Sporadic Bovine Encephalomyelitis in Canada

by G. L. Bannister[1], P. Boulanger[1], D. P. Gray[1], C. H. Chapman[2]
R. J. Avery[3] and A. H. Corner[1]

An encephalitis of young cattle was described in 1940 by McNutt (1) in the United States. He called it "Buss encephalitis" after the name of the owner of the farm where the infection was first seen. The condition was characterized by fever and depression, accompanied by staggering and weakness of the hind limbs. Other symptoms of central nervous involvement included spasm of muscle groups and lameness. Invasion of the lungs was not uncommon and was characterized by nasal discharge, laboured respiration and coughing. When the intestinal tract was involved, mild to severe diarrhea was present. The course varied from one to three weeks with mortality as high as 40 per cent. At necropsy, there was pleuritis and peritonitis with fibrinous exudate, often accompanied by complete hepatization of the apical and intermediate lobes of the lungs.

Menges (2) and Wenner (3) in 1953 studied the disease further and proved it was due to an agent belonging to the psittacosis-lymphogranuloma group of viruses. Related organisms were demonstrated in an enteritis of calves in 1950 by York and Baker (4). In 1960, Storz *et al* (5) isolated a viral agent from cases of epizootic bovine abortion which also appeared to be due to a member of this group of viruses. Other animal species such as sheep and cats (6-8) are likewise susceptible. It is now known that in addition to the psittacine birds responsible for human infection (9), other avian species such as turkeys and pigeons are carriers. Labzoffsky (10) has shown that 24 of 146 pigeons from three Canadian cities reacted serologically to this infection. A comparable situation exists on some Canadian turkey farms (11). Numerous other reports on this group of infections affecting a wide variety of animal species have appeared in American, French and Japanese literature.

1 Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec.
2 Contagious Diseases, Health of Animals Division, Canada Department of Agriculture, Sub-District Office, Lethbridge, Alta.
3 Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Animal Diseases Research Institute (Western), Lethbridge, Alta.

Clinical Investigations

On January 24, 1961, the herd of cattle under investigation was visited by two of us, accompanied by the local veterinary practitioner. Four weeks before our visit, on December 28, 1960, the owner had noticed stiffness in one calf and a week later another one was observed with the same symptoms. A local veterinary practitioner called on January 7, made a tentative diagnosis of vitamin A deficiency and treated one of the animals accordingly. On January 13, the owner called a second practitioner who submitted a calf in moribund condition to A.D.R.I. (Western) on January 20. It was destroyed January 23, and a post-mortem examination performed.

The most prominent changes were in the thoracic cavity. The apical and intermediate lobes showed various degrees of hepatization and areas of pleurisy were evidenced by the presence of fibrinous tags on both the visceral and parietal pleurae. There was a very marked fibrinous pericarditis; in some areas the pericardium was thickened being one quarter to three eighths of an inch and was showing a typical "bread and butter" appearance. Small amounts of both pericardial and pleural fluid, serosanguineous in nature, were present. The tracheal and laryngeal mucosal surfaces were normal. The major abdominal organs and intestinal tract were normal in appearance. However

TABLE I

Clinical and Serological Observations on Twelve Calves Investigated at a first visit January 24, 1961

Eartag Number	Temperatures °F	Health Status	W.B.C. Count	C.F. Titres*		
				Jan. 24	Feb. 1	March 29
4V38250	101	Moribund	5,400	1:20	dead	dead
— 248	98.2	"	12,900	1:40	1:80	"
— 251	96	"	3,000	1:10	dead	"
— 252	96.3	"	3,650	1:40	"	"
— 253	91.2	"	4,900	1:40	"	"
— 254	103.3	Recovered	8,050	1:160	1:160	1:80
— 255	103.8	Off color	5,000	1:20	1:40	1:160
— 256	103.2	Normal	7,850	—	1:10	—
— 257	102.8	Recovered	6,350	1:320	1:320	1:80
— 258	102.8	"	11,300	1:320	1:160	1:160
— 259	102.9	Normal	9,950	—	1:10	1:5
— 260	103.4	"	8,950	—	1:10	1:5

*Titres expressed as the highest serum dilution showing 50 per cent haemolysis or less in the presence of antigen.

as in the thoracic cavity, a small amount of serosanguineous fluid was observed. The brain and meninges appeared normal but there was an apparent increase in cerebrospinal fluid.

Histological sections of the liver stained with Pinkerton's adaptation of Machiavello's stain for rickettsiae revealed on the surface of the organ inclusion bodies typical of a member of the psittacosis-lymphogranuloma group of agents. Sections of mouse lung experimentally infected with the virus of enzootic abortion of ewes were used as control slides. Histological examination of the brain revealed a leptomeningitis characterized by massive infiltrations of round cells. This meningitis appeared more severe over the cerebellum. Both focal and diffuse areas of gliosis were noted throughout the brain. In the cerebellum degenerate Purkinje cells appeared to act as a nucleus for the formation of glial nodes. Severe perivascular mononuclear cuffing was general and a few areas of encephalomalacia were noted in the cerebrum. Inclusion bodies were not demonstrated in sections of the brain.

At the time of our first visit to the premises on January 24, 8 calves out of the 58 kept on the farm had died and 5 were in a moribund condition. Untreated and heparinized blood samples were taken from the 5 moribund, 3 recovered and 3 normal animals and from one which had shown signs of illness that morning. The temperature of these animals was also taken and found to vary from 91.2 to 103.8°F. as indicated in Table I. Intramuscular inoculation of aureomycin was recommended for the moribund animals and aureomycin-supplemented feed for the healthy subjects. However, despite the recommendation, actual treatment was not only delayed but was not completely adhered to and consisted of minimal dosage.

One moribund animal, eartag 2V38251 was taken to the laboratory for necropsy. It was dead upon arrival and the post-mortem examination took place two hours after death. The lesions were not as extensive as in the previous animal. Very little pleurisy was found and no fibrinous pericarditis. The lung involvement was extensive but of a different type. The right cardiac and apical lobes showed almost complete hepatization. The right diaphragmatic lobe and the left lung presented spotty hepatization. There was evidence of fibrinous exudate in the ventral portion of the thoracic cavity, particularly in the region of the diaphragmatic lobe.

These findings, together with a marked leucopenia in four of the five moribund animals, as listed in Table I, pointed to the presence of a viral agent as the cause of the condition. This was also indicated by the results of the complement-fixation tests with a psittacosis-lymphogranuloma

TABLE II

Results of Serological Tests on Paired Serum Samples from 15 Calves Showing Fever at the Time of the First Bleeding

Eartag Number	Temperatures °F	C.F. Titres		Clinical Observations
		Feb. 1	March 29	
4V48315	104	—	1:5	
— 316	105.8	1:80	1:80	
— 308	104	1:10	1:80	
— 322	104.6	1:80	1:160	
— 311	104	1:10	1:320	Nervous symptoms, diarrhea Jan. 31
— 325	106	tr. 1:10	1:160	Eye discharge Feb. 1
— 327	106	1:20	1:40	
— 334	104	1:20	1:80	
— 335	106.4	1:10	1:80	
— 310	104.2	1:20	1:5	Incoordination Jan. 31
— 337	105.6	1:20	1:320	
— 338	104.4	1:40	1:160	
— 339	104.6	1:10	1:160	
— 340	104	—	—	
— 259	104	1:10	1:5	

tr = trace of fixation

group reactive antigen, which were strongly positive with the sera of the three recovered animals. Frozen tissues from the two animals which were previously necropsied were sent to A.D.R.I. (Eastern) in order to isolate the viral agent and confirm the diagnosis.

By the time of our second visit to the premises, January 26, an additional two animals had died (-252, -253), making a total of 11 deaths. Another one (-262) had had a temperature of 105.1°F; it appeared to have no sense of direction and required assistance to the feeding area. On January 31, a third visit was made to the farm. Another animal (-250) had died making a total of 12 losses. In addition to the three remaining sick animals (-248, -255, -262) referred to previously, another seven (-307 to -313) appeared to be sick. The most prominent manifestations were stiffness and incoordination of the hind quarters. The next day, February 1, blood samples were collected from 44 calves and two in-contact bulls for serological tests. Fifteen of these animals as shown in Table II, had temperatures ranging from 104 to 106.4°F. Blood samples were also taken from 5 of the 30 contact sheep. The calves were kept with the bulls in loose housing on the home premises and had access to a surrounding corral as did the sheep. On reviewing the number of calves that had died since the infection was first noticed in the herd, it was learned from the owner that 13 instead of 12 calves had died prior to this visit. In addition a late calf which had been in poor condition was found dead at this visit, making a total of 14 losses.

A fifth visit was made to the farm February 17 in order to collect for serological tests, blood samples from 70 adult cattle which were on a winter pasture two miles from the home premises and from 15 sheep which were not bled previously. In contrast to the calves, the adult cattle and the sheep had not displayed clinical manifestations. However, on March 24, a ewe gave birth to a dead lamb a month prematurely. A blood sample was collected from this animal on this date and again on May 17. The aborted foetus was sent to A.D.R.I. (Eastern) for virus isolation. A second series of blood samples was collected from 43 calves on March 29 in order to determine possible changes in their serological status.

The report of the serological tests indicated clearly, as will be discussed later, that the calves and the sheep were experiencing an acute infection with a virus belonging to the psittacosis-lymphogranuloma group of agents. However, the origin of this infection was not readily ascertained. No addition had been made to the cattle or sheep population in the last few years except for two adult bulls which re-

TABLE III

Results of Serological Test on Paired Serum Samples from Seven Calves that did not Show Fever at the Time of the First Bleeding

Eartag Number	Temperature °F	C. F. Titres		Clinical Observations
		Feb. 1	March 29	
4V38307	103	—	1:320	Fever previous day
— 224	103	1:20	1:320	
— 321	103.8	—	1:20	
— 309	102.4	1:80	1:160	
— 262	102.8	1:5	1:80	Fever Jan. 27
— 313	101.3	1:20	1:640	Stiff previous day.
— 255	102.4	1:5	1:80	Ill Jan. 25

mained serologically negative. Both animal species had been free of disease in the previous year. There were no turkeys among the 56 poultry of the farm. However, at the beginning of December, approximately three weeks previous to the first manifestation of disease in the calves, a flock of approximately five hundred pigeons had descended on the farm buildings and remained there for the greater part of a day. These pigeons were traced to a farm within five miles distance. A hundred of them were bought for serological and viral investigations.

Serological Studies

The complement-fixation test was the serological method used in this investigation. The sera were titrated in two-fold serial dilutions as described in a previous publication (12). The period of fixation for the bovine, pigeon and the experimental guinea-pig sera was 18 hours at approximately 9°C., whereas for sheep sera an incubation period of 90 minutes at 37°C., was used to reduce the non-specific reactivity. The antigen for the complement-fixation test was a phenolized-boiled group-reactive antigen prepared from yolk sacs from embryonated eggs inoculated with the virus of enzootic abortion in ewes. Normal control antigens were prepared in a similar way from yolk-sac membranes of non-inoculated embryonated eggs. Every serum was tested in duplicate with the infected and normal antigens in order to detect non-specific reactivity to the egg tissue. In the present study, as well as in a previous survey of more than a thousand cattle sera, a good percentage of specimens gave a non-specific reaction in the 1:10 and sometimes in the 1:20 dilution with a normal egg antigen. When testing cattle serum, it is necessary to be aware of this potentiality in order to avoid misinterpretation of the results.

Among the sera collected January 24, those from the moribund calves had titres ranging from 1:10 to 1:40 whereas the titres of the sera from the three recovered animals ranged from 1:160 to 1:320 (Table I). The sera from the three normal calves were negative. The serum from the animal which had been sick for only a day when the specimen was collected, showed a 1:20 reaction which had increased to 1:40 and to 1:160 in the specimens taken February 1 and March 29 respectively. The results of the tests on these first 12 calves' sera strongly suggested that the infective agent belonged to the psittacosis-lymphogranuloma group of viruses.

On February 1st, sera from 44 calves and two bulls were tested. Twenty-two of the calves' sera gave positive reactions with titres ranging from 1:20 to 1:320. Ten sera gave a 1:10 titre (questionable reaction), and 14 others including the two bulls' sera, were negative. In the subsequent test performed March 29, out of 43 calves' sera, 26 were positive with titres ranging from 1:20 to 1:640; only three sera were slightly questionable and 14 were negative.

Table II indicates that 15 of the calves tested February 1st had a temperature ranging from 104 to 106.4°F. Their serum titres were relatively low at that time as compared to those obtained in the test performed eight weeks later. By this time, in ten cases reactions were given in serum dilutions two to six-fold higher. This suggested that the calves were at the acute phase of the disease when first tested. A similar increase in titre was also displayed by the sera of seven calves listed in Table

III, that had a temperature close to the normal range when first tested. Of the remaining paired sera tested, twelve showed a lowering of titre, four remained stationary and seven were negative in both tests.

On February 16, 70 sera from the healthy adult cattle pastured two miles from the calves, were tested by the complement-fixation test. One gave a positive reaction in the 1:20 serum dilution and 12 gave questionable reactions. Twenty-four sera were non-specific, reacting to 1:5 or 1:10 dilutions with the normal control antigen. Thirty-three sera were negative with both the normal and viral antigens.

Five of the sheep in contact with the calves were bled February 1. Their sera gave non-specific reactions in the 1:20 dilution with the normal control antigen. However, two of these sera also reacted in the 1:80 dilution with the viral antigen which was suggestive of actual infection. Fifteen additional sheep were bled two weeks later, February 16. Six of the 15 sera gave a positive reaction, 5 were questionable and 4 negative. Nine sera also gave a trace reaction with the normal control antigen. One of the ewes whose February 16 serum reacted 1:40 aborted March 24. On the day of abortion the serum titre was only 1:20 but had increased to 1:80 three weeks later on May 17.

Ninety-three sera from the large flock of pigeons which came to the farm early in December, were tested by the complement-fixation test. Ten sera were positive giving titres ranging from 1:20 to 1:160; five were questionable, reacting in a 1:10 dilution and 77 were negative.

Demonstration of the Causative Virus

Attempts to isolate the causative agent were made mainly with tissues and cerebrospinal, peritoneal and thoracic fluids from two naturally infected calves and from tissues of an aborted lamb foetus. After collection the tissues and fluids were kept frozen. The method of Kawakami *et al* (13) was used in preparing the tissue for inoculation. A ten per cent suspension of tissue was made in buffered saline and centrifuged at 4°C. for 30 min. at 3000 R.P.M. in an International PR2 centrifuge. The supernatant fluid was removed and mixed with streptomycin sulphate to make a final antibiotic content of 2 mg. per ml. supernatant. This suspension was held for one hour at 4°C., then recentrifuged at 3000 R.P.M. for 20 min. This final supernatant was used to make the various inoculations. Before inoculation, the body fluids were also treated with 2 mg. of streptomycin for each ml. of fluid.

Infected Calves' Tissue

A pool of frozen tissue (spleen, lung, liver) from the two calves necropsied January 23 and 24 respectively, was made as described above and inoculated as follows:

Chicken embryos: Twenty embryonating 8-day chicken eggs were inoculated by the yolk sac route with 0.5 ml. of the above supernatant. They were examined daily for a week. Impressions were made with yolk sacs of dead embryos and stained with Machiavello's stain as described in previous publications (12, 14). Blind passages were also made in eggs for three successive passages.

Mice: Five 21-day old mice were inoculated intraperitoneally with 0.2 ml. of the suspected tissue extract and an additional five mice were inoculated by both the intranasal and intracranial routes using 0.1 ml. and 0.03 ml. amounts respectively. Three blind passages were performed in mice at 3 or 4 day intervals.

Guinea Pigs: Two pre-bled guinea pigs (16 and 17) received 0.25 ml. inoculum intranasally and two others (18 and 19) received 1 ml. intraperitoneally. Their temperatures were taken daily and 24 days after inoculation, blood samples were collected for complement-fixation tests.

Calves: One calf (506033) after collection of a pre-inoculation blood sample, was injected intramuscularly in the hip with 3 ml. of tissue suspension and 1 ml. of thoracic fluid was administered subcutaneously in the left shoulder region. Another calf (506050) was pre-bled and received by inhalation during a 5 min. period, an aerosol generated by a "Collison" spray. The aerosol consisted of a pool of peritoneal, cerobrospinal and thoracic fluids. The temperatures of both animals were taken daily and a blood sample for the complement-fixation test collected from the first calf 20 days after inoculation. The second animal was bled 24, 55, 60, 69 and 75 days after exposure.

Due to bacterial contamination, the material inoculated in the eggs and mice did not reveal the viral agent. Three of the guinea pigs (16, 17 and 18) did not develop any increase of temperature during

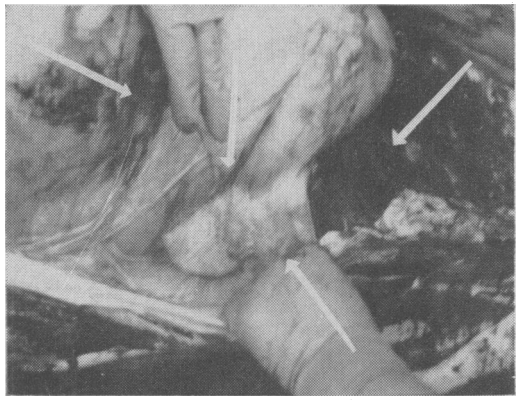

FIGURE I
Fibrinous Serositis seen in an Experimentally Infected Calf. Note Adhesion of Spleen

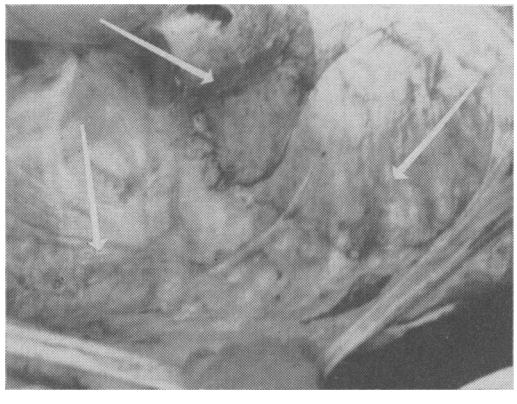

FIGURE II
Fibrinous Peritonitis Seen in an Experimentally Infected Calf. Note the Adhesions Surrounding the Spleen

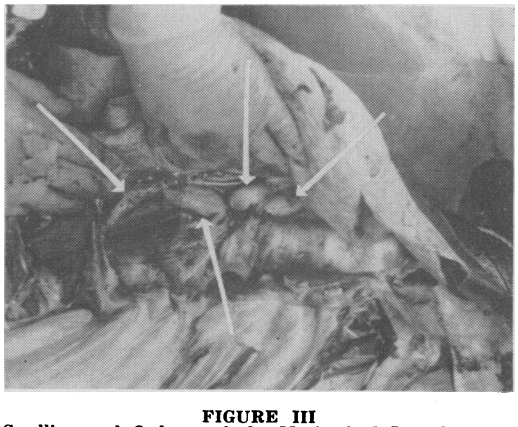

FIGURE III
Swelling and Oedema of the Mediastinal Lymph Nodes seen in an Experimentally Infected Calf

the 24 days of observation and their sera were negative in the complement-fixation test with a psittacosis-lymphogranuloma group antigen. The other guinea pig (19), however, developed a temperature rise of approximately 2°F. on the 8th and 9th days after inoculation. Twenty-four days after inoculation its serum had a complement-fixing antibody titre of 1:80. This was our first indication of successful experimental transmission of the infection. This animal was sacrificed 7 days later and portions of the liver and spleen were extracted as indicated above. This material was inoculated intraperitoneally into guinea pigs and into embryonating eggs which failed to develop the infection.

The first inoculated calf (506033) did not show a temperature rise but 20 days after injection of the suspected material its serum gave a questionable complement-fixation reaction, the titre being 1:10. This animal which was then destroyed, showed no gross post-mortem lesions and it was impossible to demonstrate the agent microscopically. The second calf (506050) which had received the aerosol inhalation did not develop an elevated temperature during the 24 days following exposure. Its serum which was negative in the complement-fixation test of the 24th day bleeding, was positive at the 55th day, titre 1:80. The titre had increased to 1:160 by the 69th day after exposure and had decreased to 1:80 on the 75th day when the animal was killed. Post-mortem examination revealed a fibrinous pleuritis and fibrinous peritonitis involving the diaphragm and the spleen as shown in Figures I and II. The mediastinal lymph nodes showed swelling and oedema, Figure III. There were some flocules of fibrin in the cerebrospinal fluid. There was also a suggestion of cystitis. Mice, embryonating eggs and guinea pigs were inoculated with a pooled extract of the liver, spleen, brain, lymph nodes and cerebrospinal fluid treated as previously indicated. The eggs and guinea pigs failed to reveal the infection after two serial passages. However, brain impressions made from third serial, intracanial mouse passage were positive when stained by Machiavello's method and examined by bright field microscopy. The inclusions appeared as clusters of reddish coloured, round granules representing the elementary bodies of the psittacosis-lymphogranuloma group of viruses.

Aborted Lamb Foetus

A pooled extract of the liver, spleen, kidney and brain of the aborted lamb foetus was made following the method previously described. This suspension was inoculated in 0.5 ml. amounts into 10 embryonating chicken eggs by the yolk sac route, and intraperitoneally in 0.2 ml. amounts into ten 21-day old mice and into a litter of suckling mice.

Six days later, two chicken embryos and one of the 21-day old mice died. Impressions stained with Machiavello's stain failed to reveal the evidence of elementary bodies by bright light microscopy. Thirteen days after inoculation one suckling mouse died and again elementary bodies were not found in impressions from the spleen. At this time an egg passage was made by faith from the living inoculated embryos. Ten days later these embryos died and suspicious elementary bodies were found in stained impressions from the yolk sacs. A third serial passage was made in chick embryos which died 11 days later. The yolk sac impressions also contained suspicious elementary bodies. This material was repassaged a fourth time and the chick embryos died in seven days. Stained yolk sac impressions examined with the dark-field condenser revealed the presence of scattered single elementary bodies as illustrated in Figure IV.

In order to show further that the agent was propagating in the embryonating eggs, the yolk sacs from the fourth egg passage were used to prepare a complement-fixation antigen as described in a previous publication (12). This antigen gave a fixation reaction to a 1:8 dilution when tested with a positive control serum known to contain psittacosis-lymphogranuloma group antibodies. No reaction was obtained with a known negative control serum. A 1:8 antigen titre is relatively low in comparison to those of antigens prepared from viruses well adapted to eggs such as the agent of enzootic abortion in ewes, but is high enough to indicate that the agent was present in the inoculated eggs in sufficient quantity to be demonstrable serologically.

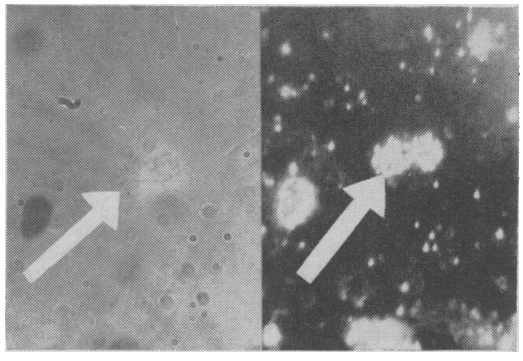

FIGURE IV

At left: Smear stained with Machiavello's stain showing twin clusters of elementary bodies.
At right: The same smear examined under dark-field. Note the granular appearance of the refractile elementary bodies.
Magnification: 427x.

Discussion

This report indicates that an infection by a virus belonging to the psittacosis-lymphogranuloma group was detected in a herd of Canadian cattle. It also points out that this infection was responsible for considerable economic loss. In the farm stock studied 24 per cent of the calf population died within a month. Even though no human infection was noted among the attendants of this herd, the possibility of it being a public health hazard should be borne in mind. The origin of the infection is obscure. There had been no additions to the cattle population during the previous year and no turkeys were raised on the farm. However, even though it cannot be proven, there is a possibility that a flock of pigeons known to have descended on the farm might have been the source of the infection. More than ten per cent of these pigeons were serologically positive.

The diagnosis of this sporadic bovine encephalomyelitis is easily made by means of the complement-fixation test using a group reactive agent. However, the results must be interpreted with caution because a significant percentage of cattle sera may fix complement with normal egg antigen. To prevent misinterpretation it is mandatory therefore to perform the test with a normal egg control antigen as well as with an infected egg antigen. Furthermore at the acute phase of the disease the titre is usually low. It increases later when the animal is recovering. Hence the testing of samples collected at three week intervals facilitates the diagnosis.

Difficulties were encountered in the demonstration of the causative agent in tissue of dead animals. The elementary bodies were few in number and did not form in as many clusters as are usually

seen with other strains of this group of viruses. In addition, they reacted only faintly to Machiavello's stain. A combination of dark-field examination with Machiavello staining greatly facilitated the demonstration of clusters as well as dispersed single elementary bodies. In the first part of our investigation the impression slides were examined by bright-field illumination only and it is conceivable that some regarded as negative might have been found to contain inclusions had they been examined by the dark-field method. By this method the stained elementary bodies appeared as clusters of round refractile granules.

It is impossible to determine if the agent isolated is the same as the one causing the infection described by McNutt (1) and the strains studied by Wenner (3). Wenner's strains were pathogenic for guinea pigs but did not infect mice. The agent we have isolated infected guinea pigs when inoculated intra-peritoneally but was also recovered in the brain of mice after three serial intracranial passages done by faith.

Summary

Clinical sporadic bovine encephalomyelitis has been diagnosed on a Canadian farm. It caused 24 per cent mortality among the calves. In addition, abortion in one ewe on this farm was related to the infection in calves.

Serum samples collected from the calves and sheep on the farm contained psittacosis-lymphogranuloma group antibodies when tested by the complement-fixation test.

The causative agent was experimentally transmitted to calves, guinea pigs, mice, and embryonating chicken eggs. The possibility of pigeons playing a ro'e in the transmission of the infection is discussed briefly.

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Résumé

Un cas d'encéphalomyélite sporadique des bovins a été diagnostiqué dans une ferme d'élevage au Canada. L'infection causa 24 pour cent de mortalité chez les veaux.

Des échantillons de sang prélevés à intervale chez les veaux et les moutons de cette ferme furent hautement positifs dans l'épreuve de la fixation du complément utilisant un antigène du groupe de la psittacose-lymphogranulomatose.

L'agent causal a été transmis par inoculations aux veaux, aux cobayes, aux souris, et aux embryons de poulet. La possibilité que le pigeon ait joué un rôle dans le déclanchement de cette infection est aussi discutée brièvement.

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