AN EPIZOOTIC OF PSEUDORABIES IN RANCH MINK

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ABSTRACT

An epizootic of pseudorabies in ranch raised mink in following the feeding of raw pork lungs. Five hundred twenty mink died (2% of the herd) during a 6-7 day period. The pseudorabies virus isolation was confirmed by inactivation with chloroform, pathogenicity for rabbit, and naturalization with specific antipseudorabies serum.

Epizootics of pseudorabies (PR) in mink has been reported in most mink-raising areas of the world (1, 2, 3). More recently, there have been reports from the Netherlands (4), Korea, (5), Sweden (6), and in North America (present report).

Pseudorabies was diagnosed on an Ohio mink farm that had a total mink population of 1100 breeder males, 5500 breeder females, and 24,000 kittens. A total of 525 mink (approximately 2%) died over a 6-7 day period. The attack rate for males, females, and kittens was similar. The case/fatality rate approached 100%.

The approximate losses recorded by the mink farmer were as follows: 58 dead (Oct. 11), 100 dead (Oct. 12), 100 dead (Oct. 13), 100 dead (Oct. 14), 100 dead (Oct. 15), 50 dead (Oct. 16), and 17 dead (Oct. 17, 18 and 19).

Affected mink were anorexic for one day prior to the onset of clinical signs. Other prodromal signs were excessive salivation and depression or excitement. Muscular tremors and varying degrees of posterior paralysis followed. Death occurred within 12 to 24 hours after the first signs were observed. Most traumatic injuries were probably caused by chewing a posterior foot, leg, or tail and was noted in less than 1% of the dead mink. From a differential diagnostic point, it appeared that almost all dead mink were found curled in their nest boxes, whereas in the instance of outbreaks of

botulism (where paralysis is a prominent clinical feature) the mink are usually found dead in the woven wire part of the pen.

The carcasses were in good nutritional condition. The necropsy findings on ten mink that were examined were similar. Small hemorrhages were seen in the lungs, heart, mediastinum, and the serosa of the stomach and intestines. The pleural cavity contained a substantial quantity of blood-tinged fluid, and the lungs were congested and edematous. Because of marked postmortem decomposition, tissues were not processed for histologic examination.

The spleens and proximal spinal cords of ~~four~~ three affected mink (nos. 1, 2, 3 and 4) were removed and used to prepare 10% w/v tissue suspensions in minimum essential medium (MEM) containing ~~100 mg~~ 500 µg/ml each of streptomycin and gentamycin and 500 IU of penicillin. Tissue suspensions were inoculated (0.5 ml) onto 25 ~~CM~~ cm^2 monolayers of the rabbit (LCCRK$_1$) kidney cell line. After 2 hours adsorption at 37°C the inocula were removed, and the monolayers washed twice with MEM and then fed with MEM containing 2% fetal calf serum. Cell cultures inoculated with the spleen and spinal cord suspensions from two of the mink (no. 1 and 2) exhibited focal cytopathic effects (CPE) between 48 and 96 hours after inoculation. No CPE was observed in the cell cultures that were inoculated with the tissues from ~~the remaining two~~ mink 3.

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Three New Zealand white

~~These~~ rabbits were inoculated intramuscularly with 0.5ml of pooled spleen and spinal cord suspensions from mink nos. 1, 2, and 3. On the third day post-inoculation (PI), two of the rabbits were found dead and ~~the third~~ rabbit no 3 was moribund and died during handling with convulsions. ~~Inocula~~ Suspensions prepared from the spleens and brains from the three rabbits ~~was~~ were inoculated onto Madin Darby bovine and rabbit kidney cell (LCcRK1) cultures. ~~Foci containing~~ focal lesions were surrounded by multinucleated polymorphic syncytia containing several nuclei were observed ~~within~~ at approximately 48 hours PI. In formalin-fixed monolayer cell cultures, stained with hematoxylin and eosin, syncytia containing intranuclear inclusions were found surrounding the lesions.

The virus isolates were rendered non-infectious after treatment with 1/10 volume of chloroform for 30 minutes, and were neutralized by a porcine hyperimmune serum against pseudorabies virus. The neutralization assays were performed by incubating mixtures of serial 2-fold dilutions of hyperimmune serum with 200 $TCID_{50}$ of each isolate for 4 hours at 22°C. The 50:1 plaque reduction titers ranged from serum-dilution 1:32 to 1:64, and were in the same range as neutralization titers obtained with a reference BUK strain of pseudorabies virus supplied by Dr. R. Skoda, Czeckoslovak Academy of Sciences, Bratislava (7).

Four virus isolates, one each from the spinal cords removed from rabbits (inoculated with tissue suspension from mink ~~nos~~. 1 and 2, ~~one tissue~~ a cell culture isolate from the spleen isolate ~~of~~ mink no. 1, and an ~~isolation~~ isolate from a rabbit spinal cord inoculated with

Four virus isolates, ~~two~~ one each from the spinal cords removed from two rabbits (inoculated with tissue suspension from mink 1 and 2), one from a cell culture isolate from the spleen of mink ~~no~~. 1, and one from a rabbit isolate ~~from a rabbit~~ inoculated with the tissue pool of mink 1 and 2, were plaque-purified and used to prepare viral DNA from infected cell cultures. Four DNA preparations were tested for restriction enzyme fragment length polymorphism (RFLP) by cleaving them with the restriction enzymes Hind III, Bam H1, and Sal 1. Electrophoresis in 0.6% agarose gels revealed identical genome structure for the four isolates. Restriction fragment polymorphism is known to occur among pseudorabies virus strains and has been used for epidemiologic tracing of pseudorabies virus strains. Since we demonstrated that the four isolates obtained from this outbreak had identical fragment profiles, we assumed that a single RFLP-type was responsible for this outbreak.

Because of a favorable price, the involved mink farmer incorporated raw pork lungs (approximately 12%) as part of the ration for his mink. The other ingredients of the ration included ocean fish, poultry byproducts, and cooked eggs. The rancher was advised that the lungs should be cooked at 190°F for 30 minutes before being added to the ration to prevent possible pseudorabies virus contamination. Cooking of the pork lungs for the prescribed time was discontinued after approximately one year because of the inconvenience of the

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the tissue pool of mink #3, 1 and 2 were plaque purified and used to prepare viral DNA from infected cell cultures. Four DNA preparations were tested for restriction enzyme fragment length polymorphism (RFLP) by cleaving them with the restriction enzymes Hind III, Bam H1, and Sal 1. Electrophoresis in 0.6% agarose gels revealed identical genome structure for the four isolates. Restriction fragment polymorphism is known to occur among pseudorabies virus strains and has been used for epidemiologic tracing of pseudorabies virus strains. Since we demonstrated that the four isolates obtained from this outbreak had identical fragment profiles, we assumed that a single RFLP-type was responsible for this outbreak.

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Because of the prevalence and persistent nature of the PK virus in hogs, we would anticipate frequent outbreaks of PR to occur in mink on ranches

which practice feeding of raw pork offals. But raw pork by-products are not commonly fed to mink in the United States. Furthermore, mink farmers are also cautioned not to feed pork by-products to pregnant mink because of the potential danger of abortions caused by *Salmonella* spp.

The feeding of pork by-products has been the suspected infectious source of most PR worldwide outbreaks with few exceptions. For example, in the Netherlands, seven outbreaks diagnosed as PR in which pork by-products were ruled out as an infectious source because only poultry offal was fed to the mink. Because of the proximity of these mink farms to large pig breeding establishments, aerosol transmission of PR-virus was suspected (4). Mink are susceptible to PR when the inoculum was given by drops into the nares (8).

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