Toxoplasmosis in Mink

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TOXOPLASMOSIS has been gaining recognition rapidly during the past few years as an important disease entity in both veterinary and human medicine. Cole et al. (1), in support of this statement, cite the world-wide distribution of toxoplasmosis in mammals and birds from the tropics to about 55 degrees north latitude, the occurrence of enzootics in several species of domestic animals, and the high incidence of toxoplasma infection in animals revealed by recent serologic surveys. Feldman (2) in a recent survey, reported significant dye-test titres in a number of rabbits, guinea pigs, swine, cats, goats, and dogs in New York State. Toxoplasmas have been identified in enzootics among swine, cattle, sheep, and goats (1, 3); and Hulland (4) states that natural occurring infection has been reported in dogs, cats, chinchillas, rabbits, swine, cattle, sheep, goats, rats, and mice; he also reports the condition in a six-week-old mink. Jacobs et al. (5), who carried out a survey of toxoplasma infection in pigeons and found them to be a possible reservoir of infection, state that it has been well established by Carini in 1911 and by Reis and Nobrega in 1936 that the pigeon is a natural host of toxoplasmas.

That toxoplasmosis is also an important disease in man is supported by Weinmann and Chandlere (6), but the significance of animal toxoplasmosis as a source of human infection is still undetermined. These workers have made an extensive investigation into a possible relationship between toxoplasmosis in man and swine. They found a more-than-chance correlation existing between persons suffering from trichinella infection and those suffering from toxoplasmosis; and as most cases of trichinella infection are caused by eating undercooked pork, one may conclude that toxoplasmosis could be spread in the same manner. They were able, under laboratory conditions, to infect pigs by the oral route, and as a result postulated a cycle of infection from rodents to swine to man. There may, however, be more than one source of toxoplasmosis in humans. Other possible modes of transmission are the intermediary of arthropods, droplet infection, handling of infected animals or carcasses, and food soiled by excreta particularly that of dogs.

There is evidence to show that toxoplasma organisms will live at 4°C up to 30 days in cuts of pork (6) and up to 25 days in body fluids (3). Available evidence indicates that toxoplasmas are killed by freezing unless special precautions are employed to preserve them (20, 21, 22). Toxoplasma infection in a boy living in close contact with 9 dogs, 2 of which had toxoplasmosis was reported by Cole et al (3). They also report significant dye-test titres in 10 out of 37 people living in close contact with their toxoplasma-infected pets. Some cases of toxoplasmosis in humans are congenital, the disease in the infant being acquired from the mother; and congenital toxoplasmosis has been shown to occur in pigs, calves, lambs, and pups (1).

Cole et al. (1) state that the cardinal symptoms of toxoplasmosis in swine, cattle, sheep, and dogs, are fever in acute cases, respiratory distress, and central nervous system disturbances; in pregnant animals premature births, still-births, and abortions are common.

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The prominent autopsy findings are pneumonia, intestinal ulceration, grey foci in and enlargement of the liver, and an increased amount of fluid in serous cavities. Histologically, focal necrotizing granulomatous lesions often containing toxoplasma organisms are frequent in the liver, spleen, lymph nodes, pancreas, adrenals, uterus, and placenta. Microglial granulomas, necrosis, and pseudocysts are common findings in the brain and spinal cord, calcification also being frequent. Organisms have been demonstrated in the colostrum and placentae and, commonly, in the serous fluids of dogs, cattle, sheep, and swine (1, 3).

Diagnosis:

Diagnosis is based routinely on serology, histopathological examination, and animal inoculation. The allergic skin test (12, 13) has also been suggested as a diagnostic aid.

In 1948 Sabin and Feldman (9) reported a new immunity phenomenon in which dyes of certain chemical composition had been found capable of indicating the presence or absence of antibody activity. This test has been used extensively in surveys and as a diagnostic tool. The complement fixation (C.F.) test is also used. During the course of the disease dye-test antibodies are reported to appear earlier and remain longer than C.F. antibodies. This phenomenon is useful in determining the status of an animal with respect to the infection (16, 17). Jacobs and Lunde (8) developed a hemagglutination (H. A.) test as a serologic method for determining the presence or absence of toxoplasma antibodies. They report results analogous to the dye-test of Sabin and Feldman and give evidence to support the statement that the H. A. test is easier and safer to perform.

Laboratory animals play an important role in the diagnosis of toxoplasmosis. The rat and mouse may be used for both the isolation of the organism and as a source of antigen for serological tests. The techniques used for diagnosis of toxoplasmosis with the aid of laboratory animals are contained in various reports, important descriptions being given by Armstrong and MacMurray (10), Kass et al. (11), and Cole et al. (3). The embryonating chicken egg has also been used as an aid in the isolation of the organism from its natural host by Kass et al. (11) and Cole et al. (3). Giemsa-stained smears of serous fluids and impression smears from various organs may be diagnostic.

The purpose of this paper is to describe toxoplasmosis as it appeared in two serious outbreaks in mink and to draw attention to the important position that toxoplasmosis is gaining in veterinary medicine.

OUTBREAK # 1.

In December, 1956, a characteristic clinical syndrome appeared in mink on four different ranches, all of which were receiving feed from a common source; this syndrome still existed in September, 1957, on the two ranches which were not entirely wiped out. The supply of food was maintained largely by local casualty cases and by the local slaughter-house; therefore, a great deal of the meat fed on these ranches was fresh. The mink on a fifth ranch, also given feed from the same source, were unaffected, but, from the middle of November until March, this rancher was in the habit of purchasing feed only once or twice a week. A sixth rancher purchased 10 mink from one of the affected ranches on December 13th, 1956; nine of these animals died with toxoplasmosis in June, 1957 and the tenth was severely ill. One of the females had a litter of 4 normal kits and 1 with hydrocephalus before she died. No other mink on this ranch were affected.

It has already been shown by Weinmann and Chandler (6) that infection can be acquired by eating contaminated meat; therefore one must suspect ingestion as a mode of transmission in this enzootic. If the infection was ac-

quired per os, one may postulate that the mink on the unaffected ranch, owing to the owner's practice of acquiring food intermittently during the winter did not receive any of the contaminated food that the other four ranchers used, or that the meat was handled in such a way (stored, frozen) that the toxoplasmas were killed.

Symptoms

To the very observant mink handlers the first sign of encephalitic toxoplasmosis in affected mink was an extreme nervousness manifested by unusual rushing in and out of the nest box. Simultaneously there occurred a staring expression or exophthalmia and an extreme shyness, which resulted in the mink not wanting to be seen, but it could be observed peeking out of the darkness of the nest box. At this stage contrary to their usual habit of defecation and urination at one spot the mink scattered feces and urine about at random. Gradual loss of condition occurred, feed consumption being very spotty. Early in the course of the disease some animals stopped drinking water entirely; in these mink death was rapid in the summer because of heat and dehydration, but in the winter a mink might live seven to ten days.

As the disease progressed central nervous system involvement became more marked. While eating, mastication was very slow and the jaws did not coordinate properly, which resulted in much of the food falling out of the mouth before it could be swallowed. Many of the mink could not locate their food unless it was placed in exactly the same spot every time. Most of them showed a reluctance to raise their heads, and were unwilling to eat from the wire above them but ate readily from a dish placed on the floor of the cage. However, the appetite did not fail completely until death was imminent. Most of the mink appeared to be unable to balance, stood with their feet spread wide apart, and carried their tails in a manner most

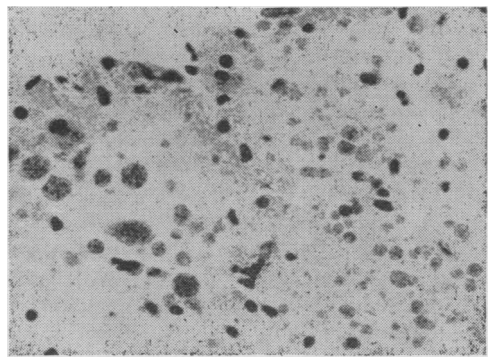

Fig. 1. Brain from mouse, second serial passage. Toxoplasma pseudocysts are present; X350.

peculiar in a mink, curving up over the back like that of a squirrel. Many would grasp the wire, drinking cup, or nest box with their teeth and then appear to be unable to open their jaws, remaining in that position for up to an hour at a time. Some circling occurred, and some mink would force their heads into a corner and die in this position. In the winter the tails and feet of many of the affected mink became frozen, and this resulted in the sloughing of these parts.

During the breeding season most of the mink mated, but many died later from toxoplasmosis. Some males, apparently normal, bred in a peculiar manner. Normally, during copulation, it is impossible to perceive the penis of the male. Affected males which, on casual observation, were copulating normally, were seen on closer examination to be riding high up on the female's back with the penis fully extended and exposed to view. These males never completed the act of coitus and invariably died later from toxoplasmosis.

Some severely affected mink underwent apparent recoveries and would seem almost normal for many days, only to relapse with severe nervous symptoms and succumb. One female made an apparent recovery from a moribund state, bred, whelped, then relapsed and died. In the whelping season many litters disappeared during the first four to five days of life. Many females, later to be

visibly affected, killed all their kits at four to five weeks of age. One rancher reported that some of his females, which ultimately died, gave birth to their kits outside and left them scattered about on the wire rather than in the nest box; these kits were reported to be deformed, many having large heads, but none were examined by the authors. None of the kits that survived to weaning time was found subsequently to be affected although many of their mothers are now dead.

No recoveries were observed. The course of the disease was generally four to six weeks. Morbidity was 100% on two ranches and up to 90% on the other ranches. All animals that were visibly affected died.

Diagnosis:

The gross postmortem picture was normal except for extreme emaciation. Occasionally the liver of a mink was yellow, but this was considered to be non-specific. The majority of the brains appeared normal in the gross, with a few showing injection of the meningeal blood vessels.

Preliminary histopathological examination in most cases showed congestion of the meninges, cerebrum and cerebellum. Perivascular cuffing, degeneration and vacuolation, and focal areas of gliosis were common in the cerebrum and cerebellum. No spinal cords were examined.

Since these changes were not pathognomonic, various conditions which might produce such lesions were considered. Distemper was suspected, but no typical inclusion bodies could be demonstrated, nor were the clinical signs typical, and injection of ferrets with saline suspensions of spleen from the affected mink failed to produce distemper. The possibility of food poisoning was also investigated but could not be proven and a complete change of diet failed to alter the course of the outbreak. Lead poisoning was suspected, but examination of blood smears failed to

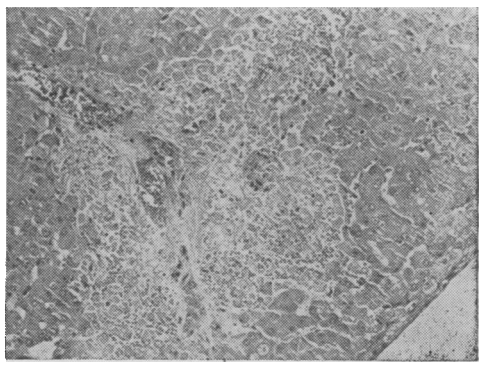

Fig. 2. Section from liver of a mink showing focal necrosis in a portal area; X350.

reveal basophilic stippling of erythrocytes (15), nor could a source of the metal be found. Some of the mink sent to the laboratory were accompanied by an excellent description of the symptoms prepared by the local veterinarian who suggested that the condition might be leptospirosis. However, guinea pigs and hamsters inoculated with blood from affected mink remained normal.

During the course of laboratory investigation some of the histopathological sections from the mink were re-examined, and a toxoplasma pseudocyst was observed in the brain of one of the mink. More living affected mink were requested from the owners so that extensive tests for toxoplasmosis could be carried out, and a series of mink was received from three of the affected ranches (ranches A, B, and C). Two of the ranchers did not submit any mink for examination, but their animals died after suffering from a clinical syndrome similar to that affecting the mink on which a diagnosis was made. Histopathological examination of additional brains, mouse passage trials, and serological tests were made in a further attempt to establish a diagnosis.

Isolation by Mouse Passage:

White laboratory mice were used for isolating the toxoplasmas from the mink by combined subcutaneous, intraperitoneal, and intracerebral routes of ino-

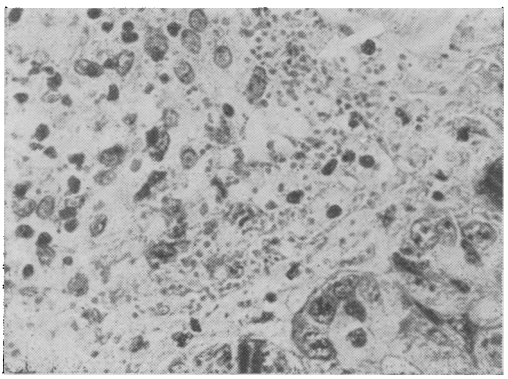

Fig. 3. Section of intestine of mink showing granulomatous thickening and numerous toxoplasma organisms (arrow) in submucosa; X350.

culation. One-half the brain, cut longitudinally, and pieces of liver and spleen from freshly killed mink were ground in a Ten Broeck grinder and a 20% suspension in saline made. This suspension was allowed to settle for one hour at refrigerator temperature and the supernatant fluid was used as inoculum. One-quarter of a cubic centimetre was given by the subcutaneous and intra-peritoneal routes with a 24-gauge needle and a tuberculin syringe, and approximately 0.05 cc of a cubic centimetre was injected intracerebrally with a 27-gauge needle and tuberculin syringe. The inoculated mice were killed two weeks after injection and small portions of their livers, spleen and brain were prepared and inoculated into additional mice as described above. After second or third passage of the material mice occasionally died, and histopathological examination of their brains revealed malacia associated with numerous toxoplasmas free and in pseudocysts. Controls were kept in all trials, killed at two week intervals and their tissues inoculated into additional mice. In no instance were toxoplasmas demonstrated in these mice.

In isolating the toxoplasmas from the mink from ranch C, a technique similar to the above was used except that 10% inactivated rabbit serum was added to the saline in which the ground tissue was suspended.

Serology:

Blood was collected aseptically by intracardial puncture under ether anaesthesia. The serum was harvested within two hours. The sera were then deep frozen and shipped to Dr. H. A. Feldman*, who examined the samples using the dye-test.

Results:

RANCH A:

Three mink were received from this ranch. Histopathological examination showed toxoplasma organisms to be present in the brains of two of the mink. No mouse passage trials or serological tests were tried on these two. The third mink was negative histopathologically, but had a dye-test titre of 1:256; and toxoplasma organisms were present in mice after the second serial passage.

RANCH B:

Eight mink were submitted from this ranch. No toxoplasma organisms were observed in the brains of any of them. Serum was examined from three of the mink, and the dye-test titres were 1:64, 1:256, and 1:256. Mouse passage trials were carried out on all eight mink, and five of them were positive.

RANCH C:

Seven mink were submitted from this ranch, six dead and one alive. Histopathological examination revealed toxoplasmas in the brain of one mink. Mouse passages were performed using tissue from the living mink, and toxoplasmas were present in the brains of mice at the end of the second serial passage.

OUTBREAK # 2.

Distemper occurred on this ranch in June, 1957, and vaccination was effective in controlling the disease. Then in August, 1957, deaths began to occur among the kits. The owner suspected

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another outbreak of distemper and brought mink to the Fur Bearing Animal Diseases Laboratory. These animals were examined, but gross lesions of distemper were not observed, nor were there any inclusion bodies in microscopic sections. There were, however, focal areas of necrosis in the liver and an occasional toxoplasma pseudocyst; therefore, a tentative diagnosis of toxoplasmosis was made.

Before the owner could be advised of our findings, he arrived at the clinic with more mink, these exhibiting signs of distemper. Histopathological examination revealed intracytoplasmic and intranuclear inclusions in epithelial tissues, and again toxoplasma pseudocysts were present in the liver. A letter was sent to the owner advising him to submit to us any carcasses that were from mink that he felt had died of a condition other than distemper. On receipt of our letter the rancher arrived at the clinic with a number of carcasses and stated that in his opinion none of them had died from distemper. At this time he reported anorexia for three to seven days, lassitude, and dyspnea in the mink prior to death. Many of the mink were affected with toxoplasmosis and distemper simultaneously, which made it impossible to attribute the signs to the specific disease from which they arose. Some of the mink died suddenly in convulsions which we feel were a result of distemper but which could possibly have been caused by a toxoplasmic encephalitis. Some had typical gross external signs of distemper, but internally showed the lesions of toxoplasmosis described below. Examination of microscopic sections from these mink confirmed a diagnosis of distemper, but also revealed an active toxoplasma infection. Campbell et al. (14) feel that latent toxoplasma infections in dogs are activated by distemper, and state that they have never observed a primary toxoplasma infection causing sickness in dogs. Therefore, it is possible that distemper could also activate a latent toxoplasma infection in mink. Two other ranchers purchased food from the same sources but neither reported any disease among their mink.

Diagnosis:

Necropsy of many carcasses revealed the following pathological changes. There was severe injection of meningeal blood vessels. The lungs appeared like liver owing to severe congestion and edema, and small white nodules, which were necrotic areas, were scattered throughout the parenchyma. Momberg-Jorgensen (19) observed similar lesions. An unusual appearance was imparted to the liver by focal necrosis, congestion, and focal haemorrhages. Massive haemorrhage and focal necrosis of the pancreas were observed. Great enlargement was coupled with massive haemorrhage and necrosis of the mesenteric lymph nodes. The mucosa of the gastro-intestinal tract was always found to be normal, but ecchymotic and paint-brush haemorrhages in the muscular layers were usual findings. The spleen was always greatly enlarged and congested, and had a mottled appearance due to hyperplastic areas. The kidneys were yellowish with a turkey-egg appearance, being spotted with cortical petechial haemorrhages. The wall of the urinary bladder of one mink was greatly thickened and haemorrhagic.

HISTOPATHOLOGY:

Lung — Congestion and marked edema were constant. There was an increase in mononuclear cells in interstitial tissues. Focal necrosis with no accompanying cellular reaction was observed in some cases. Distemper inclusions were present in the bronchiolar epithelium of some of the sections examined. Individual toxoplasma organisms were observed in interstitial areas.

Liver — Focal necrosis was present throughout the organ with numerous toxoplasma organisms at the periphery of the necrotic areas. Pseudocysts were observed occasionally; however, in some

instances the focal necrosis was unaccompanied by discernible toxoplasma organisms. There was no cellular reaction accompanying these changes.

Spleen — Acute splenitis was common. Large focal areas of necrosis involving the splenic nodules were present occasionally. No toxoplasmas were observed in this organ.

Kidney — Hyaline casts were present in the lumen of a few of the convoluted and collecting tubules. There were some fatty changes in the cortex, and focal haemorrhages in the cortex and medulla.

Intestine — An acute inflammatory reaction involved areas of the submucosa. Necrosis and some fibroblastic activity were present in a few cases. The submucosa was greatly thickened and contained numerous toxoplasma organisms. These were associated with haemorrhage in and necrosis of muscular layers, and neutrophil infiltration which extended to the serosal layer. Occasionally toxoplasma pseudocysts were observed in the muscle fibres.

Pancreas — There was haemorrhagic necrosis of the peri-pancreatic tissue with lymphocyte and mononuclear cell infiltration. Thrombosis occurred in a few of the blood vessels.

Lymph glands — Haemorrhage and massive necrosis were constant findings.

Brain — Toxoplasma pseudocysts were observed in the cerebrum and cerebellum. Some non-specific degeneration and perivascular cuffing occurred. Occasionally a focal area of gliosis was observed.

Diagnosis was based on histopathological findings only.

Treatment:

Treatment was tried on this ranch in accordance with the recommendations of Eyles and Coleman (23, 24), who state sulfonamides, and sulfones, especially Daraphin*, are the most active agents, and a combination of daraprim and sulfa-diazine showed a marked synergistic effect. Daraprim was given in the feed at the rate of 1 milligram per mink once a week, and sulphamethazine was administered in the feed at the rate of one grain per lb. of body weight for five days, and then given for a three-day period every ten days.

Losses dropped from twenty a week to three a week in about seven days and stayed at that lower rate. As controls were not kept the significance of this reduction in mortality may be questioned. Many of the mink that died after treatment was commenced had distemper. The owner had lost about ten percent of his mink when this paper was written.

DISCUSSION

The source of the infection in the first enzootic has not been determined. However, it has already been shown by Weinmann and Chandler (6) that infection in swine can be acquired by eating contaminated meat; therefore, one must suspect ingestion as a mode of transmission in this enzootic. If the infection was acquired per os, one may postulate that mink on the unaffected ranch in the first outbreak, owing to the owner's practice of acquiring food intermittently during the pelting season, did not receive any of the contaminated food that the other four ranchers used or that the meat was handled in such a way (stored, frozen) that the toxoplasmas were killed.

The source of the infection in the second outbreak has also been undetermined. Three different ranchers were purchasing food materials from the same sources, but only one was affected. Perhaps the presence of distemper on the affected ranch rendered the mink more susceptible to toxoplasmosis.

SUMMARY

During the period December 1956 to September 1957 toxoplasmosis was recognized on six mink ranches in Ontario.

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The diagnosis was made in the first outbreak by histopathological examination of sections of brain and verified by serological tests and the isolation of the organism in mice, and by gross and histopathological examination only, in the second outbreak. Toxoplasmas were observed in the brain, liver, intestines, pancreas, and lung sections of affected animals. Mortality ranged up to 100 percent.

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