# Malignant Catarrhal Fever I. Response of American Cattle to Malignant Catarrhal Virus Isolated in Kenya

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## ABSTRACT

Fifty-three American cattle were inoculated with malignant catarrhal fever virus isolated from a wildebeest in Kenya. Three animals showed the mild form of the disease and recovered, and 47 showed the severe form of the disease. The other three did not react. Of the 47 cattle, 28 died, 16 were killed for the collection of specimens and three recovered. The incubation period for the 47 cattle ranged from 16 to 29 days and the course of the fatal disease for 28 cattle averaged three to 23 days.

Virus titration of specimens from nine infected steers yielded a mean titer of $10^4/TCID_{50}$ per gm for lymph nodes, $10^3$ $TCID_{50}$ per mL for buffy coats and $10^{2.3}$ $TCID_{50}$ per gm for spleens. Smaller amounts of virus were found in the liver, kidneys, adrenals and thyroids. Malignant catarrhal fever virus was also found in nasal secretions and saliva of viremic cattle. Viral infectivity was shown in bovine buffy coat cells stored at 4°C for two days but was immediately destroyed upon freezing even when glycerine or dimethylsulfoxide was added. Viral particles were not found in infected animal tissues by electron microscopy.

The disease was successfully transmitted in steers by intratracheal intubation and by aerosol inhalation but not by contact.

## RÉSUMÉ

Cette expérience consistait à inoculer 53 bovins des États-Unis avec une souche du virus de la fièvre catarrhale maligne, isolée d'un gnou du Kenya. Trois de ces bovins ne développèrent que la forme bénigne de la maladie, contrairement à 47 autres; quant aux trois derniers, ils ne manifestèrent aucune réaction. Des 47 sujets qui développèrent la forme grave de la maladie, 28 moururent et on en sacrifia 16 desquels on préleva des échantillons; les trois autres guérirent. La période d'incubation varia de 16 à 29 jours, chez ces 47 bovins; le cours de la forme fatale de la maladie varia par ailleurs de trois à 23 jours, chez les 28 sujets qui en moururent.

La recherche de la concentration virale, dans divers échantillons provenant de neuf bouvillons, donna les résultats suivants: ganglions lymphatiques, $10^4TCID_{50}$ au gramme; rate, $10^{2.3}TCID_{50}$ au gramme; couenne d'échantillons de sang, $10^3TCID_{50}$ au mL. Le foie, les reins, les surrénales et les thyroïdes contenaient moins de virus; au cours de la virémie, les sécrétions nasales et la salive recelaient aussi le virus. Les cellules de la couenne d'échantillons de sang qu'on entreposa à 4°C, durant deux jours, s'avérèrt virulentes; leur congélation, même après l'addition de glycérine ou de diméthylsulfoxyde, leur fit cependant perdre toute virulence. La microscopie électronique ne révéla pas de virions, dans les tissus des sujets infectés.

L'infection intra-trachéale et l'inhalation d'aérosols permirent de transmettre la maladie à d'autres bovins, contrairement au simple contact.


## INTRODUCTION

Malignant catarrhal fever (MCF) is a disease of cattle characterized by high fever, profuse nasal and ocular discharges, severe hyperemia and necrosis of mucosae, corneal opacity and enlargement of lymph nodes. The disease observed in Africa is caused by a herpesvirus that has its reservoir in the wildebeest (16, 17). Sheep are reservoir hosts for MCF reported mainly outside Africa (16).

The disease is transmitted from the reservoir hosts to cattle and other susceptible animals during lambing season of sheep or calving season of wildebeest (1, 3, 16). In cattle, MCF is noncontagious and therefore is sporadic, however, epizootics resulting from exposure of large numbers of susceptible animals to sheep or wildebeests

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have been reported (4, 5, 9, 10, 13, 15).

Although the wildebeest-associated MCF is considered an African disease, the possibility remains that the disease could be introduced to other countries by the importation of infected wildebeest. The need for testing the susceptibility of American cattle to the disease is therefore apparent. In the experiments to be reported here, we successfully infected 50 American cattle with a virulent MCF virus (MCFV) isolated from a wildebeest in Kenya. A successful transmission of MCF in cattle by aerosol inhalation or intratracheal intubation is also described.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS

Sixteen Holstein calves, two to nine months old, and 37 Hereford steers, 15 to 24 months old, were tested. These animals were held in quarantine for at least two weeks. Their preinoculation sera did not contain neutralizing antibodies against MCFV.

After quarantine, all animals were held in isolation rooms. They were examined and rectal temperatures were taken daily. Any rise to 39.3°C and above was an indication of disease.

### VIRUSES

A virulent strain of MCFV[1] that was isolated from a tonsil of a wildebeest cow (18) was used in the inoculation studies. This herpesvirus was used after five to eight passages in bovine thyroid (BTh) cells after the original isolation. Strain WC11 of MCFV (17) was used in the neutralization, complement fixation and immunodiffusion tests.

### CELL CULTURES

Malignant catarrhal fever virus was grown in primary or secondary BTh cells (17) seeded either in plastic flasks or Leighton tubes. Thyroid cells were grown in a medium developed by this laboratory which consists of one part of Hank's salt solution[2] supplemented with 0.5% lactalbumin hydrolysate[2] and two part of minimum essential medium with Earle's salts.[2] The growth medium contained 10% bovine serum (BS) penicillin (200 units/mL), dihydrostreptomycin (200 $\mu$g/mL), mycostatin (60 units/mL) and kanamycin (80 $\mu$g/mL). The maintenance medium containing 2% BS was changed every four to six days.

### INOCULATIONS

Animals were inoculated into the prescapular lymph nodes or intravenously (IV) with $10^2$ to $10^4$ $TCID_{50}$ of the virus derived either from bovine blood or infected cell culture. Intratracheal and aerosol exposure used in the transmission experiments will be described below.

### RECOVERY OF VIRUS FROM INOCULATED CATTLE

Blood for buffy coat cells and serum was collected from cattle weekly before the onset of the clinical disease and every two to four days thereafter. Recovered animals were bled monthly. Buffy coat cells were prepared from 45 mL of blood collected into a vial containing 5 mL of 1% ethylenediaminetetraacetic acid (EDTA) in 0.85% NaCl. Buffy coats were obtained by centrifugation of blood at 500 x g for 30 to 45 minutes. White blood cells were then collected, washed once in phosphate buffered saline (PBS) and centrifuged for 15 minutes. The resulting buffy coat cells were collected, suspended in 2 mL of the maintenance medium and immediately inoculated into replicated monolayers of BTh cells. On the next day, the inoculated cultures were washed two to three times with PBS and then fed with fresh medium. Inoculated cultures were examined every two to three days for 21 days. Serum obtained from clotted blood was stored in vials at -20°C.

### STUDIES ON SPECIMENS FROM KILLED STEERS

Nine steers were killed three to nine days after the onset of the clinical disease for viral assay and for electron microscopical (EM) and histopathological examinations. Tissue specimens collected included various lymph nodes, Peyer's patches, spleens, livers, adrenals, kidneys, thyroids and salivary glands.

For viral titration, 10% tissue suspensions were prepared with Ten Broeck grinders. These suspensions were assayed for MCFV in BTh cells. Occasionally in attempts to isolate a virus from infected tissues, thyroids and adrenals were minced, trypsinized and grown in the medium described for thyroid cell cultures.

Specimens for EM were minced to 1 to 2 $mm^3$ fragments, fixed in 2% glutaraldehyde and embedded in (EPON).[3] Sections from each preparation were cut and examined. Tissue specimens collected for histopathological examination were fixed in 10% formalin contained in neutral buffered saline. Sections four to six microns thick were prepared and stained with hematoxylin and eosin as described (8).

### PRESERVATION OF MCFV IN BOVINE SPECIMENS

In an attempt to preserve MCFV in specimens from clinical cases, 100 mL of blood in EDTA was collected from each of ten diseased cattle. Each blood sample was divided into four aliquots. The aliquot one was unfrozen, two was frozen (-70°C) without additive and three and four were frozen (-70°C) with 15% of either glycerine or dimethylsulfoxide (DMSO). Freezing and thawing were repeated three times. Buffy coat layers were then prepared and

[1]Received from the Director of East African Veterinary Research Organization, Muguga, P.O. 32, Kikuyu, Kenya.
[2]Grand Island Biological Company, Grand Island, New York.
[3]Shell Development Corporation, Stamford, Connecticut.

assayed in BTh cultures for infectivity. Cell suspensions of the spleen and lymph nodes were divided into four aliquots and treated similarly.

### SEROLOGICAL TESTS

Sera from experimental animals were tested by virus neutralization and complement fixation (CF). For the virus neutralization test, serum samples were heat inactivated at 56°C for 30 minutes and then diluted in twofold series. Beginning with a 1:2 dilution, 0.6 mL of each serum dilution was mixed with an equal volume of the cell-free virus containing about 1000 $TCID_{50}$ per mL of final dilution. After the serum-virus mixtures were held for one hour at 37°C four culture tubes were each inoculated with 0.25 mL of every serum-virus mixture. Inoculated cultures were examined for 14 days. The 50% virus neutralization titer was calculated by the Karber's method (7).

The CF test was conducted as described (2). Viral antigens were concentrated by dialysis against polyethylene glycol (Carbo-wax 20M).[4]

### STUDIES ON MCF TRANSMISSION IN CATTLE

Swab specimens were obtained weekly from inoculated animals in an attempt to isolate MCFV from excretions. Nasal secretions were collected by gently swabbing the nasal mucosa. Oral specimens were collected either from the ventral aspects of the tongue or as saliva dripped from the mouth. Swab specimens were also collected from the eye, rectum and vulva. Swabs were dipped into 3 mL of the growth medium containing three times the concentrations of antibiotics described for culture fluid. Secretions were then removed from the swabs by agitation. The resulting suspensions were approximated as 1:10 dilution of the appropriate secretions. They were assayed for infectivity in BTh cells.

After the virus was detected in nasal secretions by the cell culture technique, steers were inoculated with nasal swab specimens for further confirmation of viral infectivity. Nasal swabs from an infected steer were inoculated into two steers. Each received 3 mL into the prescapular lymph nodes. For a second animal passage, two steers were inoculated with nasal swabs collected from diseased animals of the first passage.

### INFECTION OF CATTLE WITH MCFV BY THE RESPIRATORY TRACT

The failure to demonstrate contagious infection in MCF in previous trials (18) prompted us to inoculate steers by intratracheal and by aerosol exposure. For intratracheal inoculation, four steers were anesthetized IV with sodium pentothal. When animals became recumbent, a 3.5 foot piece of tubing (outside diameter 0.25 inch) was introduced into the trachea via the mouth. Then 10 mL of cell culture suspension containing $10^{4.2}$ $TCID_{50}$ was administered rapidly into the tubing. For aerosol exposure a large plastic bag was fitted over the heads of the four restrained steers, after which 10 mL of infected cell suspension containing $10^{4.2}$ $TCID_{50}$ was sprayed into this plastic bag for 15 seconds. An aerosol was created by blowing compressed air (2 lb/sq inch) into a glass nebulizer containing the inoculum. The plastic bag was left in place for two minutes. For inoculum control, four steers received the same dose of virus into the prescapular lymph node.

### ATTEMPTS TO TRANSMIT MCF IN CATTLE BY CONTACT

Five steers in groups of two and three were in contact with diseased cattle for 47 to 68 days, respectively. Contact animals were then challenged with the virulent virus at 88 and 150 days after exposure, respectively.

## RESULTS

### CLINICAL RESPONSES OF CATTLE TO MCFV

Of 53 cattle inoculated with MCFV, 50 became infected. Three of the infected animals showed the mild form and recovered and 47 showed the severe form of MCF. These forms have been described in detail by Jubb and Kennedy (6). Among the 47 animals displaying the severe disease, 28 died, 16 were killed for specimen collection and three recovered. There were no significant differences in the incubation period or course of the disease between calves and steers (Table I).

The first clinical signs were nasal and lacrimal discharges as well as an enlargement of lymph nodes. Nasal discharges were at first serous and mild, then gradually increased in quantity and density and became mucoid, mucopurulent and, later, purulent. The purulent excretions partly or completely blocked nasal passages and frequent sneezing and dyspnea resulted. Blocked nostrils forced animals to breathe through the mouth and to discharge frothy saliva. Foul breath was marked. Heavy encrustation of the muzzle

**TABLE I. Response of U.S. Cattle to African Malignant Catarrhal Fever Virus**

| | Incubation Period in Days[a] | | | | Course of Disease in Days | | | |
|---|---|---|---|---|---|---|---|---|
| Age Group | No. of Animals | Range | Mean | S.D.[b] | No. of Animals | Range | Mean | S.D. |
| Calves | 16 | 14-26 | 17.9 | ±3.4 | 14 | 4-52 | 12.5 | ±11.9 |
| Steers | 31 | 16-46 | 24.6 | ±6.7 | 14 | 5-42 | 13.2 | ± 8.9 |
| Total: calves and steers | 47 | 14-46 | 22.3 | ±6.6 | 28 | 4-52 | 12.9 | ±10.3 |

[a]Cattle showing the severe form of the disease only are given
[b]S.D. = Standard deviation

[4]Carbie and Carbon Chemicals, Union Carbide and Carbon Corp., New York.

TABLE II. Summary of the Clinical Response of U.S. Cattle to African Malignant Catarrhal Fever Virus

| Sign | No. of Animals[a] | Percent % |
|---|---|---|
| Pyrexia | 50 | 100 |
| Discharges: oral and nasal | 50 | 100 |
| Congestion of mucosae | 50 | 100 |
| Corneal opacity | 50 | 100 |
| Encrustation of the muzzle | 50 | 100 |
| Enlargement of lymph nodes | 50 | 100 |
| Nervous signs | 11 | 22 |
| Submandibular edema | 8 | 16 |
| Petechiation | 2 | 4 |
| Recovery | 3 | 6 |

[a]Three animals that did not show clinical or serological reactions are not included

and nares were common features (Table II). All peripheral lymph nodes were palpable at this time. At the onset of the febrile reaction, lymph nodes, together with corresponding hemolymph nodes, rapidly enlarged up to ten times the normal size. They usually attained this size after two to five days of fever but they regressed if the course of disease was prolonged.

Two to seven days after the onset of the clinical reaction, a febrile reaction was observed. The temperature fluctuated between 40°C and 41.7°C until shortly before death, when it declined to subnormal.

The oral mucosa was hyperemic and edematous with increased salivation. Oral congestion first appeared in patches. It rapidly became diffuse and severe and resulted in superficial necrosis of lips, gums, both divisions of the palate and the cheeks. The congestion of buccal papillae started at the tips and progressed to the base. The affected areas became necrotic, sloughed off and left blunted papillae or stumps. The pathological change in the mouth caused severe pain that was expressed by difficulties in chewing food and reluctance to submit to examination of the oral cavity. Petechiae in oral and nasal mucosae were observed in two animals.

Changes in the eye included lacrimation that became purulent in the latter stages. Ophthalmia, prominent scleral veins, swollen eyelids and photophobia were also observed. These changes were accompanied by corneal opacity that started at the corneoscleral junction and progressed centripetally. Corneal opacity resulted in either impaired vision or blindness, depending on its extent and intensity.

Constipation was common but terminal diarrhea was observed in two animals. Submandibular edema and nervous signs were also observed (Table II). Nervous signs were shivering, incoordinated movements, paralysis of cervical muscles and terminal nystagmus. Cutaneous lesions were not observed.

Signs of the mild form of the disease were slight to moderate temperature, congestion of mucosae, nasal and ocular discharges and enlarged lymph nodes. These changes regressed after three to nine days.

TABLE III. Summary of the Pathological Response of Cattle to African MCFV

| Lesion | No. of Animals[a] | Percent % |
|---|---|---|
| Necrosis of mucosae: oral and nasal | 50 | 100 |
| Erosions and ulcers in abomasum | 42 | 84 |
| Erosions in esophagus | 32 | 64 |
| Pericarditis | 25 | 50 |
| Foci in kidney | 10 | 20 |
| Gelatinous infiltration around the lymph nodes | 5 | 10 |
| Cystitis | 3 | 6 |
| Ulcers in forestomachs | 2 | 4 |

[a]Three animals that did not show the clinical or serological reactions are not included

GROSS PATHOLOGICAL CHANGES

For the severe form of the disease the carcasses were emaciated if the course was prolonged. Oral lesions have been described under clinical signs.

Erosions often found in the esophagus were covered by a yellowish, necrotic pseudomembrane. The forestomachs were usually normal. The abomasal mucosa was hyperemic, with edematous abomasal folds. Hemorrhagic erosions or ulcers were also observed around the pyloric region (Table III). Congested areas in the small intestine were covered with blood tinged contents in the lumen. Intestinal walls were thickened with edema and Peyer's patches showed minimal changes. Lines of congestion characteristic of other mucosal infections were often observed along the longitudinal mucosal rugae of the large intestine.

Necrotic lesions in the nasal cavity were covered by thick cheesy exudate. The pharyngeal and laryngeal mucosae swollen and often covered by necrotic tissues. The mucosae of the trachea and bronchioles were covered with diphtheritic membranes. The lungs were often emphysematous in six cattle.

Renal foci were observed in ten cattle. They occurred either as many and small (2 to 4 mm) or few and large (5 to 15 mm) lesions. They were whitish, rounded projections contained in the renal cortex. Cystitis were observed in three cattle.

Lymph nodes with hemolymph nodes were edematous, enlarged and occasionally hemorrhagic. Gelatinous infiltration around these nodes was found in five cattle.

The liver and spleen were slightly enlarged and congested. Petechiae in the trachea and coronary groove were seen (Table III). Generalized petechiae were observed in one animal. In 25 animals, particularly those with submandibular edema, about 200 mL of serous or blood tinged fluid was found in the pericardial sac.

Two chronically ill animals died

52 and 130 days after the onset of the clinical disease. The necropsy showed multiple button-like ulcers in the forestomachs, abomasum, liver and lungs. Focal lesions were observed in the kidneys of one of three cattle that showed the mild form of the disease.

### HISTOPATHOLOGICAL EXAMINATIONS

There were histological lesions in the kidneys, livers, adrenals and brains. Kidney lesions were those of glomerulonephritis and multifocal interstitial nephritis similar to those already described by Jubb and Kennedy (6). Many mitotic stages of mononuclear cells were observed. Venous thrombosis, together with endothelial proliferation of muscular arteries, was also seen. Sinusoids were dilated. Adrenals showed essentially the changes described for the liver. Blood vessels in the brain, particularly the cerebellum, were infiltrated with mononuclear cells. Histological lesions were not seen in six uninoculated steers that were examined.

### RECOVERY OF VIRUS FROM INOCULATED CATTLE

Malignant catarrhal fever virus was isolated from blood of all infected cattle. The virus was first isolated in the buffy coat cells two to seven days before the onset of the clinical disease and was demonstrable throughout the course of the disease. The titer of virus varied from $10^{2.5}$ to $10^4$ $TCID_{50}$ per mL of buffy coat cells. For cattle that recovered the titer declined to about $10^{0.5}$ $TCID_{50}$ and then to zero, two to three weeks after the disappearance of the clinical syndrome.

Syncytia developed in monolayers of BTh cells four to ten days after inoculation with buffy coat specimens. Cultures inoculated with a 1:10 dilution of the buffy coat cells showed more extensive cytopathic effects (CPE) than those given the undiluted sample.

Virus titration from nine reacting animals showed a mean titer of $10^4$ $TCID_{50}$ per gm for lymph nodes, $10^3$ $TCID_{50}$ per mL for buffy coats cells and $10^{2.3}$ $TCID_{50}$ per gm for spleens. Various lymph nodes from the same animal contained similar titers. The amount of virus recovered did not vary with the severity of the clinical disease. Small amounts of the virus were detected in 1:10 dilutions of the livers, kidneys, adrenals and thyroids. Primary cultures from infected thyroids and adrenals produced typical CPE.

### PRESERVATION OF MCFV IN BOVINE SPECIMENS

Malignant catarrhal fever virus was isolated from blood held on ice for two days. In contrast, the virus was not isolated by cell culture methods in any blood sample frozen with or without additives.

### ELECTRON MICROSCOPIC STUDY OF BOVINE SPECIMENS

Viral particles were not seen in any of the 138 specimens that were carefully examined. However, cytological changes consisting of condensation and fragmentation of chromatin, aggregation of mitochrondria and loss of cytoplasmic organelles were seen in a few cells.

### SEROLOGICAL RESPONSE OF INFECTED CATTLE

Neutralizing antibodies were detected in 13 animals and CF antibodies only in eight (Table IV). Four cattle sera in which neutralizing antibodies were detected at a dilution of 1:8 or 1:16 were CF negative.

**TABLE IV. Neutralizing (NT) and CF Antibodies in Sera of Cattle Infected with MCF Virus**

| Nt Antibody unit/0.1 mL[a] | CF Antibody units/0.1 mL[c] |
|---|---|
| 8 (1)[b] | None |
| 16 (3) | None |
| 32 (5) | 16 (4) |
| 64 (3) | 32 (1); 64 (2) |
| 128 (1) | 128 (1) |

[a]Reciprocal of highest serum dilution that neutralized 100 $TCID_{50}$ of MCFV
[b]No. of animals displaying the serum titer
[c]Reciprocal of highest serum dilution that inhibited 70% or more hemolysis

### STUDIES ON MCF TRANSMISSION IN CATTLE

Malignant catarrhal fever virus was isolated by cell culture technique in 32 of 53 nasal swab specimens collected in early stages of the clinical disease. Specimens collected in later stages of the disease were toxic to BTh cells. On inoculation into cattle, nasal secretions from sick animals produced a disease similar to that produced by infected blood. The incubation period, however, was longer by ten days in animals infected with nasal swabs (Table V).

Malignant catarrhal fever virus was isolated also in eight of ten saliva samples from diseased animals. Virus was not isolated from

**TABLE V. Clinical Reactions of Cattle to Inoculations of Blood or Nasal Swabs from Steers Infected with MCF Virus**

| Donor Animal No. | Recipient Animal No. | Inoculum | Incubation Period (DPI)[a] | Form of Disease | Day of Death (DPI) |
|---|---|---|---|---|---|
| 1 | 4 | Blood | 16 | severe | 25 |
| 1 | 5 | Blood | 21 | severe | 26 |
| 2 | 6 | Blood | —[b] | — | 52 (K)[c] |
| 2 | 7 | Blood | 23 | severe | 34 (K) |
| 3 | 9 | Blood | 22 | severe | 34 (K) |
| Mean | | | 20.5 | | |
| 1 | 2 | Swabs | 25 | severe | 48 (R)[d] |
| 1 | 3 | Swabs | 28 | mild | 48 (K) |
| 2 | 8 | Swabs | 31 | severe | 45 (K) |
| 3 | 10 | Swabs | 38 | severe | 50 (K) |
| Mean | | | 30.5 | | |

[a]DPI = day postinoculation
[b]— = no clinical response
[c]K = killed during the course of disease
[d]R = killed after recovery

the eye, rectum, vulva and urine of viremic animals. Titration of ten nasal secretions and four saliva samples yielded a mean of $10^{1.5}$ $TCID_{50}$ per mL for both groups.

The results of experimental infection of steers by the respiratory tract showed that eight steers were readily infected by this route (Table VI). The clinical response of these animals was comparable with that elicited by inoculating the virus into the prescapular lymph nodes (Table VI).

Five steers exposed to these animals by contact did not produce clinical signs of the disease of seroconversions for up to 150 days after exposure.

## DISCUSSION

Clinical and pathological observations in 50 American cattle that became infected with MCFV were strikingly similar to those of European and African breeds of cattle given various strains of the same virus (11, 12). These findings indicated that the response of cattle to this virus is apparently not influenced by the origin or breed of cattle.

Malignant catarrhal fever virus was readily isolated from lymphoid tissues of reacting animals by use of BTh cells. Thus, virus isolation is a rapid procedure for confirming the diagnosis of the African MCF. To date, virus isolation from clinical MCF serves to differentiate the African disease (wildebeest derived) from the clinically similar disease described in various countries (16). The latter is derived from sheep. Attempts to isolate a virus from the sheep-associated disease have not yet been successful (5, 14, 19).

Results in the present study indicate that the virus in buffy coats of infected cattle survived for two days at the usual refrigerator temperature but lost infectivity when frozen with or without additive. Thus, if there is an unavoidable lapse of time between the collection of the material and its inoculation into cell culture or susceptible animals the specimens should not be frozen but should be left at refrigerator temperature (4°C). In contrast, in a separate study to be reported elsewhere (Kalunda *et al*, manuscript in preparation) we found that once the virus was isolated from animal specimen by cell culture it withstood freezing temperature.

The low viral titer detected in animal tissue was considered to be the cause for the failure to detect viral particles by electron microscopy. Tests by previous investigators were negative for detectable antibodies. Viral isolation and identification as well as histopathological findings are the reliable methods for confirming the diagnosis of an acute case of the African MCF.

Detection of MCFV in both nasal and oral secretions indicated that MCFV is excreted by infected cattle. Our failure to transmit the disease in cattle by exposing five animals to infected steers for over two months suggested that not enough virus was excreted or the excreted virus was not in a stable state capable of contact transmission. However, steers were infected by the respiratory route, suggesting this could be the natural route of infection. Previous trials failed to show contagious infection in this disease (14, 16).

**TABLE VI. Clinical Response of Steers Infected with MCFV by the Respiratory and Glandular Routes**

| Route of Inoculation | No. of Animals | Mean Incub. Period (days) | Mean Course of Disease (days) |
|---|---|---|---|
| Aerosol | 4 | 24.5 | 20.7 |
| Endotracheal | 4 | 19.3 | 10 |
| Intraglandular | 4 | 19.5 | 11.8 |

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