11 isolate, and VM cultures for the IG30359 isolate. Briefly, nearly confluent cell culture flasks (two days) were infected with cell-free virus and incubated at 34C until 90% of the cell monolayer showed viral cpe. Uninfected culture flasks were processed identically for production of negative CF antigens. The flasks were put through a single freeze-thaw cycle (-70C). Cell debris was removed by centrifugation at 2000 rpm for 15 minutes. The clarified viral supernatant fluid was concentrated 10-fold by dialysis against polyethylene glycol (Carbowax[a]). The concentrated viral and negative supernatant fluids were used as antigens in the complement fixation assay.

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[a]Fisher Scientific, Fairlawn, New Jersey.

Complement fixation procedure

The complement fixation assay was performed as previously described by Jenney et al. (1982). Veronal buffered (VBS) saline (pH 7.2 to 7.4) was used as diluent. Antisera were diluted 1:5 in VBS and heat inactivated at 56C for 30 minutes. Optimal antigen dilutions were determined by block titrations. A three percent suspension of washed sheep red blood cells (SRBC) was prepared and standardized to give replicate spectrophotometric absorbance readings between 0.33 and 0.35 at 550nm. Rabbit hemolysin (0.09 ml) containing two units of activity for sensitization of SRBC was added to 100 ml of VBS, and mixed with 100 ml of standardized SRBC suspension. The mixture was incubated for 10 minutes in a 37C water bath to sensitize the SRBC. Optimal guinea pig complement dilution (1:31) containing 1.9 CH_{100} units was determined by complement titration. Complement fixation at 37C for three hours and overnight at 4C were tried.

Determination of Identity between exotic species IgG and Bovine IgG

Antisera from 59 exotic species were tested by the double diffusion technique of Ouchterlony to determine complete or partial identity between immunoglobulins from exotic species and bovine IgG. One percent Noble agar[a] in borate buffer (Appendix VII) was prepared and poured into petri dishes and allowed to harden to a thickness of 2.8mm. Seven well patterns with a center well surrounded by six outside wells were cut using a template with cutters for wells 5.3mm in diameter and

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[a]Difco Laboratories, Detroit, Michigan.

2.4mm apart. Undiluted IgG fraction of rabbit anti-bovine (RAB) IgG (H + L)-(Organon Teknika Corp., West Chester, PA, Cat. No.-0202-0082) was placed in the center well of each pattern. Purified bovine IgG (IgG_1 and IgG_2)-(Organon Teknika Corp., West Chester, PA, Cat.No.-6002-0080) diluted 1:40 with phosphate buffered saline (PBS, 0.01M, pH 7.2) was placed in three alternate outside wells of each pattern. Antisera from different species diluted 1:40 with PBS were placed into the remaining three outside wells of each pattern. The dilution of antisera and bovine IgG which gave the best definition of precipitin lines had been determined by titration against undiluted IgG fraction of RAB. The plates were incubated in a 22C incubator overnight. Patterns were read for precipitin lines of identity or nonidentity with bovine IgG.

Antigenic Relationships Between AHV-1, BHV 1-3, and PHV-1

Antisera used to examine antigenic relationships between AHV-1, BHV 1-3, and PHV-1 as detected by the IFA, the VN, and the ELISA were primarily hyperimmune antisera produced in cattle, gnotobiotic calves, and pigs by the personnel of the Diagnostic Virology Laboratory, NVSL. Sera 4808301[a], VS512[a], and 4808601[a] were negative control antisera. Serum 3548102[a] was a hyperimmune antiserum produced against the IG30359 virus. Sera C5763[a], 3508201[a], pig #1, pig #3, pig #4, and GC 33[a] were hyperimune antisera produced against the Colorado strain of infectious bovine rhinotracheitis virus. Bovine sera 32, 56B, and 61 were BHV-2 VN positive field sera submitted to NVSL for BHV-2 serology. Hyperimmune BHV-2

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[a]Antisera produced in cattle; GC = production of antiserum in a gnotobiotic calf.

antisera were 3518201[a], and GC 36[a]. Sera 3528201[a], and GC 35[a] were hyperimmune antisera produced against DN599 virus. The Shope strain of PHV-1 was used to produce porcine antisera 3389, 19289, A266, and R239. Positive DN599 antisera 8549[a], 41[a], 51[a], and 52[a] were provided by Dr. Van der Maaten, NADC, Ames, Iowa. Positive MCF antisera WAP43 (Formosan sika deer), 831041 and 84037 (wildebeest) were provided by Dr. W.P. Heuschele, San Diego Zoo, San Diego, California. Positive antisera 30359 (Indian gaur) and 30282A (bovine) were provided by Dr. A. E. Castro, Oklahoma State University, Stillwater, Oklahoma.

Indirect Immunofluorescence Assay

Viral cover slip preparation

Confluent BT cultures were trypsinized and resuspended in F-15 medium containing 10% FBS at a 1:3 ratio. Cell suspension was seeded into racks of sterile Leighton tubes (1.5 ml/tube) containing removable glass cover slips. The racks were incubated at 37C for three days. Tubes in different Leighton racks were inoculated with BHV-1, BHV-2, BHV-3, or PHV-1. The infected cell monolayers were incubated at 37C until early viral cpe was detected. The glass cover slips were removed, rinsed twice in 0.01M PBS, fixed in acetone, air dried, and stored in glass vials at -70C. Cover slips could be stored at -70C for over 12 months without loss of viral antigen reactivity.

Cell-associated IG30359 virus was used for AHV-1 cover slip preparation during the initial phase of this study. Briefly, confluent

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[a]Antisera produced in cattle; GC = production of antiserum in a gnotobiotic calf.

four to seven day VM cultures were trypsinized and resuspended in F-15 medium containing 10% FBS at a 1:3 ratio. Cell-associated IG30359 virus was added to the cell suspension at a 1:300 dilution. The infected cell suspension was seeded into racks of sterile Leighton tubes (1.5 ml/tube) containing removable glass cover slips. The Leighton racks were incubated at 37C until early viral cpe developed (two to four days) in the cell monolayers. Cover slips were processed and stored at -70C as previously described. The cell-associated nature of this virus made it difficult to achieve uniform infectivity of cover slips. False negatives were occasionally seen due to lack of viral antigen on some cover slips.

With the development of cell-free WC11 virus stocks, coverslips were prepared using BT cultures. Confluent seven day BT cultures were trypsinized and resuspended in F-15 medium containing 10% FBS at a 1:1 ratio. Cell-free WC11 virus was added to the cell suspension at a 1:1000 dilution. The infected cell suspension was seeded into racks of sterile Leighton tubes (1.5 ml/tube) with removable glass cover slips. The Leighton racks were incubated at 37C until small plaques of viral cpe were present (five days). Cover slips were fixed and stored as previously described.

Indirect immunofluorescence procedure

Viral cover slips were removed from -70C and laid cell-side-up on IFA staining boards and warmed to room temperature. Test sera, and known reference positive and negative control sera were diluted 1:20 in cold PBS (0.01M). Each cover slip was overlaid with a diluted test serum. The cover slips were incubated in a humidified 37C CO_2 incubator for 30 minutes. Excess serum was removed using two PBS rinses. Cover slips were

again placed on staining boards cell-side-up and overlaid with the appropriate fluorescein isothiocyanate (FITC)-labeled anti-species conjugate (Reagent Section, Scientific Services Laboratory, NVSL). The cover slips were incubated for 30 minutes in a humidified 37C CO_2 incubator. Excess conjugate was removed using two PBS rinses followed by a distilled water rinse. Coverslips were air dried and mounted in 50% glycerol, and read for the presence of specific viral immunofluorescence using a UV microscope[a]. Sera tested positive at the 1:20 dilution were retested at a 1:100 dilution.

Virus Neutralization Assay

Standardized microtiter plate VN assays were performed with each AHV-1 isolate. Antisera were diluted 1:2 in F-15 medium and heat-inactivated at 56C for 30 minutes. Serial two-fold serum dilutions (1:2 to 1:256) were made in duplicate rows of a 96 well microtitration plate with 0.025μl diluter loops. Cell-free virus (100-300 $TCID_{50}$/well) was added to the test wells. The plates were covered and incubated at 37C in a humidified CO_2 chamber for one hour. Vero-Maru cell suspension (1:3) was added to each well (100μl/well). Plates were covered with plastic lids and incubated at 37C in a humidified incubator with four percent CO_2 for 10 days. Plates were read for viral cpe. The reciprocal of the highest serum dilution demonstrating 100% neutralization was considered the antiserum titer. Controls used included cell controls, positive and

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[a]Leitz, Wetzlar, Germany, (200W ultra-high-pressure mercury lamp HBO; BG 23, BG 12, BG 38 filters).

negative serum controls, test serum controls, and a virus back titration by half log_{10} dilutions to determine viral dose ($TCID_{50}$/well).

Enzyme-linked Immunosorbent Assay

Antigen preparation

Positive (WC11) and normal host cell (NHC) ELISA antigens were prepared using a modification of the procedure described by Snyder and Erickson (1981) for pseudorabies ELISA antigen production. Differentiation of negative and weak positive antisera was not possible with the first two lots of ELISA antigens. The antigen production procedure was modified, and third lots of WC11 and NHC antigens were prepared. Briefly, for the WC11 antigen, medium was decanted from 20 confluent three day old BT flasks ($150cm^2$), and the cell monolayers were rinsed with F-15 medium to remove residual FBS. Twenty-five milliliters of F-15 medium containing one percent MPS^{tm} Concentrate (100X)[a] was added to each flask. The flasks were inoculated with cell-free WC11 virus (1 ml/flask), and incubated at 34C. When the cell monolayers developed 100% viral cpe (14 days) they were aseptically scraped and decanted into 50 ml centrifuge tubes, and centrifuged at 3000 rpm for 30 minutes at 4C. The clarifed viral supernatant fluids were collected and pooled to yield a final volume of 550 ml. Cellular pellets were discarded. The clarified supernatant fluid was filtered through a 0.45μm millipore filter to remove additional host cell debris. The filtrate was placed in sterile

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[a]K C Biological, Lenexa, Kansas.

polyallomer tubes[a] and centrifuged through a 8 ml 40% sucrose cushion (Appendix VII) at 25,000 rpm for one hour at 4C using a SW28 rotor. The supernatant fluid was decanted from each tube, and 0.5 ml of sterile PBS (0.01M) added. The tubes were covered with Parafilm[b], and placed at 4C overnight to resuspend the viral pellets. The virus suspensions were collected into a glass flask, and the tubes were rinsed three times with sterile PBS to harvest residual virus. The final volume of resuspended virus was 11 ml. Examination of the concentrated virus by electron microscopy revealed only herpesvirus enveloped virions and naked nucleocapsids. An equal volume of antigen solubilization buffer (Appendix VII) was added to the concentrated virus and the mixture was stirred continuously for 24 hours at 4C. The solubilized antigen was centrifuged at 38,000 rpm for 90 minutes at 4C using a type 40.2 small fixed angle rotor. The tubes contained clear supernatant fluid and a small pellet of cell and viral debris. The top 0.2 ml of each tube was discarded to remove any lipoproteins. The remaining fluid containing the solubilized viral proteins (22 ml), was harvested and dispensed in 0.5 ml aliquots in sterile glass serum vials and stored at -70C.

The NHC antigen was produced by an identical procedure, except the BT flasks were not infected with virus. The resulting solubilized NHC antigen (8 ml) was dispensed in 0.5 ml aliquots in sterile glass serum vials and stored at -70C.

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[a]Beckman Instruments, Inc., Palo Alto, California.

[b]American Can Company, Greenwich, Connecticut.

Antigens were evaluated for specificity. Optimal working dilutions were determined by block titrations against four standard controls: serum diluent control, negative serum control, weak positive serum control, and a strong positive serum control. The optimal working dilution of the WC11 antigen was determined to be 1:400. The NHC antigen was then titrated around the WC11 optimal dilution. The dilution of NHC antigen (1:500) that produced mean absorbance values for the serum diluent and negative serum control closest to those of the WC11 antigen was selected.

Enzyme labeled antispecies conjugates

Three ELISA conjugates were used in this study. Optimal working dilutions for each conjugate were determined by block titrations against the standard control sera. Peroxidase labeled affinity purified rabbit antibody to sheep IgG (H + L)-(Cat. No.-04-23-06) was purchased from Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, and used at a 1:400 dilution for testing sera from domestic and exotic ovine species. Peroxidase labeled rabbit anti-goat ELISA conjugate (176R28601) produced by the Reagent Section of the Scientific Services Laboratory, NVSL, was used at a 1:1000 working dilution for testing sera from domestic goats and African pygmy goats. Commercial IgG fraction rabbit anti-bovine IgG (H + L)-(Cat. No.- 0202-0082) which recognized both subclasses of bovine IgG (IgG_1 and IgG_2) was purchased from Organon Teknika Corp., West Chester, PA, and conjugated with horseradish peroxidase by the Reagent Section of Scientific Services Laboratory, NVSL. The conjugate was used at a 1:150 dilution for exotic species and at a 1:200 dilution for members of the genera Bison and Bos.

Coating ELISA microplates

For the screen ELISA, the WC11 antigen was diluted 1:400 in coating buffer (Appendix VII) in a glass flask and kept chilled (4C) while being dispensed into 96 well Immulon I microplates[a] with a high precision pipetting device (100μl/well). The plates were covered with plastic lids, double sealed in plastic bags (three plates/bag), and incubated overnight at 37C on a variable rotator[b] set at 60 rpm. The plates were then placed in a 4C cooler and stored at least overnight prior to use. In the verification ELISA, alternate vertical rows were filled with WC11 and NHC antigen using the same methodology.

Enzyme-linked immunosorbent procedure

The ELISA was performed with modifications by the procedure described by Snyder and Erickson (1981) for the pseudorabies ELISA. Antigen coated microplates were removed from 4C storage and warmed to room temperature. Excess antigen was removed using three consecutive washes with ELISA wash solution (Appendix VII) and a automatic plate washer[a]. Following aspiration of the final wash solution, the plates were inverted and tapped on absorbent material to remove residual wash solution. Test sera and control sera (not heat-inactivated) were diluted 1:20 in serum diluent (0.01M PBS with 0.4% tween-20, and one percent ovalbumin[c] for the genera Bos, genera Bison, and domestic and exotic sheep or 0.01M PBS with 0.4%

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[a]Dynatech Laboratories, Inc., Alexandria, Virginia.

[b]American Dade, Miami, Florida.

[c]Organon Teknika Corp., West Chester, Pennsylvania.

tween-20 for the remaining animal species). Each control serum and the serum diluent control (100µl/well) was placed into four replicate wells of each plate in a standard configuration. Each diluted test serum (100µl/well) was placed into two replicate wells. The plates were covered with plastic lids and incubated at 37C for 30 minutes on a variable rotator set at 60 rpm. Excess serum was removed using three wash cycles with a three minute soak period between the dispensing and aspiration of each wash cycle. Following final aspiration of wash solution, the plates were inverted, and lightly tapped to remove residual wash solution. The appropriate antispecies ELISA conjugate diluted to its working dilution in conjugate diluent (0.01M PBS with 0.4% tween-20, and one percent ovalbumin) was dispensed into each well (50µl/well) with a multichannel pipetting device used exclusively for conjugates. The plates were covered with plastic lids and incubated at 37C for 30 minutes on a variable rotator set at 60 rpm. Excess conjugate was removed using three washes with three minute soak cycles. Substrate (ABTS) solution (Appendix VII) was dispensed into each well (100µl/well) with a multichannel pipetting device used exclusively for substrate. Uncovered plates were incubated at room temperature on a variable rotator set at 60 rpm for the appropriate time dependent on the anti-species conjugate being used. Sixty minutes was used for the rabbit anti-bovine conjugate, 30 minutes screen and 15 minutes verification for the rabbit anti-goat conjugate, and 20 minutes screen and 15 minutes verification for the rabbit anti-sheep conjugate. The Dynatech MR-580 ELISA reader was allowed to warm up at least 30 minutes prior to calibration. The reader was set for a dual wavelength reading with a test wavelength of 405nm and a reference wavelength of

450nm. The reader was calibrated using the substrate solution shortly before the plates were read to eliminate the effect of substrate color. Color change was proportional to time, temperature, and reagent volumes which were all stringently controlled. The 50% positive threshold value was calculated for each plate as the midpoint value between the mean absorbance value of the negative serum control and mean absorbance value of the weak positive serum control. Sera tested and found to be greater than or within five percent of the 50% positive threshold were retested using the verification ELISA. The methodology for the verification ELISA was identical to that used with the screen ELISA except each test serum was placed into two vertical replicate wells for each antigen (WC11 and NHC). The mean WC11 antigen absorbance value was divided by the mean NHC antigen absorbance value to give a signal to noise (S/N) ratio for each serum. For a serum to be considered positive the mean absorbance value had to be above the 50% positive threshold and the S/N ratio had to be greater than or equal to 1.8.

Statistical Analysis

The arithmetic mean, standard deviation, and coefficient of variation were calculated for each serum tested by the screen or verification ELISA. The sensitivity and specificity of each serologic assay as compared to the other assays were calculated as follows:

Sensitivity

$$= \frac{\text{true positives (1st assay) - false positives (1st assay)}}{\text{true positives (2nd assay)}} \times 100$$

Specificity

$$= \frac{\text{true negatives (1st assay) - false negatives (1st assay)}}{\text{true negatives (2nd assay)}} \times 100$$

RESULTS

Development of Antibodies against the WC11 Virus in Experimentally Inoculated Calves

Sera from daily bleeding of calf 9471 and weekly bleeding of gnotobiotic calf #16 were tested by the IFA, the VN, and the screen ELISA to follow antibody development to the WC11 virus (Table 1). Antibodies against the WC11 virus were first detected in calf 9471 at day 11 post-inoculation by all three assays. Antibody titers continued to rise until day 20. A second intravenous inoculation of cell-free WC11 virus on day 23 produced a marked anamnestic response by day 30. The calf was exsanguinated on day 34. Control sera for the ELISA assay were selected from those obtained from the daily bleeding of calf 9471. Days 0, 1, and 2 were used as negative serum controls. Days 14, 15, 16, 17, and a pool of day 13 and day 15 (13/15) were used as weak positive controls, and day 34 was used as the strong positive control. Gnotobiotic calf #16 was first detected as seropositive on day 14 post-inoculation by the IFA and VN assays. Seroconversion by the ELISA was first detected at day 28 post-inoculation, and probably represented a marked anamnestic response to the second intravenous inoculation of cell-free WC11 virus.

Complement Fixation Assay

Block titrations of the WC11, IG30359, and the two normal (BT and VM) complement fixation (CF) antigens against sera from calf 9471 and gnotobiotic calf #16 diluted 1:5 in VBS showed strong (4+) anticomplementary activity with all four undiluted CF antigens, and complete hemolysis with all four antigens at the 1:5 dilution. All test controls reacted appropriately. Antigens were used at the 1:5 dilution in

Table 1. Comparison of antibody development by three serologic assays in calves inoculated intravenously with the WC11 strain of alcelaphine herpesvirus-1

Serial bleedings[b]	Serologic Assays[a] IFA	WC11 VN	Screen ELISA 1	2	3	4	Mean	+/-	SD	CV(%)
calf 9471										
Day 0[c]	<1:20	<1:4	0.063	0.076	0.067	0.085	0.073	-	0.010	13.5
1	<1:20	<1:4	0.054	0.077			0.066	-	0.016	24.8
2	<1:20	<1:4	0.069	0.082			0.076	-	0.009	12.2
3	<1:20	<1:4	0.068	0.077			0.073	-	0.006	8.8
4	<1:20	<1:4	0.108	0.064			0.086	-	0.031	36.2
5	<1:20	<1:4	0.082	0.082			0.082	-	0.000	0.0
6	<1:20	<1:4	0.089	0.091			0.090	-	0.001	1.6
7	<1:20	<1:4	0.077	0.065			0.071	-	0.008	12.0
8	<1:20	<1:4	0.071	0.077			0.074	-	0.004	5.7
9	<1:20	<1:4	0.081	0.093			0.087	-	0.008	9.8
10	<1:20	<1:4	0.102	0.086			0.094	-	0.011	12.0
11	1:20	1:4	0.107	0.115			0.111	+	0.006	5.1
12	1:40	1:4	0.144	0.124			0.134	+	0.014	10.6
13	1:80	1:8	0.143	0.159			0.151	+	0.011	7.5
14	1:160	1:8	0.173	0.164			0.169	+	0.006	3.8
15	1:320	1:16	0.186	0.163			0.175	+	0.016	9.3
16	1:320	1:16	0.202	0.194			0.198	+	0.006	2.9
17	1:640	1:32	0.177	0.188			0.183	+	0.008	4.3
18	1:640	1:32	0.201	0.191			0.196	+	0.007	3.6
19	1:1280	1:32	0.218	0.218			0.218	+	0.000	0.0
20	1:1280	1:32	0.230	0.241			0.236	+	0.008	3.3
21	1:1280	1:32	0.275	0.273			0.274	+	0.001	0.5
23[c]	1:1280	1:64	0.290	0.323			0.307	+	0.023	7.6
30	1:10240	1:512	0.551	0.582			0.567	+	0.022	3.9
34	1:5120	1:512	0.672	0.445	0.590	0.758	0.616	+	0.133	21.6
13/15[d]	ND	ND	0.128	0.141	0.169	0.146	0.146	+	0.017	11.7
Dil	ND	ND	0.012	0.010	0.013	0.008	0.011	-	0.002	20.6

gnotobiotic calf 16								
Day 0[c]	<1:20	<1:4	0.013	0.018	0.016	-	0.004	22.8
7	<1:20	<1:4	0.012	0.017	0.015	-	0.004	24.4
14	1:80	1:4	0.026	0.038	0.032	-	0.008	26.5
21[c]	1:640	1:8	0.053	0.061	0.057	-	0.006	9.9
28	1:2560	1:32	0.226	0.189	0.208	+	0.026	12.6
32	1:2560	1:32-64	0.212	0.212	0.212	+	0.000	0.0

[a]IFA = indirect immunofluorescence assay (WC11 antigen); WC11 VN = virus neutralization assay (100 $TCID_{50}$); Screen ELISA = screen enzyme-linked immunosorbent assay-3rd WC11 antigen 1:400, RAB ELISA conjugate 920BPV8402 1:150, ABTS substrate 60 minutes; Mean = arithmetic mean; SD = standard deviation; CV(%) = coefficienct of variation.

[b]Thirty milliliters of whole blood was drawn each serial bleeding.

[c]Indicates days the calves were inoculated intravenously with WC11 strain AHV-1.

[d]Weak positive ELISA control serum.

two further CF assays using IFA positive field sera. The first assay used a three hour complement fixation period at 37C, and the second an overnight 4C complement fixation period. All of the sera tested were negative for complement fixing antibodies. Since it wasn't known if any of the sera tested actually contained complement fixing antibodies, it could not be determined if the CF antigens were functional. Review of the literature on use of the CF assay for detection of AHV-1 antibody revealed the CF assay to be less sensitive than either the VN or the IFA (Kalunda, 1975; Hamdy et al., 1980). Further work to develop this assay was not done.

Determination of Identity between Exotic Species IgG and Bovine IgG

The double diffusion technique demonstrated precipitin lines of complete or partial identity between bovine IgG and the immunoglobulins of 55 of the animal species tested (Table 2). All 55 species demonstrated one strong precipitin line of identity with bovine IgG, and at least one weak precipitin line of identity or partial identity with bovine IgG. Precipitin lines of nonidentity with bovine IgG were demonstrated with members of the family Equidae and family Camelidae.

Based on these data, it was deemed acceptable to use rabbit anti-bovine conjugate to test the 55 species in both the IFA and the ELISA.

Table 2. Determination of identity between immunoglobulins from exotic species and bovine IgG[a] by double diffusion using IgG fraction of rabbit anti-bovine IgG[b]

Species which have good identity with Bovine IgG:

Family Cervidae
- Reeves muntjac (Muntiacus reevesi)
- Elds deer (Cervus eldi thamin)
- Formosan sika deer (Cervus nippon taiwanus)
- Brown elk (Cervus elaphus)
- Tule elk (Cervus elaphus nannodes)
- Barasingha (Cervus duvauceli)
- Axis deer (Cervus axis)
- Pere Davids deer (Elaphurus davidianus)
- Chinese water deer (Hydropotes inermis)
- White-tailed deer (Odocoileus virginianus)
- Reindeer (Rangifer tarandus)

Family Giraffidae
- Giraffe (Giraffa camelopardalis)
- Okapi (Okapia johnstoni)

Family Antilocapridae
- Pronghorn (Antilocapra americana)

Family Bovidae
- American bison (Bison bison)
- European bison/Wisent (Bison bonasus)
- Indian gaur (Bos gaurus)
- Ankole (Bos taurus)
- Banteng (Bos javanicus)
- Scottish highland cattle (Bos taurus)
- Domestic cattle (Bos taurus)
- Yak (Bos mutus)
- Cape buffalo (Syncerus caffer caffer)
- Eland (Taurotragus oryx)
- Nyala (Tragelaphus angasi)
- Sitatunga (Tragelaphus spekei)

Family Bovidae continued
- Greater kudu (Tragelaphus strepsiceros)
- Black duiker (Cephalophus niger)
- Waterbuck (Kobus ellipsiprymnus)
- Nile lechwe (Kobus megaceros)
- Cape hartebeest (Alcelaphus buselaphus caama)
- Wildebeest (Connochaetes sp.)
- Hunters hartebeest (Damaliscus hunteri)
- Blesbok (Damaliscus dorcas phillipsi)
- Addax (Addax nasomaculatus)
- Sable antelope (Hippotragus niger)
- Scimitar-horned oryx (Oryx dammah)
- Beisa oryx (Oryx gazella beisa)
- Gemsbok (Oryx gazella gazella)
- Fringe-eared oryx (Oryx gazella callotis)
- Arabian oryx (Oryx leucoryx)
- Blackbuck (Antilope cervicapra)
- Grants gazelle (Gazella granti)
- Thomsons gazelle (Gazella thomsoni)
- Persian gazelle (Gazella subgutterosa)
- Dama gazelle (Gazella dama)
- Spekes gazelle (Gazella spekei)
- Gerenuk (Litocranius walleri)
- Dik dik (Madoqua sp.)
- Suni (Neotragus moschatus)
- Klipspringer (Oreotragus oreotragus)
- Markhor (Capra falconeri)
- Nubian ibex (Capra ibex nubiana)
- Chamois (Rupicapra rupicapra)
- Saiga antelope (Saiga tatarica)

Species which do not have good identity with Bovine IgG:

Family Equidae
Zebra (Equus sp.)
Family Camelidae
Camel (Camelus sp.)
Llama (Lama glama glama)
Alpaca (Lama glama pacos)

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[a]Bovine IgG (Cat. No. 6002-0080), Organon Teknika Corp., West Chester, PA.

[b]IgG fraction-RAB IgG (H + L)-(Cat. No. 0202-0082), Organon Teknika Corp., West Chester, PA. This product recognized both subclasses of bovine IgG (IgG1 and IgG2).

Antigenic Relationships Between AHV-1, BHV 1-3, and PHV-1

Antigenic relationships detected by the indirect immunofluorescence assay

All antisera were diluted 1:20 in cold PBS (0.01M) and screened against each of the six viral antigens (Table 3). Sera determined to be IFA positive against any of the six viral antigens at the 1:20 dilution were titrated to determine the endpoint titer. Positive AHV-1 antisera did not demonstrate antigenic cross reactivity with any of the other viral antigens. Antigenic cross reactivity between BHV-1 antisera of bovine origin and the PHV-1 viral antigens were seen at endpoint dilutions 16 to 64-fold lower than their respective homologous BHV-1 endpoint titers. Antigenic cross reactivity between BHV-1 antisera of porcine origin and the PHV-1 viral antigens were not demonstrated. Antigenic cross reactivity between BHV-2 antisera and other viral antigens were not demonstrated. Positive BHV-3 antisera demonstrated antigenic cross reactivity with both the WC11 and the IG30359 viral antigens up to a dilution of 1:100. Calf 51 was seropositive for BHV-1 prior to inoculation with DN599 virus. Antigenic cross reactivity between PHV-1 antisera and the other viral antigens were not demonstrated.

Antigenic relationships detected by the virus neutralization assay

The same antisera were tested by standard virus neutralization assays for each of the six viral antigens to detect antigenic relationships between the AHV-1 group of viruses and other herpesvirus. Homologous virus neutralizing antibody titers were determined for each antiserum. Antisera against the BHV-3 virus did not demonstrate positive homologous neutralizing antibody titers. Antigenic cross reactivity between the six

Table 3. Antigenic relationships among alcelaphine (AHV-1) herpesvirus-1, bovine (BHV 1-3), and porcine (PHV-1) herpesviruses as detected by the indirect immunofluorescence assay

	Virus Infected Cover Slips[a,b]					
	AHV-1		BHV-1	BHV-2	BHV-3	PHV-1
Antisera[c]	WC11	IG30359	IBR	BHM	DN599	PRV
AHV-1						
3548102	5120	5120	<20	<20	<20	<20
30359	320	640	<20	<20	<20	<20
30282A	160	320	<20	<20	<20	<20
WAP 43	5120	10240	<20	<20	<20	<20
831041	320	320	<20	<20	<20	<20
84037	1280	1280	<20	<20	<20	<20
BHV-1						
C5763	<20	<20	10240	<20	<20	160
3508201	<20	<20	5120	<20	<20	320
Pig #1	<20	<20	2560	<20	<20	<20
Pig #3	<20	<20	5120	<20	<20	<20
Pig #4	<20	<20	10240	<20	<20	<20
GC[a]33	<20	<20	5120	<20	<20	160
BHV-2						
3518201	<20	<20	<20	20480	<20	<20
32	<20	<20	<20	1280	<20	<20
56B	<20	<20	<20	2560	<20	<20
61	<20	<20	<20	640	<20	<20
GC36	<20	<20	<20	5120	<20	<20

BHV-3						
3528201	<20	<20	<20	<20	20480	<20
8549	100	100	<20	<20	5120	<20
41	<20	20	<20	<20	2560	<20
51	<20	<20	160	<20	320	<20
52	<20	<20	<20	<20	1280	<20
GC35	100	100	<20	<20	5120	<20
PHV-1						
3389	<20	<20	<20	<20	<20	2560
B302	<20	<20	<20	<20	<20	1280
19289	<20	<20	<20	<20	<20	5120
A266	<20	<20	<20	<20	<20	2560
R239	<20	<20	<20	<20	<20	2560
Controls						
4808301	<20	<20	<20	<20	<20	<20
VS512	<20	<20	<20	<20	<20	<20
4808601	<20	<20	<20	<20	<20	<20

[a]WC11 = blue wildebeest AHV-1 isolate (Plowright 1965b); IG30359 = Indian gaur AHV-1 (Castro et al., 1981); BHV-1 (IBR) = infectious bovine rhinotracheitis; BHV-2 (BHM) = bovine herpes mammillitis; BHV-3 (DN599) = movar bovine herpesvirus; PHV-1 (PRV) = pseudorabies; GC = gnotobiotic calf.

[b]Virus infected cover slips were prepared using bovine turbinate monolayers.

[c]Antisera were diluted 1:20 in cold phosphate buffered saline (0.01M, pH 7.2).

herpes viruses was not demonstrated using virus neutralization assays (Table 4).

Antigenic relationships detected by the enzyme-linked immunosorbent assay

Bovine origin BHV 1-3 antisera, and normal bovine antisera were tested by the screen WC11 ELISA. Antigenic cross reactivity between the WC11 antigen and BHV 1-3 antisera were not demonstrated (Table 5).

Indirect Immunofluorescence Assay

Sera from 63 different animal species were tested by the indirect immunofluorescence assay for antibodies against alcelaphine herpesvirus-1. Of the 1089 animals tested, 236 (21.7%) were found to be IFA positive at the 1:20 serum dilution. Twenty-six different species were represented among the positive animals. The 236 IFA positive animals were retested at the 1:100 serum dilution. Of the 236 animls retested, 178 (75.4%) were IFA positive at the 1:100 dilution. Twenty-two species were represented. Positive animals included both AHV-1 infected animals as confirmed by virus isolation, and histopathologically confirmed cases of sheep-goat-associated MCF. A summary of the IFA data at both the 1:20 and 1:100 dilutions is presented by family and subfamily in Table 6, and by species in Table 7.

Virus Neutralization Assay

The same 1089 animals were tested by standard virus neutralization (VN) assays with both AHV-1 viral isolates. Neutralizing antibodies against the WC11 virus were detected in 116 animals (10.7%) representing 15 different species with VN titers ranging from 1:4 to 1:512. Sixteen different species, and 119 animals (10.9%) were IG30359 VN positive with

Table 4. Antigenic relationships among alcelaphine (AHV-1) herpesvirus-1, bovine (BHV 1-3), and porcine (PHV-1) herpesviruses as detected by the virus neutralization assay

	Virus Neutralization Titers[a,b]					
	AHV-1		BHV-1	BHV-2	BHV-3	PHV-1
Antisera[c]	WC11	IG30359	IBR	BHM	DN599	PRV
AHV-1						
3548102	128	128	<4	<4	<4	<4
30359	<4	<4	<4	<4	<4	<4
30282A	<4	4	<4	NTIS[d]	<4	<4
WAP 43	512	512	<4	<4	<4	<4
831041	32	32	<4	<4	<4	<4
84037	128	128	<4	<4	<4	<4
BHV-1						
C5763	<4	<4	256	<4	<4	<4
3508201	<4	<4	256	<4	<4	<4
Pig #1	<4	<4	64	<4	<4	<4
Pig #3	<4	<4	256	<4	<4	<4
Pig #4	<4	<4	128	<4	<4	<4
GC[a]33	<4	<4	32	<4	<4	<4
BHV-2						
3518201	<4	<4	<4	256	<4	<4
32	<4	<4	<4	16	<4	<4
56B	<4	<4	<4	16	<4	<4
61	<4	<4	<4	8	<4	<4
GC36	<4	<4	<4	4	<4	<4

BHV-3						
3528201	<4	<4	<4	<4	<4	<4
8549	<4	<4	<4	<4	<4	<4
41	<4	<4	<4	NTIS	<4	<4
51	<4	<4	4	NTIS	<4	<4
52	<4	<4	<4	NTIS	<4	<4
GC35	<4	<4	<4	<4	<4	<4
PHV-1						
3389	<4	<4	<4	<4	<4	64
B302	<4	<4	<4	<4	<4	64
19289	<4	<4	<4	<4	<4	128
A266	<4	<4	<4	<4	<4	64
R239	<4	<4	<4	<4	<4	64
Controls						
4808301	<4	<4	<4	<4	<4	<4
VS512	<4	<4	<4	<4	<4	<4
4808601	<4	<4	<4	<4	<4	<4

[a]WC11 = blue wildebeest AHV-1 isolate (Plowright 1965b); IG30359 = Indian gaur AHV-1 (Castro et al., 1981); BHV-1 (IBR) = infectious bovine rhinotracheitis; BHV-2 (BHM) = bovine herpes mammillitis; BHV-3 (DN599) = movar bovine herpesvirus; PHV-1 (PRV) = pseudorabies; GC = gnotobiotic calf.

[b]Standard microtiter plate virus neutralization assays were performed for each virus.

[c]Antisera were heat inactivated at 56C for 30 minutes.

[d]NTIS = no test insufficient serum.

Table 5. Antigenic relationships between the WC11 ELISA antigen and bovine herpesviruses (BHV 1-3) as detected by the enzyme-linked immunosorbent assay

	WC11 Enzyme-Linked Immunosorbent Assay[a,b]						
Antisera[c,d]	1	2	Mean	PTH[b]	+/-	SD[b]	CV(%)[b]
BHV-1							
C5763	0.059	0.064	0.062	0.107	-	0.004	5.7
3508201	0.032	0.035	0.034	0.098	-	0.002	6.3
GC[d]33	0.008	0.006	0.007	0.098	-	0.001	20.2
BHV-2							
3518201	0.020	0.017	0.019	0.098	-	0.002	11.5
32	0.035	0.032	0.034	0.114	-	0.002	6.3
56B	0.055	0.064	0.060	0.098	-	0.006	10.7
61	0.045	0.049	0.047	0.107	-	0.003	6.0
GC36	0.005	0.006	0.006	0.098	-	0.001	12.9
BHV-3							
3528201	0.042	0.044	0.043	0.107	-	0.001	3.3
8549	0.058	0.055	0.057	0.098	-	0.002	3.8
41	0.036	0.036	0.036	0.107	-	0.000	0.0
51	0.018	0.019	0.019	0.098	-	0.001	3.8
52	0.027	0.026	0.027	0.098	-	0.001	2.7
GC35	0.009	0.009	0.009	0.098	-	0.000	0.0
Controls							
4808301	0.032	0.034	0.033	0.098	-	0.001	4.3
VS512	0.026	0.025	0.026	0.098	-	0.001	2.8
4808601	0.005	0.004	0.005	0.099	-	0.001	15.7

[a]WC11 ELISA-3rd WC11 antigen 1:400, RAB ELA conjugate 920BPV8402 1:200, ABTS substrate 60 minutes; WC11 = blue wildebeest AHV-1 isolate (Plowright 1965b).

[b]PTH = 50% positive threshold; SD = standard deviation; CV(%) = coefficient of variation.

[c]Antisera were diluted 1:20 in PBS (0.01M) with 0.4% tween-20, and one percent ovalbumin.

[d]BHV-1 = infectious bovine rhinotracheitis; BHV-2 = bovine herpes mammillitis; BHV-3 = movar group of bovine herpesvirus; GC = gnotobiotic calf.

VN titers ranging from 1:4 to 1:512. A Summary of the VN data for both AHV-1 isolates is presented by family and subfamily in Table 6, and by species in Table 7.

Enzyme-Linked Immunosorbent Assay

The same 1089 animals were tested by the screen ELISA. Two hundred and thirty-two animals (21.3%) representing 27 species were positive for antibodies against the WC11 antigen. The positive sera were retested using the WC11 verification ELISA. Two hundred and eighteen of the 232 animals (94%) representing 25 species were verified as ELISA positive. ELISA positive sera were from animals with wildebeest-derived MCF, animals with histopathologically confirmed clinical cases of sheep-goat-associated MCF, and from reservoir host species. A summary of the data by family and subfamily is presented in Table 6, and by species in Table 7.

Direct Comparison of the Serologic Assays

Direct comparison of the serologic assays was completed using the 1089 animals representing 63 different species from which serum had been tested for the presence of antibodies against the WC11 virus by all three assays (Table 8). Eight hundred and three animals (73.7%) representing 33 different species were seronegative by all three assays. One hundred and fourteen animals (10.5%) representing 15 different species were seropositive by all three assays. The serological results differed assay to assay for the remaining 172 animals (15.8%). Serological results of the 172 animals were as follows: 50 IFA negative, 122 IFA positive, 136 VN negative, two VN positive, 34 VN no test, 68 ELISA negative, and 104 ELISA positive. Pairs of serologic assays were compared for sensitivity and specificity. Comparison of the indirect immunofluorescence assay and

Table 6. Serological data summarized by family and subfamily

Family subfamily	Number of animals	Serologic Assays[a] IFA 1:20 number pos	IFA 1:100 number pos	IG30359 VN number pos	WC11 VN number pos
Family Equidae	7	0	NDb	0	0
Mean ± SDb				<4	<4
(range)					
Family Camelidae	10	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Family Cervidae					
subfamily Muntiacinae	17	0	ND	0	0
Mean ± SD				<4	<4
(range)					
subfamily Cervinae	23	5	5	4	4
Mean ± SD				177.0 ± 140.3	177.0 ± 140.3
(range)				(4-512)	(4-512)
subfamily Odocoileinae	101	4	3	0	0
Mean ± SD				<4	<4
(range)					
Family Giraffidae	3	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Family Antilocapridae	1	1	0	0	0
Mean ± SD				NTIS	<4
(range)					
Family Bovidae					
subfamily Bovinae	393	32	24	6	6
Mean ± SD				8.7 ± 11.4	6.7 ± 4.8
(range)				(4-32)	(4-16)
subfamily Reduncinae	8	0	ND	0	0
Mean ± SD				<4	<4
(range)					
subfamily Alcelaphinae	138	96	88	83	82
Mean ± SD				50.0 ± 54.2	45.9 ± 57.0
(range)				(4-256)	(4-256)
subfamily Hippotraginae	97	21	17	16	14
Mean ± SD				14.4 ± 11.3	21.9 ± 20.1
(range)				(4-32)	(4-64)
subfamily Antilopinae	50	1	1	1	1
Mean ± SD				8	8
(range)					
subfamily Caprinae	241	76	40	9	9
Mean ± SD				18.7 ± 41.0	18.2 ± 41.2
(range)				(4-128)	(4-128)

[a]IFA = indirect immunofluorescence assay; VN = virus neutralization assay; ELISA = enzyme-linked immunosorbent assay.

[b]Mean ± SD = mean VN titer or absorbance value ± one standard deviation; ND = not done; NTIS = no test insufficient serum.

Screen ELISA		Verification Enzyme-Linked Immunosorbent Assay			
number neg	number pos	number neg		number pos	
WC11 antigen	WC11 antigen	WC11 antigen	NHC antigen	WC11 antigen	NHC antigen
7					
0.043 ± 0.020					
(0.026-0.086)					
10					
0.021 ± 0.011					
(0.007-0.037)					
17					
0.061 ± 0.011					
(0.037-0.084)					
19	4			4	
0.064 ± 0.018	0.510 ± 0.253			0.455 ± 0.215	0.026 ± 0.007
(0.032-0.097)	(0.170-1.072)			(0.156-0.777)	(0.018-0.034)
96	5			5	
0.057 ± 0.019	0.305 ± 0.215			0.286 ± 0.194	0.047 ± 0.030
(0.015-0.106)	(0.165-0.676)			(0.146-0.608)	(0.026-0.097)
3					
0.034 ± 0.007					
(0.029-0.042)					
1					
0.059 ± 0.0					
351	42	14		28	
0.057 ± 0.018	0.236 ± 0.124	0.174 ± 0.066	0.160 ± 0.065	0.248 ± 0.104	0.055 ± 0.025
(0.014-0.098)	(0.105-0.618)	(0.108-0.378)	(0.097-0.365)	(0.113-0.450)	(0.031-0.138)
8					
0.053 ± 0.014					
(0.026-0.071)					
47	91			91	
0.045 ± 0.021	0.391 ± 0.161			0.385 ± 0.167	0.045 ± 0.029
(0.008-0.111)	(0.161-0.995)			(0.165-0.932	(0.002-0.150)
77	20			20	
0.048 ± 0.015	0.304 ± 0.132			0.302 ± 0.126	0.043 ± 0.023
(0.017-0.089)	(0.136-0.592)			(0.117-0.531)	(0.019-0.104)
48	2			2	
0.047 ± 0.015	0.167 ± 0.031			0.171 ± 0.058	0.020 ± 0.005
(0.011-0.083)	(0.145-0.189)			(0.130-0.212)	(0.016-0.023)
173	68			68	
0.066 ± 0.039	0.256 ± 0.092			0.273 ± 0.095	0.045 ± 0.022
(0.013-0.170)	(0.119-0.516)			(0.127-0.515)	(0.011-0.109)

Table 7. Serological data summarized by species

Family subfamily species	Number of animals	Serologic Assays[a] IFA 1:20 number pos	IFA 1:100 number pos	IG30359 VN number pos	WC11 VN number pos
Family Equidae					
Zebra	7	0	NDb	0	0
Mean ± SDb				<4	<4
(range)					
Family Camelidae					
Camel	7	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Llama	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Alpaca	2	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Family Cervidae					
subfamily Muntiacinae					
Reeves muntjac	17	0	ND	0	0
Mean ± SD				<4	<4
(range)					
subfamily Cervinae					
Elds deer	2	2	2	1	1
Mean ± SD				256	256
(range)					
Formosan sika deer	5	2	2	2	2
Mean ± SD				162 ± 223.4	162 ± 223.4
(range)				(4-512)	(4-512)
Brown elk	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Tule elk	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Barasingha	7	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Axis deer	3	1	1	1	1
Mean ± SD				128	128
(range)					
Pere Davids deer	4	0	ND	0	0
Mean ± SD				<4	<4
(range)					

[a]IFA = indirect immunofluorescence assay; VN = virus neutralization assay; ELISA = enzyme-linked immunosorbent assay.

[b]Mean ± SD = mean VN titer or absorbance value ± one standard deviation; ND = not done; NT = no test; NTIS = insufficient serum.

Screen ELISA		Verification Enzyme-Linked Immunosorbent Assay			
number neg	number pos	number neg		number pos	
WC11 antigen	WC11 antigen	WC11 antigen	NHC antigen	WC11 antigen	NHC antig
7 0.043 ± 0.020 (0.026-0.086)					
7 0.020 ± 0.011 (0.007-0.037)					
1 0.009 ± 0.0					
2 0.030 ± 0.002 (0.028-0.031)					
17 0.061 ± 0.011 (0.037-0.084)					
1 0.062 ± 0.0	1 0.553 ± 0.0			1 0.441 ± 0.0	0.022 ± 0
3 0.064 ± 0.014 (0.048-0.074)	2 0.477 ± 0.433 (0.170-1.072)			2 0.390 ± 0.330 (0.156-0.777)	0.025 ± 0 (0.018-0.
1 0.097 ± 0.0					
1 0.072 ± 0.0					
7 0.060 ± 0.019 (0.033-0.094)					
2 0.081 ± 0.008 (0.075-0.087)	1 0.534 ± 0.0			1 0.598 ± 0.0	0 .034 ±
4 0.052 ± 0.019 (0.032-0.078)					

Table 7. (continued)

Family subfamily species	Number of animals	Serologic Assays IFA 1:20 number pos	IFA 1:100 number pos	IG30359 VN number pos	WC11 VN number pos
subfamily Odocoileinae					
Chinese water deer	2	1	0	0	0
Mean ± SD[b]				<4	<4
(range)					
White-tailed deer	92	3	3	0	0
Mean ± SD				<4	<4
(range)					
Reindeer	7	0	ND[b]	0	0
Mean ± SD				<4	<4
(range)					
Family Giraffidae					
Giraffe	2	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Okapi	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Family Antilocapridae					
Pronghorn	1	1	0	0	0
Mean ± SD				NTIS[b]	<4
(range)					
Family Bovidae					
subfamily Bovinae					
American bison	12	1	0	0	0
Mean ± SD				<4	<4
(range)					
European bison/Wisent	5	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Indian gaur	14	4	3	1	0
Mean ± SD				4	<4
(range)					
Ankole	4	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Bantang	11	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Scottish highland	2	1	1	0	0
Mean ± SD				<4	<4
(range)					
Yak	2	0	ND	0	0
Mean ± SD				<4	<4
(range)					

Screen ELISA		Verification Enzyme-Linked Immunosorbent Assay			
number neg WC11 antigen	number pos WC11 antigen	number neg WC11 antigen	number neg NHC antigen	number pos WC11 antigen	number pos NHC antig
2 0.051 ± 0.043 (0.020-0.081)					
87 0.058 ± 0.019 (0.015-0.106)	5 0.305 ± 0.215 (0.165-0.676)			5 0.286 ± 0.194 (0.146-0.608)	5 0.047 ± 0 (0.026-0.
7 0.048 ± 0.014 (0.025-0.071)					
2 0.031 ± 0.002 (0.029-0.032)					
1 0.042 ± 0.0					
1 0.059 ± 0.0					
10 0.062 ± 0.013 (0.032-0.086)	2 0.121 ± 0.023 (0.015-0.137)	1 0.179 ± 0.0	1 0.188 ± 0.0	1 0.114 ± 0.0	1 0.055 ± 0
5 0.037 ± 0.011 (0.019-0.048)					
12 0.034 ± 0.007 (0.020-0.052)	2 0.166 ± 0.031 (0.144-0.189)			2 0.200 ± 0.035 (0.175-0.254)	2 0.037 ± 0 (0.029-0.
3 0.038 ± 0.013 (0.024-0.050)	1 0.157 ± 0.0	1 0.191 ± 0.0	1 0.167 ± 0.0		
11 0.043 ± 0.015 (0.025-0.074)					
1 0.031 ± 0.0	1 0.176 ± 0.0			1 0.205 ± 0.0	1 0.072 ± 0
2 0.047 ± 0.006 (0.039-0.062)					

Table 7. (continued)

Family subfamily species	Number of animals	Serologic Assays IFA 1:20 number pos	IFA 1:100 number pos	IG30359 VN number pos	WC11 VN number pos
subfamily Bovinae					
Domestic cattle	251	26	20	5	6
Mean ± SD				9.6 ± 12.5	6.7 ± 4.8
(range)				(4-32)	(4-16)
Cape buffalo	15	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Eland	30	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Nyala	15	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Sitatunga	21	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Greater kudu	11	0	ND	0	0
Mean ± SD				<4	<4
(range)					
subfamily Reducinae					
Waterbuck	7	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Nile lechwe	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
subfamily Alcelaphinae					
Hartebeest	26	23	20	18	18
Mean ± SD				18 ± 16.4	17 ± 16.6
(range)				(4-64)	(4-64)
Wildebeest	107	73	68	65	64
Mean ± SD				59 ± 57.5	3.9 ± 61.7
(range)				(4-256)	(4-256)
Hunters hartebeest	4	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Blesbok	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
subfamily Hippotraginae					
Addax	35	8	7	6	4
Mean ± SD				5.7 ± 2.0	7.5 ± 3.4
(range)				(4-8)	(4-16)

Screen ELISA		Verification Enzyme-Linked Immunosorbent Assay			
number neg	number pos	number neg		number pos	
WC11 antigen	WC11 antigen	WC11 antigen	NHC antigen	WC11 antigen	NHC antig
216	35	11		24	
0.060 ± 0.018	0.254 ± 0.129	0.174 ± 0.075	0.160 ± 0.072	0.259 ± 0.107	0.056 ± 0
(0.014-0.097)	(0.117-0.618)	(0.108-0.378)	(0.097-0.365)	(0.113-0.450)	(0.031-0.
15					
0.040 ± 0.016					
(0.016-0.075)					
30					
0.052 ± 0.015					
(0.031-0.095)					
15					
0.063 ± 0.017					
(0.026-0.097)					
20	1	1			
0.069 ± 0.017	0.126 ± 0.0	0.155 ± 0.0	0.118 ± 0.0		
(0.042-0.098)					
11					
0.044 ± 0.009					
(0.023-0.062)					
7					
0.053 ± 0.015					
(0.024-0.071)					
1					
0.050 ± 0.0					
5	21			21	
0.066 ± 0.044	0.267 ± 0.074			0.236 ± 0.060	0.020 ± 0
(0.008-0.111)	(0.166-0.419)			(0.165-0.392)	(0.012-0.
37	70			70	
0.044 ± 0.016	0.429 ± 0.161			0.430 ± 0.163	0.053 ± 0
(0.012-0.083)	(0.161-0.995)			(0.167-0.932)	(0.002-0.
4					
0.037 ± 0.006					
(0.029-0.043)					
1					
0.038 ± 0.0					
27	8			8	
0.046 ± 0.014	0.244 ± 0.048			0.261 ± 0.053	0.060 ± 0
(0.017-0.096)	(0.173-0.342)			(0.178-0.359)	(0.019-0.

Table 7. (continued)

Family subfamily species	Number of animals	Serologic Assays IFA 1:20 number pos	IFA 1:100 number pos	IG30359 VN number pos	WC11 VN number pos
subfamily Hippotraginae					
Sable antelope*	26	1	0	0	0
Mean ± SD				<4	<4
(range)					
Scimitar-horned oryx	2	2	2	2	2
Mean ± SD				10 ± 8.5	10 ± 8.5
(range)				(4-16)	(4-16)
Beisa oryx	7	6	6	6	6
Mean ± SD				24 ± 12.4	33 ± 25
(range)				(8-32)	(4-64)
Gemsbok	15	2	1	1	1
Mean ± SD				16	24
(range)					
Fringe-eared oryx	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Arabian oryx	11	2	1	1	1
Mean ± SD				16	32
(range)					
subfamily Antilopinae					
Blackbuck	2	1	1	1	1
Mean ± SD				8	8
(range)					
Grants gazelle	30	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Thomsons gazelle	2	0	ND	NT[b]	NT
Mean ± SD					
(range)					
Persian gazella	9	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Dama gazelle	5	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Spekes gazelle	1	0	ND	0	0
Mean ± SD				NTIS	<4
Suni	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					

Screen ELISA		Verification Enzyme-Linked Immunosorbent Assay			
number neg	number pos	number neg		number pos	
WC11 antigen	WC11 antigen	WC11 antigen	NHC antigen	WC11 antigen	NHC antig
25	1				1
0.057 ± 0.015	0.136 ± 0.0			0.117 ± 0.0	0.032 ± 0
(0.024-0.89)					
	2				2
	0.228 ± 0.018			0.211 ± 0.016	0.029 ± 0
	(0.215-0.240)			(0.199-0.222)	
1	6				6
0.044 ± 0.0	0.434 ± 0.143			0.420 ± 0.131	0.035 ± 0
	(0.237-0.592)			(0.250-0.531)	(0.022-0.
13	2				2
0.038 ± 0.011	0.247 ± 0.127			0.237 ± 0.144	0.024 ± 0
(0.023-0.072)	(0.157-0.349)			(0.135-0.372)	(0.016-0.
1					
0.069 ± 0.0					
10	1				1
0.045 ± 0.015	0.449 ± 0.0			0.434 ± 0.0	0.026 ± 0
(0.024-0.067)					
1	1				1
0.083 ± 0.0	0.189 ± 0.0			0.212 ± 0.0	0.016 ± 0
30					
0.052 ± 0.013					
(0.011-0.082)					
1	1				1
0.036 ± 0.0	0.145 ± 0.0			0.130 ± 0.0	0.023 ± 0
9					
0.036 ± 0.01					
(0.022-0.061)					
5					
0.037 ± 0.012					
(0.025-0.057)					
1					
0.028 ± 0.0					
1					
0.032 ± 0.0					

Table 7. (continued)

Family subfamily species	Number of animals	Serologic Assays IFA 1:20 number pos	IFA 1:100 number pos	IG30359 VN number pos	WC11 VN number pos
subfamily Caprinae					
Aoudad/Barbary sheep	3	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Markhor	28	5	2	0	0
Mean ± SD				<4	<4
(range)					
Nubian ibex	35	27	11	2	2
Mean ± SD				6 ± 2.8	6 ± 2.8
(range)				(4-8)	(4-8)
Domestic goat	50	18	14	2	1
Mean ± SD				6 ± 2.8	4
(range)				(4-8)	
African pygmy goat	37	5	3	0	0
Mean ± SD				<4	<4
(range)					
Four horn sheep	7	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Lincoln lamb	1	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Dalls sheep	3	0	ND	0	0
Mean ± SD				<4	<4
(range)					
Mouflon	6	2	1	0	0
Mean ± SD				<4	<4
(range)					
Domestic sheep	49	17	7	5	6
Mean ± SD				29 ± 55.5	25 ± 50.6
(range)				(4-128)	(4-128)
Chamois	17	2	2	0	0
Mean ± SD				<4	<4
(range)					
Saiga antelope	5	0	ND	0	0
Mean ± SD				<4	<4
(range)					

Screen ELISA		Verification Enzyme-Linked Immunosorbent Assay			
number neg	number pos	number neg		number pos	
WC11 antigen	WC11 antigen	WC11 antigen	NHC antigen	WC11 antigen	NHC antig
3					
0.071 ± 0.024					
(0.054-0.099)					
22	6			6	
0.052 ± 0.023	0.237 ± 0.051			0.213 ± 0.082	0.027 ± 0
(0.023-0.102)	(0.151-0.289)			(0.136-0.353)	(0.022-0.
9	26			26	
0.058 ± 0.015	0.235 ± 0.093			0.242 ± 0.086	0.034 ± 0
(0.028-0.093)	(0.119-0.477)			(0.126-0.465)	(0.024-0.
37	13			13	
0.065 ± 0.040	0.239 ± 0.097			0.308 ± 0.083	0.066 ± 0
(0.017-0.146)	(0.134-0.516)			(0.186-0.505)	(0.037-0.
36	1			1	
0.041 ± 0.025	0.446 ± 0.0			0.429 ± 0.0	0.100 ± 0
(0.013-0.121)					
7					
0.043 ± 0.013					
(0.028-0.060)					
1					
0.088 ± 0.0					
3					
0.081 ± 0.013					
(0.066-0.090)					
4	2			2	
0.097 ± 0.026	0.289 ± 0.055			0.263 ± 0.018	0.046 ± 0
(0.074-0.133)	(0.250-0.328)			(0.250-0.275)	(0.030-0.
32	17			17	
0.116 ± 0.032	0.288 ± 0.090			0.308 ± 0.104	0.054 ± 0
(0.056-0.170)	(0.184-0.507)			(0.140-0.515)	(0.011-0.
14	3			3	
0.055 ± 0.018	0.291 ± 0.061			0.270 ± 0.051	0.023 ± 0
(0.021-0.086)	(0.221-0.333)			(0.240-0.329)	(0.020-0.
5					
0.040 ± 0.012					
(0.029-0.061)					

the virus neutralization assay is presented in Table 9. The calculated sensitivity of the immunofluorescence assay, compared with that of the virus neutralization, was 99.1%. The specificity of the IFA was 87.6%. The calculated sensitivity of the virus neutralization, compared with that of the indirect immunofluorescence assay, was 49.8%. The specificity of the VN was 99.9%. Comparison of the indirect immunofluorescence assay and the enzyme-linked immunosorbent assay is presented in Table 10. The calculated sensitivity of the indirect immunofluorescence assay, compared with that of the enzyme-linked immunosorbent assay, was 96.3%. The specificity of the IFA was 97.0%. The calculated sensitivity of the enzyme-linked immunosorbent assay, compared with that of the indirect immunofluorescence assay, was 89.0%. The specificity of the ELISA was 99.1%. Comparison of the enzyme-linked immunosorbent assay and the virus neutralization is presented in Table 11. The calculated sensitivity of the enzyme-linked immunosorbent assay, compared with that of the virus neutralization, was 98.3%. The specificity of the ELISA was 89.7%. The calculated sensitivity of the virus neutralization assay, compared with the enzyme-linked immunosorbent assay, was 54.0%. The specificity of the VN was 99.8%.

Table 8. Comparison of serologic techniques for the detection of antibodies to alcelaphine herpesvirus-1 in domestic and exotic species.

Order Family Subfamily Genus Species (Scientific Name)	Number of Sera	Number of Animals
PERISSODACTYLA		
Equidae		
Equus		
Zebra (*Equus sp.*)	7	7
ARTIODACTYLA		
Camelidae		
Camelus		
Camel (*Camelus sp.*)	7	7
Lama		
Llama (*Lama glama glama*)	1	1
Alpaca (*Lama glama pacos*)	2	2
Cervidae		
Muntiacinae		
Muntiacus		
Reeves muntjac (*Muntiacus reevesi*)	25	17
Cervinae		
Cervus		
Elds deer (*Cervus eldi thamin*)	2	2
Formosan sika deer (*Cervus nippon taiwanus*)	6	5
Brown elk (*Cervus elaphus*)	1	1
Tule elk (*Cervus elaphus nannodes*)	1	1
Barasingha (*Cervus duvauceli*)	8	7
Axis deer (*Cervus axis*)	3	3
Elaphurus		
Pere Davids deer (*Elaphurus davidanus*)	4	4
Odocoileinae		
Hydropotes		
Chinese water deer (*Hydropotes inermis*)	2	2
Odocoileus		
White-tailed deer (*Odocoileus virginianus*)	95	92
Rangifer		
Reindeer (*Rangifer tarandus*)	7	7

[a]IFA = indirect immunofluorescence assay.

[b]VN = virus neutralization assay; Neg = negative; Pos = positive.

[c]ELISA = screen enzyme-linked immunosorbent Assay.

[d]Ver = verification enzyme-linked immunosorbent assay.

IFA[a] Negative						IFA Positive				
VN[b] Neg ELISA[c]			VN Toxic ELISA		VN Pos ELISA	VN Neg ELISA		VN Toxic ELISA		VN Pos ELISA
Neg	Pos Ver[d] Neg	Pos Ver Pos	Neg	Pos Ver Pos	Neg	Neg	Pos Ver Pos	Pos Ver Pos	Neg	Pos Ver Pos
7	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	1
3	0	0	0	0	0	0	0	0	0	2
1	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	1
4	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	0	0	0	0
70	0	1	17	1	0	0	1	2	0	0
7	0	0	0	0	0	0	0	0	0	0

Table 8. (continued)

Order / Family / Subfamily / Genus / Species (Scientific Name)	Number of Sera	Number of Animals
Giraffidae		
Giraffa		
Giraffe (Giraffa camelopardalis)	2	2
Okapia		
Okapi (Okapia johnstoni)	1	1
Antilocapridae		
Antilocapra		
Pronghorn (Antilocapra americana)	1	1
Bovidae		
Bovinae		
Bison		
American bison (Bison bison)	18	12
European bison/Wisent (Bison bonasus)	5	5
Bos		
Indian gaur (Bos gaurus)	22	14
Ankole (Bos taurus)	4	4
Banteng (Bos javanicus)	12	11
Scottish highland cattle (Bos taurus)	3	2
Yak (Bos mutus)	3	2
Domestic cattle (Bos taurus)	299	251
Syncerus		
Cape buffalo (Syncerus caffer caffer)	18	15
Taurotragus		
Eland (Taurotragus oryx)	41	30
Tragelaphus		
Nyala (Tragelaphus angasi)	21	15
Sitatunga (Tragelaphus spekei)	22	21
Greater kudu (Tragelaphus strepsiceros)	14	11
Reduncinae		
Kobus		
Waterbuck (Kobus ellipsiprymnus)	9	7
Nile lechwe (Kobus megaceros)	1	1
Alcelaphinae		
Alcelaphus		
Hartebeest (Alcelaphus buselaphus)	28	26
Connochaetes		
Wildebeest (Connochaetes sp.)	111	107
Damaliscus		
Hunters hartebeest (Damaliscus hunteri)	4	4
Blesbok (Damaliscus dorcas phillipsi)	1	1

IFA Negative						IFA Positive				
	VN Neg ELISA		VN Toxic ELISA		VN Pos ELISA	VN Neg ELISA		VN Toxic ELISA	VN Pos ELISA	
Neg	Pos Ver Neg	Pos Ver Pos	Neg	Pos Ver Pos	Neg	Neg	Pos Ver Pos	Pos Ver Pos	Neg	Pos Ver Pos
2	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
10	1	0	0	0	0	0	1	0	0	0
5	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	2	2	0	0	0
3	1	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	0	0	0
2	0	0	0	0	0	0	0	0	0	0
207	11	0	7	0	0	2	18	0	0	6
15	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0
20	1	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	2	3	0	0	18
34	0	0	0	0	0	3	6	0	0	64
4	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0

Table 8. (continued)

Order Family Subfamily Genus Species (Scientific Name)	Number of Sera	Number of Animals
Hippotraginae		
Addax		
Addax (*Addax nasomaculatus*)	67	35
Hippotragus		
Sable antelope (*Hippotragus niger*)	34	26
Oryx		
Scimitar-horned oryx (*Oryx dammah*)	2	2
Beisa oryx (*Oryx gazella beisa*)	7	7
Gemsbok (*Oryx gazella gazella*)	19	15
Fringe-eared oryx (*Oryx gazella callotis*)	1	1
Arabian oryx (*Oryx leucoryx*)	11	11
Antilopinae		
Antilope		
Blackbuck (*Antilope cervicapra*)	2	2
Gazella		
Grants gazelle (*Gazella granti*)	36	30
Thomsons gazelle (*Gazella thomsoni*)	2	2
Persian gazelle (*Gazella subgutturosa*)	9	9
Dama gazelle (*Gazella dama*)	5	5
Spekes gazelle (*Gazella spekei*)	1	1
Neotragus		
Suni (*Neotragus moschatus*)	1	1
Caprinae		
Ammotragus		
Aoudad/Barbary sheep (*Ammotragus lervia*)	3	3
Capra		
Markhor (*Capra falconeri*)	29	28
Nubian ibex (*Capra ibex nubiana*)	74	35
Domestic goat (*Capra hircus*)	66	50
African pygmy goat (*Capra aegagrus hircus*)	37	37
Ovis		
Four horn sheep (*Ovis sp.*)	7	7
Lincoln lamb (*Ovis sp.*)	1	1
Dalls sheep (*Ovis dalli*)	3	3
Mouflon (*Ovis musimon*)	6	6
Domestic sheep (*Ovis aries*)	49	49
Rupicapra		
Chamois (*Rupicapra rupicapra*)	29	17
Saiga		
Saiga antelope (*Saiga tatarica*)	5	5
Totals	1317	1089

IFA Negative						IFA Positive				
VN Neg ELISA			VN Toxic ELISA		VN Pos ELISA	VN Neg ELISA		VN Toxic ELISA	VN Pos ELISA	
Neg	Pos Ver Neg	Pos Ver Pos	Neg	Pos Ver Pos	Neg	Neg	Pos Ver Pos	Pos Ver Pos	Neg	Pos Ver Pos
27	0	0	0	0	0	0	4	0	0	4
25	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	2
1	0	0	0	0	0	0	0	0	0	6
13	0	0	0	0	0	0	1	0	0	1
1	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	1	0	0	0	1
1	0	0	0	0	0	0	0	0	0	1
30	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
21	0	2	0	0	0	1	3	1	0	0
8	0	0	0	0	0	1	24	0	0	2
32	0	0	0	0	0	5	10	2	0	1
30	0	0	2	0	0	4	1	0	0	0
7	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	2	0	0	0
29	0	2	0	0	1	1	11	0	1	4
14	0	1	0	0	0	0	2	0	0	0
5	0	0	0	0	0	0	0	0	0	0
803	14	6	27	2	1	25	91	5	1	114

Table 9. Comparison of the indirect immunofluorescence assay and virus neutralization assay in the detection of antibodies to alcelaphine herpesvirus-1

	Number of sera tested by the indirect immunofluorescence assay (IFA)	
	Antibodies detected[a] (236)	Antibodies not detected (853)
Number of sera tested by the virus neutralization assay (VN)		
Antibodies detected[b] (116)	115	1
Antibodies not detected (939)	116	823
Sera toxic to tissue cultures (34)	5	29

Calculations[c]:

$$\text{Sensitivity of VN compared to the IFA} = \frac{116 - 1}{231} \times 100 = 49.8\%$$

$$\text{Specificity of VN compared to the IFA} = \frac{939 - 116}{824} \times 100 = 99.9\%$$

$$\text{Sensitivity of IFA compared to the VN} = \frac{231 - 116}{116} \times 100 = 99.1\%$$

$$\text{Specificity of IFA compared to the VN} = \frac{824 - 1}{939} \times 100 = 87.6\%$$

[a]Titer > 1:20. Total number of sera in parentheses.

[b]Titer > 1:4.

[c]Sera toxic to tissue cultures were not used in calculations of sensitivity and specificity.

Table 10. Comparison of the indirect immunofluorescence assay and enzyme-linked immunosorbent assay in the detection of antibodies to alcelaphine herpesvirus-1

	Number of sera tested by the indirect immunofluorescence assay (IFA)	
	Antibodies detected[a] (236)	Antibodies not detected (853)
Number of sera tested by the enzyme-linked immunosorbent assay (ELISA)		
Antibodies detected[b] (218)	210	8
Antibodies not detected (871)	26	845

Calculations:

$$\text{Sensitivity of ELISA compared to the IFA} = \frac{218 - 8}{236} \times 100 = 89.0\%$$

$$\text{Specificity of ELISA compared to the IFA} = \frac{871 - 26}{853} \times 100 = 99.1\%$$

$$\text{Sensitivity of IFA compared to the ELISA} = \frac{236 - 26}{218} \times 100 = 96.3\%$$

$$\text{Specificity of IFA compared to the ELISA} = \frac{853 - 8}{871} \times 100 = 97.0\%$$

[a]Titer > 1:20. Total number of sera in parentheses.

[b]Positive serum had a mean absorbance (405/450 nm) value greater than the 50% positive threshold absorbance value, and a signal to noise ratio greater than 1.8.

Table 11. Comparison of the enzyme-linked immunosorbent assay and virus neutralization assay in the detection of antibodies to alcelaphine herpesvirus-1

	Number of sera tested by the enzyme-linked immunosorbent assay (ELISA)	
	Antibodies detected (218)	Antibodies not detected (871)
Number of sera tested by the virus neutralization assay (VN)		
Antibodies detected (116)	114	2
Antibodies not detected (939)	97	842
Sera toxic to tissue cultures (34)	7	27

Calculations:

$$\text{Sensitivity of VN compared to the ELISA} = \frac{116 - 2}{211} \times 100 = 54.0\%$$

$$\text{Specificity of VN compared to the ELISA} = \frac{939 - 97}{844} \times 100 = 99.8\%$$

$$\text{Sensitivity of ELISA compared to the VN} = \frac{211 - 97}{116} \times 100 = 98.3\%$$

$$\text{Specificity of ELISA compared to the VN} = \frac{844 - 2}{939} \times 100 = 89.7\%$$

[a]Positive serum had a mean absorbance (405/450 nm) value greater than the 50% positive threshold absorbance value, and a signal to noise ratio greater than 1.8; Total number of sera in parentheses.

[b]Titer $\geq$ 1:4.

[c]Sera toxic to tissue cultures were not used in calculations of sensitivity and specificity.

DISCUSSION

In order to provide laboratory diagnostic assistance for MCF diagnosis, three serologic assays, the indirect immunofluorescence assay, the virus neutralization assay, and the enzyme-linked immunosorbent assay were compared for detection of antibodies to MCF virus in clinically affected animals with either epidemiological form of the disease, and in potential reservoir host species.

A fourth serologic assay, the complement fixation assay, was unsuccessful in detecting MCF complement fixing antibodies in sera from clinically affected animals, reservoir host species, or in either of the two calves (calf 9471, GC #16) used to follow MCF antibody development secondary to intravenous inoculation with the WC11 strain of AHV-1. Failure of the CF assay could have several plausible explanations. Insufficient antigen or inadequate antigenic mass may have resulted in the formation of insufficient quantities of antigen-antibody complexes to fix the first component of complement and initiate the classical pathway of complement fixation. Complement fixing antibodies may not have been present in the sera at levels that could be detected by the CF assay used in this study. The use of a less stringent CF assay such as that described by Kalunda (1975) or Hamdy et al. (1980) may have had better success, but even these CF assays failed to detect complement fixing antibodies prior to development of neutralizing antibodies. Bovine IgG1 and IgM are the only bovine immunogobulins capable of fixing guinea pig complement, but even these immunoglobulins have a low complement fixing efficiency. Excess levels of noncomplement fixing antibodies in these

sera may have inhibited the formation of antigen-antibody complexes thus preventing complement fixation. Anticomplementary activity of the test sera did not appear to be a contributing factor as the serum controls reacted appropriately in all of the CF assays completed. The use of a modifying factor in the AHV-1 CF assay was not investigated. Review of the literature on the use of the CF assay for detection of MCF antibodies found the assay to be less sensitive then either the IFA or the VN (Kalunda, 1975; Hamdy et al., 1980). Further work on the CF assay was not done.

The indirect immunofluorescence assay was shown to have the greatest sensitivity for detection of antibodies directed against MCF virus, with calculated sensitivities of 99.1% as compared to the VN, and 96.3% as compared to the ELISA. The IFA detected antibodies against AHV-1 antigens in clinically affected animals with either epidemiological form of the disease earlier and with higher titers than the VN assay. This finding was in agreement with Rossiter et al. (1977) who had previously reported that antibodies against AHV-1 virus could be detected earlier and with higher titers by the IFA than by the VN in cattle and rabbits experimentally infected with AHV-1. Rossiter et al. (1977) hypothesized that antibodies detected by the IFA were not directed against the same surface viral antigens as neutralizing antibodies, but were directed against viral antigens expressed earlier in the disease process. Rossiter (1983) demonstrated IFA antibodies to the AHV-1 virus in cattle with histopathologically confirmed cases of sheep-goat-associated MCF. He hypothesized that the etiological agent of the sheep-goat-associated form

is antigenically related to the AHV-1 group of viruses. Other investigators have also reported on the presence of IFA antibodies to the AHV-1 virus in cattle with sheep-goat-associated MCF (Harkness 1985; Heuschele et al., 1984a). The pattern of specific IFA fluorescence is the same using sera from either epidemiological form of the disease, making it impossible to distinguish the epidemiological forms on the basis of IFA serology. Advantages of the IFA include minimal maintenance, short assay time, high degree of sensitivity for detection of MCF antibodies, and strong negative correlation with the VN and ELISA. Large numbers of WC11 infected cover slips can be prepared and stored at -70C for over 12 months without loss of viral antigen reactivity. Readily visible WC11 viral cpe in the cell monolayers of infected coverslips makes it easy to detect uninfected cover slips, thus eliminating false negative results. The IFA procedure can be completed in three to four hours. The strong negative correlation (>99%) between the three assays, along with the high degree of sensitivity for detection of antibodies makes this assay the assay of choice for screening large numbers of animals for antibodies to MCF virus. IFA negative animals can be reported as seronegative with a high degree of confidence. The IFA would work well for laboratories which have limited capabilites to maintain cell cultures. It would also be the assay of choice for laboratories which do not want to expend a lot of time or money on MCF diagnosis, but want to employ a simple serologic assay to assist field veterinarians in diagnosing MCF. Disadvantages of the IFA assay include, antigenic cross reactivity with other bovine herpesvires, the need to determine the appropriate anti-species IFA conjugate for use with

each species being tested, and subjective differentiation of specific fluorescence from nonspecific fluorescence in the cell monolayers of infected cover slips. Antigenic cross reactivity of the WC11 IFA antigens with antisera produced against BHV-3 was detected in this study. The antigenic cross reactivity was unidirectional, as no cross reactivity between BHV-3 viral antigens and hyperimmune antisera produced against AHV-1 viruses were detected at the 1:20 serum dilution. The unidirectional cross reactivity makes it possible to use BHV-3 infected coverslips to identify BHV-3 positive antisera which have the potential of cross reactivity with the AHV-1 viral antigens. Antigenic cross reactivity among AHV-1, BHV-1 and BHV-2 (Rossiter et al., 1977; Heuschele, 1982; Rossiter, 1983; Osorio et al., 1985) has also been reported to occur, but was not demonstrated in this study. Potential antigenic cross reactivity among AHV-1, unrecognized sheep and goat herpesviruses, and bovine cytomegalovirus detected by the IFA will need to be evaluated as isolates become available. The degree of antigenic relationship between the sheep-goat-associated etiological agent and the AHV-1 group will be able to be determined only after the causative agent for the sheep-goat-associated MCF has been identified and characterized. The double diffusion technique demonstrated precipitin lines of complete or partial identity between bovine IgG and the immunoglobulins of most of the ruminants of the family Bovidae, family Cervidae, family Giraffidae, and family Antilocapridae making it possible to use anti-bovine IFA conjugate with these families. Sera from IFA positive animals were tested by the VN and the ELISA to confirm the serologic status of the animal. Nonspecific

fluorescence of the WC11 infected BT monolayers could be mistaken for specific fluorescence by inexperienced readers causing false positive IFA results.

The enzyme-linked immunosorbent assay was second in sensitivity for detection of antibodies to MCF virus with calculated sensitivities as compared to the IFA of 89.0%, and as compared to the VN of 98.3%. The ELISA assay detected antibodies against the WC11 solubilized antigen in clinically affected animals with either epidemiological form of the disease. This concurred with the findings of Wan and Castro (1986) with their AHV-1 ELISA. Two hundred and forty sera could be screened for the presence of MCF antibodies in approximately five hours. Antigenic cross reactivity of the WC11 ELISA antigen with antisera produced against other bovine herpesviruses were not seen. This again was in agreement with the findings of Wan and Castro (1986). This lack of antigenic cross reactivity makes the ELISA more specific than the IFA assay. Potential antigenic cross reactivity with unrecognized sheep and goat herpesviruses and bovine cytomegalovirus remains to be evaluated as isolates become available. The range of the ELISA absorbance values was from 0.007 to 1.072, with the majority of the absorbance values under 0.500. The low specific ELISA absorbance values seen in this assay can be attributed primarily to two factors, the marginal antigenic mass of the ELISA antigen, and secondly the cell-associated nature of the virus which results in delayed antibody development and lower specific antibody titers than generally noted with most herpesviruses. The specific ELISA absorbance values for exotic species were also affected by the relative

binding affinity of the RAB ELISA conjugate for exotic species immunoglobulins. The late seroconversion of gnotobiotic calf #16 by the ELISA (day 28) represents a phenomenon commonly seen with gnotobiotic sera used in ELISA assays. Lack of normal background immunoglobulins in the gnotobiotic serum results in low absorbance values even when high specific antibody titers are present as detected by other serologic assays. It has been hypothesized that specific antigen-antibody reactions incorporate normal background immunoglobulins into the ELISA reactions system thus intensifying the colormetric reaction. In the gnotobiotic sera adequate background immunoglobulins are not present to facilitate the reaction thus accounting for the lower absorbance values. Falsely elevated ELISA absorbance values were seen with bacterially contaminated antisera, sera with severe hemolysis, and in serum with antibodies directed against host cell proteins. These were differentialed from the true ELISA positives by use of the verification ELISA. The ELISA assay requires more time and equipment to maintain as compared to the requirements for the other serologic assays. Enzyme-linked immunosorbent assay microplates needed to be coated at least two days prior to use. Buffers and solutions needed to be properly maintained. The appropriate anti-species horseradish peroxidase-labeled conjugate and the optimal conjugate dilution have to be determined for each species tested. Production of monoclonal antibodies directed against the WCll virus and conversion of the ELISA assay to a competitive ELISA would eliminate many of the current disadvantages of the ELISA system. Sera from species of the family Equidae and family Camelidae did not demonstrate identity with bovine IgG. Validity of the

ELISA results for these species could therefore be questioned. The ELISA is the assay of choice for confirmation of IFA positives as it is a rapid specific assay that can be used to detect antibodies against AHV-1 antigens from either epidemiological form of the disease. This assay could be used to eliminate potential reservoir hosts from valuable zoological collections, and as a diagnostic tool to assist with the diagnosis of both epidemiological forms of the disease when the serological findings are taken along with epidemiological information, clinical signs, gross lesions, and histopathological lesions to complete the diagnostic picture. The use of this assay would probably be limited to well equipped laboratories and research laboratories willing to spend the time necessary to maintain this assay.

The virus neutralization assay, was the least sensitive of the three assays, with calculated sensitivites for the WC11 VN of 54.0% as compared to the ELISA, and 49.8% and compared to the IFA. The assay is generally considered the most specific assay for detection of antibodies directed against MCF virus (Heuschele et al., 1984a). The WC11 VN had a calculated specificity of 99.9% as compared to the IFA, and 99.8% as compared to the ELISA. Neutralizing antibodies are rarely detected in clinically affected cattle with either epidemiological form of the disease (Plowright, 1968; Kalunda, 1975; Rossiter et al., 1977; Heuschele et al., 1984a; Wan and Castro, 1986). Neutralizing antibodies can be readily detected in reservoir hosts of the AHV-1 virus, but are rarely detected in reservoir hosts of the sheep-goat-associated MCF. Malignant catarrhal fever neutralizing antibodies were detected in 116 (10.7%) of the animals by the

WC11 VN, and 119 (10.9%) of the animals by the IG30359 VN with the majority of the VN positive animals being clinically normal exotic species primarily of the subfamily Alcelaphinae and subfamily Hippotraginae. The difference between the number of seropositive animals detected by the two VN assays was most likely related to variance in test dose virus between the assays, and not reflective of MCF strain difference, as antibody titers were shown by Mushi and Plowright (1979) to have a strong correlation to the level of test dose virus used. Individual animals considered seropositive by one VN and negative by the other generally had antibody titers that variedby only a two-fold serum dilution. This would appear to support the idea of a single serotype for the AHV-1 group of viruses as hypothesized by Rossiter et al. (1977). Thirty-four sera tested were toxic for cell cultures and therefore could not be tested (NT). Antigenic cross reactivity of the WC11 virus with antisera produced against BHV 1-3 and PHV-1 by virus neutralization was not demonstrated in this study. This agreed with the previous findings of Kalunda (1975), Heuschele (1982), and Hamblin and Hedger (1984). Potential antigenic cross reactivity with unrecognized sheep and goat herpesviruses and bovine cytomegalovirus remain to be evaluated as isolates become available. Addition of guinea pig complement to the serum virus mixture prior to neutralization did not enhance neutraliztion (Rossiter et al., 1977). In this study, serum from clinically affected animals occasionally demonstrated enhanced neutralization titers when they were tested without heat inactivation. This may be due to enhanced neutralization of the AHV-1 virus in the presence of bovine complement, which is a normal

component of sera destroyed by heat inactivation. The addition of a standard amount of bovine complement to the VN system prior to neutralization, if a source of readily available bovine complement could be found, might enhance the neutralization titers in clincally affected animals. Disadvantages to the VN assay include the need for maintenance of tissue cultures, and failure of the assay to detect antibodies in most clinically affected animals. The VN also rarely detects antibodies against the sheep-goat-associated form of MCF. One hypothesis is that antigenic differences between the AHV-1 group of viruses and the etiological agent of the sheep-goat-associated form of MCF may account for the failure of the AHV-1 VN assays to detect neutralizating antibodies against the sheep-goat-associated form. This will have to be proven when the etiological agent is finally isolated.

Direct comparison of the serologic assays demonstrated a strong negative correlation between the three assay (> 99%). A strong positive correlation was demonstrated between the IFA and ELISA (96.3%). Sera tested positive by the VN were positive by the IFA and ELISA assays in greater than 98% of the sera. The VN failed to confirm IFA positive test results in 50.2% of the sera. The majority of the IFA postive sera that were SVN negative were either from clinically affected animals, or from species of the subfamily Caprinae. Separation of the epidemiological forms of MCF, and the development of specific serological assays for the sheep-goat-associated MCF will be accomplished only with the advent of the isolation and identification of the causative agent.

Conclusion

The serologic assays described in this study can be used to detect antibodies against MCF virus. The indirect immunofluorescence assay had the greatest sensitivity for detection of antibodies in clinically affected animals with either epidemiological form of the disease. Antigenic cross reactivity between the IFA antigens and antisera against BHV-3 viruses, makes serodiagnosis by IFA alone questionable. The IFA assay is a valuable tool to rapidly screen large numbers of animals with a high degree of sensitivity for the presence of antibodies against MCF virus. The virus neutralization assay had the greatest specificity, but lowest sensitivity for antibody detection. Neutralizing antibodies were rarely detected in clinically affected animals. The primary value of the VN was its use for confirming the seropositive status of reservoir host species. The enzyme-linked immunosorbent assay appears to be the assay of choice for detection of antibodies to MCF virus. The sensitivity and specificity of the ELISA places it between the other assays with a greater sensitivity than the VN, and a higher degree of specificity than the IFA. Antigenic cross reactivity between the WC11 ELISA antigen and antisera produced against BHV 1-3 were not demonstrated making the assay more reliable for serodiagnosis than the IFA. The ELISA detected antibodies in clinically affected animals with either epidemiological forms of the disease, and readily detected the presence of antibodies in reservoir host species. The short assay time makes the ELISA the best assay for confirmation of positive IFA results. The use of one or more of the serological assays for detection of antibodies to MCF virus when taken

along with the epidemiological profile, clinical signs, gross lessions, and histopathologic lessions should markedly enhance MCF diagnosis, and provide valuable diagnostic assistance to field veterinarians, as well as provide valuable information to epidemiologists studying both forms of the disease.

Summary

Three serologic assays, the IFA, VN, and ELISA were compared for detection of MCF antibodies against either epidemiological form of the disease. Sera from 1089 animals representing 63 different species were tested by the three assays. The IFA was determined to be the most sensitive, but least specific assay. Antigenic cross reactivity between AHV-1 IFA antigens and bovine antisera produced against BHV-3 were demonstrated. The IFA detected 236 positive animals (21.7%) representing 26 different species at the 1:20 serum dilution. The IFA detected 178 positive animals (16.3%) representing 22 species at the 1:100 dilution. Antibodies against MCF virus were detected by the IFA in histopathologically confirmed cases of sheep-goat-associated MCF, in clinical cases of wildebeest-derived MCF, and in reservoir host species. The ELISA detected MCF antibodies in 218 animals (20.0%) representing 25 different species. Antigenic cross reactivity between the WC11 ELISA antigen and bovine antisera produced against BHV 1-3 were not demonstrated. The ELISA assay also detected antibodies in clinically affected animals with either epidemiological form of the disease. Seropositive reservoir host species were readily identified by the ELISA. The WC11 VN was the least sensitive assay, detecting neutralizing

antibodies in 116 animals (10.7%) representing 15 different species. Antigenic cross reactivity between the WC11 virus, and antisera produced against BHV 1-3 and PHV-1 were not demonstrated using the VN assay. Neutralizing antibodies against MCF virus were rarely detected in clinically affected animals. Neutralizing antibodies were rarely detected in histopathologically confirmed cases of sheep-goat-associated MCF. The ELISA appears to be the assay of choice for detection of antibodies against MCF when equipment and time to maintain the assay are available. All three serologic assays can be used for detection of MCF antibodies. Serologic assays will provide valuable diagnostic assistance in the diagnosis of malignant catarrhal fever, and provide valuable information for use in epidemiological studies of the disease.

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APPENDIX I

Transmission of Malignant Catarrhal Fever

Wildebeest-derived malignant catarrhal fever:
- black wildebeest to cattle
  - Mettam, 1923 (cited by Daubney and Hudson, 1936)
- blue wildebeest to cattle
  - Daubney and Hudson (1936)
  - Plowright (1965b)
  - Mushi and Wafula (1983)
- wildebeest to rabbit
  - Mushi and Wafula (1983)
- hartebeest to cattle
  - Reid and Rowe (1973)
- cattle to cattle
  - Mettam, 1923 (cited by Daubney and Hudson, 1936)
  - Daubney and Hudson (1936)
  - Piercy, 1952a (cited by Plowright, 1968)
  - Plowright (1965b)
  - Orsborn et al. (1977)
  - Kalunda et al. (1981a)
  - Whitenack et al. (1981)
- cattle to blue wildebeest
  - Plowright (1965b)
- cattle to rabbit
  - Daubney and Hudson (1936)
  - Plowright, 1953 (cited by Plowright, 1968)
  - Piercy, 1955 (cited by Hoffmann et al., 1984b)
  - Orsborn et al. (1977)
  - Edington et al., 1979 (cited by Edington and Patel, 1981)
- cattle to white-tailed deer
  - Whitenack et al. (1981)
- cattle to neonatal sheep
  - Kalunda (1975)
- gaur to cattle
  - Whitenack et al. (1981)
  - Castro et al. (1982)
- greater kudu to cattle
  - Whitenack et al. (1981)
  - Castro et al. (1982)
- sheep to cattle
  - Kalunda (1975)
- rabbit to cattle
  - Daubney and Hudson (1936)
- rabbit to rabbit
  - Orsborn et al. (1977)
- rabbit to rat
  - Jacoby et al. (1988)
- rabbit to neonatal guinea pig
  - Jacoby et al. (1988)
- rabbit to neonatal hamster
  - Jacpby et al. (1988)
- guinea pig to guinea pig
  - Reid and Buxton (1985)
- hamster to hamster
  - Reid and Buxton (1985)
- rat to rat
  - Reid and Buxton (1985)

Sheep-goat-associated malignant catarrhal fever:

- cattle to cattle
  - Goetze and Liess, 1929 (cited by Daubney and Hudson, 1936)
  - Jacotot, 1932 (cited by Plowright, 1968)
  - Rinjard, 1935 (cited by Plowright, 1968)
  - Daubney and Hudson (1936)
  - Magnusson, 1939 (cited by Plowright, 1968)
  - Duncan and Pearson, 1956 (cited by Hoffmann et al., 1984b)
  - Roderick, 1958 (cited by Plowright, 1968)
  - Blood et al., 1960 (cited by Plowright, 1968)
  - Pierson et al. (1974)
  - Horner et al. (1975)
  - Hamdy et al. (1978)
  - Pierson et al. (1978)
  - Liggitt et al., 1978 (cited by Liggitt and DeMartini, 1980)
  - Selman et al. (1978)
  - Pierson et al. (1979)
  - Ramachandra et al. (1982)
  - Reid et al. (1986)
- cattle to bison
  - Liggitt et al. (1980)
- cattle to rabbit
  - Daubney, 1959 (cited by Buxton and Reid, 1980)
  - Buxton and Reid (1980)
  - Reid et al. (1986)
- swamp buffalo to cattle
  - Mansjoer, 1957 (cited by Hoffmann et al., 1984b)
  - Hoffmann et al. (1984b)
- swamp buffalo to rabbit
  - Mansjoer, 1957 (cited by Hoffmann et al., 1984b)
- rusa deer to rabbit
  - Westbury and Denholm (1982)
- red deer to red deer
  - Oliver et al. (1983)
  - Reid et al. (1986)
- red deer to roe deer
  - Reid et al. (1986)
- red deer to rabbit
  - Buxton and Reid (1980)
  - Reid et al. (1986)
- red deer to neonatal sheep
  - Buxton et al. (1985)
- rabbit to rabbit
  - Buxton and Reid (1980)
  - Westbury and Denholm (1982)
  - Reid et al. (1986)
- rabbit to cattle
  - Buxton and Reid (1980)
- rabbit to red deer
  - Buxton and Reid (1980)
  - Reid et al. (1986)
- rabbit to roe deer
  - Reid et al. (1986)
- rabbit to hamster
  - Reid et al. (1986)
  - Jacoby et al. (1988)
- hamster to hamster
  - Reid et al. (1986)

APPENDIX II

Species with Reported Clinical Malignant Catarrhal Fever

Species (Scientific name)	Epidem. form[a]	Reference(s)
Camelidae		
Lama		
Llama (Lama glama guanicoe)	AHV	Colly (1977[b])
Cervidae		
Cervinae		
Cervus		
Axis deer (Cervus axis)	AHV	Heuschele (1982)
	SGA	Clark et al. (1970)
Barasingha (Cervus duvauceli)	AHV	Hatkin (1980)
		Heuschele (1982)
Elds deer (Cervus eldi thamin)	AHV	Heuschele (1982)
Luzon sambar deer (Cervus mariannus)	AHV	Colly (1977)
		Heuschele (1982)
Malayan sambar deer (Cervus unicolor)	AHV	Colly (1977)
Rusa deer (Cervus timorensis)	SGA	Denholm and Westbury (1982)
Sika deer (Cervus nippon)	SGA	Wilson et al. (1983)
Formosan sika (C. nippon taiwanus)	AHV	Heuschele (1982)
		Heuschele et al. (1985b)
Japanese sika (C. nippon nippon)	SGA	Sanford et al. (1977)
Dybowski sika (C. nippon dybowskii)	AHV	Sanford et al. (1977)
European red deer (Cervus elaphus)	SGA	Reid et al. (1979)
		McAllum et al. (1982)
		Oliver et al. (1983)
Elaphurus		
Pere Davids deer (Elaphurus davidanus)	AHV	Ashton (1982)
		Heuschele (1982)
	SGA	Heuschele (1982)
	UNK	Huck et al. (1961)
		Tong et al. (1961)
		Senior et al. (1962)

[a]AHV = alcelaphine herpesvirus; SGA = sheep-goat-associated malignant catarrhal fever; UNK = unkown; (?) = questionable diagnosis.

[b]Colly, 1977 (cited by Heuschele, 1982).

Odocoileinae		
Alces		
Shiras moose (Alces alces shirasi)	SGA	Williams et al. (1984)
Capreolus		
Roe deer (Capreolus capreolus)	SGA	Reid and Buxton (1984)
Odocoileus		
Mule deer (Odocoileus hemionus)	SGA	Pierson et al. (1974) Jessup (1985)
White-tailed deer (O. virginianus)	SGA	Clark et al. (1970) Clark et al. (1972) Wobeser et al. (1973)
	UNK	Wyand et al. (1971)
Bovidae		
Bovinae		
Bison		
American bison (Bison bison)	SGA	Ruth et al. (1977) Wallman and Thompson (1982)
European bison/Wisent (Bison bonasus)	AHV	Straver and Van Bekkum (1979) Ashton (1982)
Bos		
Indian gaur (Bos gaurus)	AHV	Hatkin (1980) Castro et al. (1981)
Banteng (Bos javanicus)	AHV	Hatkin (1980)
	SGA	Ramachandra et al (1982) Hoffmann et al. (1984b)
Watusi/Ankole (Bos taurus)	AHV	Heuschele et al. (1984a)
Domestic cattle (Bos taurus)	AHV	Mettam, 1923 (cited by Daubney and Hudson (1936) Daubney and Hudson (1936) Plowright (1964)
	SGA	Daubney and Hudson (1936) Murray and Blood (1961) Gray and Anthony (1969) Pierson et al. (1973) Selman et al. (1974) James et al. (1975) Orsborn et al. (1977) Mare (1977) Hamdy et al. (1978) Hutchinson (1979) Weaver (1979) Harkness (1985)
Boselaphus		
Nilgai antelope (B. tragocamelus)	AHV	Colly (1977) Heuschele (1982)

Bubalus		
Water buffalo (Bubalus bubalis)	SGA	Parihar et al. (1975) Singh et al. (1979) Vanselow (1980) Hoffmann et al. (1984a)
Syncerus		
Cape buffalo (S. caffer caffer)	AHV	Ashton (1982)
Congo buffalo (S. caffer nanus)	AHV	Pettit (1965)
Tragelaphus		
Bongo (T. eurycerus isaaci)	AHV	Heuschele (1982) (?)
Bushbuck (Tragelaphus scriptus)	AHV	Colly (1977)
Greater kudu (T. strepsiceros)	AHV	Boever and Kurk (1974) Colly (1977) Castro et al. (1981)
Sitatunga (Tragelaphus spekei)	AHV	Colly (1977) Ashton (1982)
Cephalophinae		
Cephalophus or Sylvicapra		
Duiker (species unknown)	AHV	Colly (1977)
Alcelaphinae		
Alcelaphus		
Red hartebeest (A. buselaphus)	AHV	Colly (1977) (?)
Connochaetes		
Wildebeest (Connochaetes sp.)	AHV	Ashton (1982) (?)
Damaliscus		
Blesbok (Damaliscus dorcas phillipsi)	AHV	Colly (1977)
Hippotraginae		
Oryx		
Arabian oryx (Oryx leucoryx)	AHV	Heuschele (1982) (?)
Scimitar-horned oryx (Oryx dammah)	AHV	Ashton (1982) (?)
Reduncinae		
Kobus		
Ellipsen waterbuck (K. ellipsiprymnus)	AHV	Heuschele (1982)
Nile lechwe (Kobus lechwe)	AHV	Colly (1977)
Antilopinae		
Antilope		
Blackbuck (Antilope cervicapra)	AHV	Heuschele (1982)
Gazella		
Slender horned gazelle (G. leptoceros)	AHV	Heuschele (1982)
Neotragus		
Zulu suni (N. moschatus zuluensis)	AHV	Heuschele (1982)
Caprinae		
Hemitragus		
Nilgiri tahr (Hemitragus hylocrius)	AHV	Heuschele (1982) (?)
Ovis		
Domestic sheep (Ovis aries)	SGA	Schmitz and Grumbein (1981) (?)

APPENDIX III

Species from which Alcelaphine Herpesvirus (AHV) or AHV-like Virus has been Isolated

Isolations from clinically normal animals:

- Connochaetes
- Blue wildebeest (Connochaetes taurinus taurinus)-Plowright et al. (1960)
- Whitebearded wildebeest (Connochaetes taurinus albojubatus)-Heuschele et al. (1984a)
- Whitetailed wildebeest (Connochaetes gnou)-Castro et al. (1981)
- Alcelaphus
- Cokes hartebeest (Alcelaphus buselaphus cokei)-Reid and Rowe (1973)
- Cape hartebeest (Alcelaphus buselaphus caama)-Heuschele et al. (1984a)
- Damaliscus
- Topi (Damaliscus lunatus korrigum)-Mushi et al. (1981a)
- Jimela topi (Damaliscus lunatus jimela)-Heuschele et al. (1984a)
- Oryx
- Scimitar-horned oryx (Oryx dammah)-Heuschele et al. (1984a)
- Capra
- Siberian ibex (Capra ibex sibirica)-Heuschele and Worley (1986)

Isolations from animals with clinical malignant catarrhal fever:

- Cervus
- Formosan sika deer (Cervus nippon taiwanus)-Heuschele (1982)
- Axis deer (Cervus axis)-Heuschele (1982)
- Barasingha (Cervus duvauceli)-Heuschele (1982)
- Boselaphus
- Nilgai antelope (Boselaphus tragocamelus)-Heuschele (1982)
- Tragelaphus
- Greater kudu (Tragelaphus strepiceros)-Castro et al. (1981)
- Bos
- European bison/Wisent (Bos bonasus)-Straver and Van Bekkum (1979)
- Indian gaur (Bos gaurus)-Castro et al. (1981)
- Watusi/Ankole (Bos taurus)-Heuschele et al. (1984a)
- Domestic cattle (Bos taurus)-Plowright (1965b)

APPENDIX IV

Species Tested Positive for Neutralizing Antibodies to Alcelaphine Herpesvirus-1

ARTIODACTYLA
- Hippopotamidae
  - Hippopotamus
    - Hippopotamus (Hippopotamus amphibius)-Hamblin and Hedger (1984) (1:4 to 1:45)
- Tragulidae
  - Tragulus
    - Asiatic mouse deer (Tragulus meminna)-Heuschele et al. (1984a)
- Cervidae
  - Muntiacinae
    - Muntiacus
      - Reeves muntjac (Muntiacus reevesi)-Heuschele et al. (1984a) (1:2 to 1:64)
  - Cervinae
    - Cervus
      - Axis deer (Cervus axis)-Heuschele (1982)
      - Elds deer (Cervus eldi thamin)-Heuschele et al. (1984a) (1:2 to 1:64)
      - Formosan sika deer (Cervus nippon taiwanus)-Heuschele et al. (1984a) (1:2 to 1:512)
- Giraffidae
  - Giraffa
    - Giraffe (Giraffa camelopardalis)-Heuschele et al. (1984a)
- Bovidae
  - Bovinae
    - Bison
      - American bison (Bison bison)-Heuschele (1982)
    - Bos
      - Indian gaur (Bos gaurus)-Heuschele (1982)
      - Domestic cattle (Bos taurus)-Plowright (1968)
        Kalunda (1975)
        Rossiter et al. (1980)
        Heuschele et al. (1984a)
        (1:2 to 1:256)
    - Boselaphus
      - Nilgai (Boselaphus tragocamelus)-Heuschele (1982)
    - Bubalus
      - Water buffalo (Bubalus bubalis)-Heuschele et al. (1984a) (1:2 to 1:3)
    - Syncerus
      - Cape buffalo (Syncerus caffer caffer)-Heuschele et al. (1984a) (1:2 to 1:8)
    - Taurotragus
      - Eland (Taurotragus oryx)-Heuschele (1982)

Tragelaphus
Sitatunga (Tragelaphus spekei)-Heuschele et al. (1984a)
(1:2 to 1:8)
Greater kudu
(Tragelaphus strepsiceros)-Hamblin and Hedger (1984)
(1:6 to 1:22)

Hippotraginae
Addax
Addax (Addax nasomaculatus)-Heuschele et al. (1984a)
(1:2 to 1:160)
Hippotragus
Roan antelope (Hippotragus equinus)-Heuschele et al. (1984a)
(1:2 to 1:6)
Sable antelope (Hippotragus niger)-Hamblin and Hedger (1984)
(1:4 to 1:32)
Heuschele et al. (1984a)
Barnard (1985)
Oryx
Scimitar-horned oryx (Oryx dammah)-Heuschele et al. (1984a)
(1:2 to 1:256)
Gemsbok (Oryx gazella gazella)-Hamblin and Hedger (1984)
(1:4 to 1:45)
Heuschele et al. (1984a)
(1:2 to 1:256)
Barnard (1985)
Beisa oryx (Oryx gazella beisa)-Heuschele et al. (1984a) (1:2)
Fringe-eared oryx
(Oryx gazella callotis)-Reid et al. (1975)
Mushi and Karstad (1981)
Heuschele et al. (1984a)
(1:2 to 1:256)
Arabian oryx (Oryx leucoryx)-Heuschele et al. (1984a)
(1:2 to 1:48)

Alcelaphinae
Alcelaphus
Cape hartebeest
(A. buselaphus caama)-Heuschele et al. (1984a)
(1:4 to 1:256)
Cokes hartebeest (A. buselaphus cokei)-Reid et al. (1975)
Connochaetes
Whitetailed wildebeest
(Connochaetes gnou)-Ramsay et al. (1982)
Heuschele et al.(1984a)
(1:4 to 1:256)
Barnard (1985)

Blue wildebeest
(C. taurinus taurinus)-Plowright (1967)
Kalunda (1975)
Reid et al. (1975)
Hamblin and Hedger (1984)
(1:4 to 1:256)
Heuschele et al. (1984a)
(1:12 to 1:256)
Barnard (1985)
Whitebearded wildebeest
(C. taurinus albojubatus)-Heuschele et al. (1984a)
(1:2 to 1:256)
Damaliscus
Blesbok (Damaliscus dorcas phillipsi)-Heuschele et al. (1984a)
(1:2 to 1:6)
Tsessebe (Damaliscus lunatus lunatus)-Hamblin and Hedger (1984)
(1:4 to 1:16)
Topi (Damaliscus lunatus jimela)-Heuschele et al. (1984a)
(1:2 to 1:128)
Topi (Damaliscus lunatus korrigum)-Reid et al. (1975)
Barnard (1985)

Reduncinae
Kobus
Lechwe (Kobus leche)-Hamblin and Hedger (1984)
(1:16)
Waterbuck (Kobus ellipsiprymnus)-Hamblin and Hedger (1984)
(1:45 to 1:1024)
Heuschele (1982)
Defassa waterbuck (Kobus defassa)-Hamblin and Hedger (1984)
(1:128 to 1:708)
Heuschele et al. (1984a)
(1:8 to 1:16)
Uganda kob (Kobus kob)- Heuschele et al. (1984a)
(1:2 to 1:12)
Redunca
Reedbuck (Redunca arundinum)-Hamblin and Hedger (1984)
(1:90 to 1:1024)

Antilopinae
Aepyceros
Impala (Aepyceros melampus)-Reid et al. (1975)
Barnard (1985)
Hamblin and Hedger (1984)
(1:4 to 1:22)
Heuschele et al. (1984a)
(1:2 to 1:48)
Antidorcas
Springbok (Antidorcas marsupialis)-Heuschele et al. (1984a)
(1:2 to 1:20)
Barnard (1985)

Antilope
Blackbuck (Antilope cervicapra)-Heuschele (1982)
Gazella
Dorcas gazelle (Gazella dorcas)-Heuschele et al. (1984a)
(1:4 to 1:8)
Roosevelts gazelle
(G. granti roosevelti)-Heuschele et al. (1984a)
(1:2 to 1:4)
Persian gazelle (Gazella subgutturosa)-Heuschele (1982)
Caprinae
Ammotragus
Aoudad/Barbary sheep (A. lervia)-Heuschele et al. (1984a)
(1:16 to 1:256)
Capra
Domestic goat (Capra hircus)-Heuschele et al. (1984a)
(1:3 to 1:16)
Turkomen markhor (Capra falconeri)-Heuschele et al. (1984a)
(1:3 to 1:32)
Alpine ibex (Capra ibex ibex)-Heuschele et al. (1984a)
(1:2 to 1:8)
Nubian ibex (Capra ibex nubiana)-Heuschele et al. (1984a)
(1:2 to 1:24)
West caucasian tur (C. ibex caucasica)-Heuschele et al. (1984a)
(1:2 to 1:8)
Cretan goat (Capra aegagrus cretensis)-Heuschele et al. (1984a)
(1:2 to 1:8)
Hemitragus
Nilgiri tahr (Hemitragus hylocrius)- Heuschele (1982)
Himalayan tahr (Hemitragus jemlahicus)-Heuschele et al. (1984a)
(1:2 to 1:40)
Ovis
Afghan urial (Ovis cycloceros)-Heuschele (1982)
Bighorn sheep (Ovis canadensis)-Heuschele et al. (1984a)
(1:2 to 1:4)
Dall sheep (Ovis dalli)-Heuschele et al. (1984a)
(1:2 to 1:8)
Domestic sheep (Ovis aries)-Kalunda et al. (1981a)
(1:4 to 1:16)
European mouflon (Ovis musimon)-Heuschele et al. (1984a)
(1:2 to 1:16)
Rupicapra
Chamois (Rupicapra rupicapra)-Heuschele et al. (1984a)
(1:2 to 1:8)

APPENDIX V

Table 12. Previously reported antigenic relationships between alcelaphine herpesvirus-1 (AHV-1) and other herpesviruses as detected by the indirect immunofluorescence assay

Hyperimmue Antisera	Viral Antigens[a]								
	AHV-1			BHV-1	BHV-2	BHV-3		Penn	PHV-1
	WC11	C500	IG30359	IBR	BHM	DN599	33/63	47	PRV
AHV-1									
WC11									
Rossiter et al. (1977)	512	512	ND[b]	8	8	8	ND	ND	<8
Rossiter et al. (1977)	2048	1024	ND	8	8	8	ND	ND	<8
Rossiter (1983)	1024	ND	ND	<8	<8	<8	ND	ND	<8
C500									
Rossiter et al. (1977)	512	512	ND	8	8	8	ND	ND	<8
Rossiter et al. (1977)	256	256	ND	8	<8	<8	ND	ND	<8
IG30359									
Osorio et al. (1985)	ND	ND	160	<5	<5	10	<5	40	ND
SGA									
Rossiter (1983)	256	ND	ND	8	32	<8	ND	ND	<8
Rossiter (1983)	32	ND	ND	<8	<8	<8	ND	ND	<8
Rossiter (1983)	512	ND	ND	<8	<8	8	ND	ND	<8
BHV-1									
Rossiter et al. (1977)	16	8	ND	512	8	<8	ND	ND	<8
Rossiter et al. (1977)	64	16	ND	2048	8	<8	ND	ND	16
Heuschele (1982)	40	ND	ND	ND	ND	ND	ND	ND	ND
Osorio et al. (1985)	ND	ND	<5	160	<5	<5	<5	<5	ND
BHV-2									
Rossiter et al. (1977)	<8	<8	ND	<8	512	<8	ND	ND	<8
Rossiter et al. (1977)	<8	<8	ND	<8	1024	<8	ND	ND	<8
Heuschele (1982)	80	ND	ND	ND	ND	ND	ND	ND	ND
Osorio et al. (1985)	ND	ND	<5	<5	80	<5	<5	<5	ND

BHV-3									
Rossiter et al. (1977)	128	32	ND	<8	<8	2048	ND	ND	<8
Heuschele (1982)	80	ND	ND	ND	ND	ND	ND	ND	ND
Osorio et al. (1985)	ND	ND	<5	<5	<5	160	320	20	ND
Penn 47									
Osorio et al. (1985)	ND	ND	20	<5	<5	20	20	160	ND
PHV-1									
Rossiter et al. (1977)	<8	<8	ND	<8	<8	<8	ND	ND	256
Rossiter et al. (1977)	<8	<8	ND	<8	8	8	ND	ND	512

[a]AHV-1 (WC11, C500, IG30359) = wildebeest-derived malignant catarrhal fever; SGA = sheep-goat-associated malignant catarrhal fever; BHV-1 (IBR) = infectious bovine rhinotracheitis; BHV-2 (BHM) = bovine herpes mammillitis; BHV-3 (DN599, Movar 33/63) = movar group bovine herpesvirus; Penn 47 = cell-associated herpesvirus isolated from cow with lymphosarcoma-Van der Maaten and Boothe (1972) PHV-1 (PRV) = pseudorabies.

[b]ND = not done.

APPENDIX VI

Species with Alcelaphine Herpesvirus-1 Antibodies Detected by the Indirect Immunofluorescence Assay (IFA)

ARTIODACTYLA
- Tragulidae
  - Tragulus
    - Asiatic mouse deer (Tragulus meminna)-Heuschele (1982)
- Cervidae
  - Muntiacinae
    - Muntiacus
      - Reeves muntjac (Muntiacus reevesi)-Heuschele (1982)
  - Cervinae
    - Cervus
      - Axis deer (Cervus axis)-Heuschele (1982)
      - European fallow deer (Cervus dama)-Heuschele (1982)
      - Barasingha (Cervus duvauceli)-Heuschele (1982)
      - European red deer (Cervus elaphus)-Ashton (1982)
      - Altai wapiti (Cervus elaphus sibiricus)-Heuschele (1982)
      - Elds deer (Cervus eldi thamin)-Heuschele (1982)
      - Formosan sika deer (Cervus nippon taiwanus)-Heuschele (1982) Ashton (1982)
      - Tonkin sika deer (Cervus nippon)-Heuschele (1982)
      - Indian hog deer (Cervus porcinus)-Heuschele (1982)
    - Elaphurus
      - Pere Davids deer (Elaphurus davidanus)-Heuschele (1982) Ashton (1982)
- Antilocapridae
  - Antilocapra
    - Pronghorn (Antilocapra americana)-Heuschele (1982)
- Bovidae
  - Bovinae
    - Bison
      - American bison (Bison bison)-Heuschele (1982) Ashton (1982)
      - European bison/Wisent (Bison bonasus)-Ashton (1982)
    - Bos
      - Indian gaur (Bos gaurus)-Heuschele (1982)
      - Domestic cattle (Bos taurus)-Rossiter et al. (1980)
    - Boselaphus
      - Nilgai (Boselaphus tragocamelus)-Heuschele (1982) Ashton (1982)
    - Syncerus
      - Cape buffalo (Syncerus caffer caffer)-Heuschele (1982) Ashton (1982)
    - Taurotragus
      - Eland (Taurotragus oryx)-Heuschele (1982)

Tragelaphus
Sitatunga (Tragelaphus spekei)-Heuschele (1982)
Ashton (1982)
Ramsay et al. (1982)
Greater kudu (Tragelaphus strepsiceros)-Heuschele (1982)
Hippotraginae
Addax
Addax (Addax nasomaculatus)-Heuschele (1982)
Ramsay et al. (1982)
Hippotragus
Roan antelope (Hippotragus equinus)-Heuschele (1982)
Oryx
Scimitar-horned oryx (Oryx dammah)-Heuschele (1982)
Ashton (1982)
Gemsbok (Oryx gazella gazella)-Heuschele (1982)
Fringe-eared oryx (Oryx gazella callotis)-Heuschele (1982)
Arabian oryx (Oryx leucoryx)-Heuschele (1982)
Alcelaphinae
Alcelaphus
Cape hartebeest (A. buselaphus caama)-Heuschele (1982)
Ramsay et al. (1982)
Connochaetes
Whitetailed wildebeest (C. gnou)-Heuschele (1982)
Ramsay et al. (1982)
Blue wildebeest (C. taurinus taurinus)-Heuschele (1982)
Ashton (1982)
Rossiter (1981a)
Whitebearded wildebeest
(C. taurinus albojubatus)-Heuschele (1982)
Damaliscus
Topi (Damaliscus lunatus jimela)-Heuschele (1982)
Reduncinae
Kobus
Ellipsen waterbuck (Kobus ellipsiprymnus)-Heuschele (1982)
Defassa waterbuck (Kobus ellipsiprymnus)-Heuschele (1982)
Uganda kob (Kobus kob)-Heuschele (1982)
Antilopinae
Aepyceros
Impala (Aepyceros melampus)-Heuschele (1982)
Antidorcas
Springbok (Antidorcas marsupialis)-Heuschele (1982)
Antilope
Blackbuck (Antilope cervicapra)-Heuschele (1982)
Gazella
Addra gazelle (Gazella dama dama)-Heuschele (1982)
Dorcas gazelle (Gazella dorcas)-Heuschele (1982)
Grants gazelle (Gazella granti)-Heuschele (1982)
Slender horned gazelle (Gazella leptoceros)-Heuschele (1982)
Persian gazelle (Gazella subgutturosa)-Heuschele (1982)
Ourebia
Cottons oribi (Ourebia ourebi cottoni)-Heuschele (1982)

Caprinae
  Capra
    Domestic goat (Capra hircus)-Heuschele (1982)
    Markhor (Capra falconeri)-Heuschele (1982)
    Nubian ibex (Capra ibex nubiana)-Heuschele (1982)
                                    Ramsay et al. (1982)
    Siberian ibex (Capra ibex sibirica)-Heuschele (1982)
    Cretan wild goat (Capra aegagrus cretensis)-Heuschele (1982)
    African pygmy goat (Capra hircus)-Heuschele (1982)
  Hemitragus
    Nilgiri tahr (Hemitragus hylocrius)-Heuschele (1982)
    Himalayan tahr (Hemitragus jemlahicus)-Heuschele (1982)
  Ovibos
    Musk ox (Ovibos moschatus)-Heuschele (1982)
                                Ashton (1982)
  Ovis
    Bighorn sheep (Ovis canadensis)-Heuschele (1982)
    Domestic sheep (Ovis aries)-Rossiter (1981a)
                                 Heuschele (1982)
    American mouflon (Ovis gmelini)-Heuschele (1982)
    European mouflon (Ovis musimon)-Heuschele (1982)
  Rupicapra
    Chamois (Rupicapra rupicapra)- Ramsay et al. (1982)

APPENDIX VII

Solutions and Buffers

40% Sucrose Solution (0.2M PBS):

Na_2HPO_4 2.38 g/100 ml distilled water
NaH_2PO_4 0.44 g/100 ml distilled water
NaCl 0.85 g/100 ml distilled water
q.s. to 100 ml with distilled water and filter sterilize (0.22 μm)
add sucrose 40 g/100 ml 0.2M PBS
Mix and store at 4C.

Antigen Solubilization Buffer (Tris/Glycine/Triton X-100):

Tris base 0.61 g/L distilled water (0.005M)
Glycine 0.75 g/L distilled water (0.01M)
Triton X-100 1.0 ml/100 ml distilled water (1%)
Adjust pH to 8.9 prior to use.

Borate Buffer:

NaOH 2 g
H_3BO_3 9 g
q.s. to 1 liter with distilled water
Adjust pH to 8.6 prior to use.

Antigen Coating Buffer:

Na_2CO_3 1.59 g
$NaHCO_3$ 2.93 g
q.s. to 1 liter with distilled water
Store at 4C. Adjust pH to 9.6 using 5M NaOH.

ELISA Wash Solution:

phosphate buffered saline 0.01M (pH 7.2) 1 liter
tween-20 0.25 ml
Stir mixture well prior to use.

ABTS Substrate Solution:

1. Substrate diluent (citric acid solution)
   citric acid 10.51 g (0.05M)
   distilled water 1 liter
   Store at 4C. Adjust pH of test volume (10 ml/plate) to 4.0 using 5M NaOH prior to use.
2. Stock 50% H_2O_2 - diluted 1:50 with distilled water.
   Add 0.04 ml diluted H_2O_2/10 ml substrate diluent.
3. Stock ABTS solution
   (2-2' Azino-di-(3-ethylbenzthiazaline-6-sulfonic acid)
   548.7 mg/25 ml distilled water (40mM)
   Store at 4C in sealed amber bottle.
   Add stock ABTS solution 0.1 ml/10 ml substrate diluent.
   Mix substrate by gently swirling the mixture