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From the State Veterinary Serum Laboratory, Copenhagen, Denmark.

ON PSEUDORABIES IN CARNIVORES IN DENMARK

I. THE RED FOX (VULPES VULPES)

By

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Pseudorabies or morbus Aujeszkyi (further on referred to as MA) has been diagnosed with rising frequency in Denmark since 1931, when it was found in cattle (*Bang* 1932, *Bendixen et al.* 1965). In 1964 it was for the first time diagnosed with certainty in swine (*Andersen et al.* 1964), and since then it has been found occasionally in dogs (*Møller* 1965), cats, and sheep (*Bendixen & Borgen* 1966).

In literature, so far, only one case of MA in free-living red foxes seems to have been reported (*Rátz* 1914). From January 1968 through February 1970, however, MA was demonstrated in Denmark in 12 red foxes (Vulpes vulpes). Eight of these cases have been briefly reviewed in a preliminary report (*Bitsch et al.* 1969). The present paper gives a description of the 12 cases as regards history, virological studies, and probable sources of infection, while their wild-life pathology aspects will be dealt with elsewhere (*Munch*, to be published).

MATERIAL AND METHODS

Foxes examined

From all over the country red foxes are received for examination at this laboratory when found dead or sick in the field. The 12 foxes dealt with in the present study were free-living, except one which was kept in captivity (Fox 1).

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Seven of the foxes (Foxes 5, 6, 8, 9, 10, 11, and 12) were submitted to the laboratory primarily for rabies examination.

Virus isolation

The diagnosis was based upon inoculation of primary pig kidney cell cultures with 10 % suspensions of nervous tissue in Earle's solution. In one case (Fox 1), in which the cell culture test turned out negative, two rabbits were injected intramuscularly with 1.5 ml of suspension.

Tissue cultures. Kidneys of pigs aged up to five weeks were used. The maintenance medium was Earle's solution with 0.5 % lactalbumin hydrolysate, 0.01 % yeast extract, 2 % calf serum, and 100 i.u. of penicillin and 0.1 mg of streptomycin per ml.

Inoculation of tissue cultures. Ten per cent suspensions of the organs examined were prepared in maintenance medium by grinding in a mortar with acid-washed, sterile sand. From all suspensions 0.1 ml was inoculated into six tissue culture tubes, three of which contained MA antiserum. Characteristic cytopathic changes that could be neutralized by the immune serum were taken as proof of MA virus growth.

CASE HISTORIES

The necropsy findings will be described in detail elsewhere (*Munch*, to be published). Here, only lesions indicative of intense local pruritus, and presence of specific food remnants in the digestive tract, will be briefly mentioned.

The geographical distribution of the cases is indicated in Fig. 1.

I. Northern Zealand

Fox 1. In early January 1968 a red fox which had been kept in captivity for several months was found dead. No information of clinical symptoms could be obtained, since nobody had observed the fox during the preceding 24 hrs. Lacerated skin lesions were found near the right corner of the mouth. In the stomach some pig bristles were found. During the last week before death the fox had been fed with dead piglets from a farm situated several km away. Investigations proved that swine on this farm were infected with MA.

Fox 2. In mid-February a free-living red fox was found dead

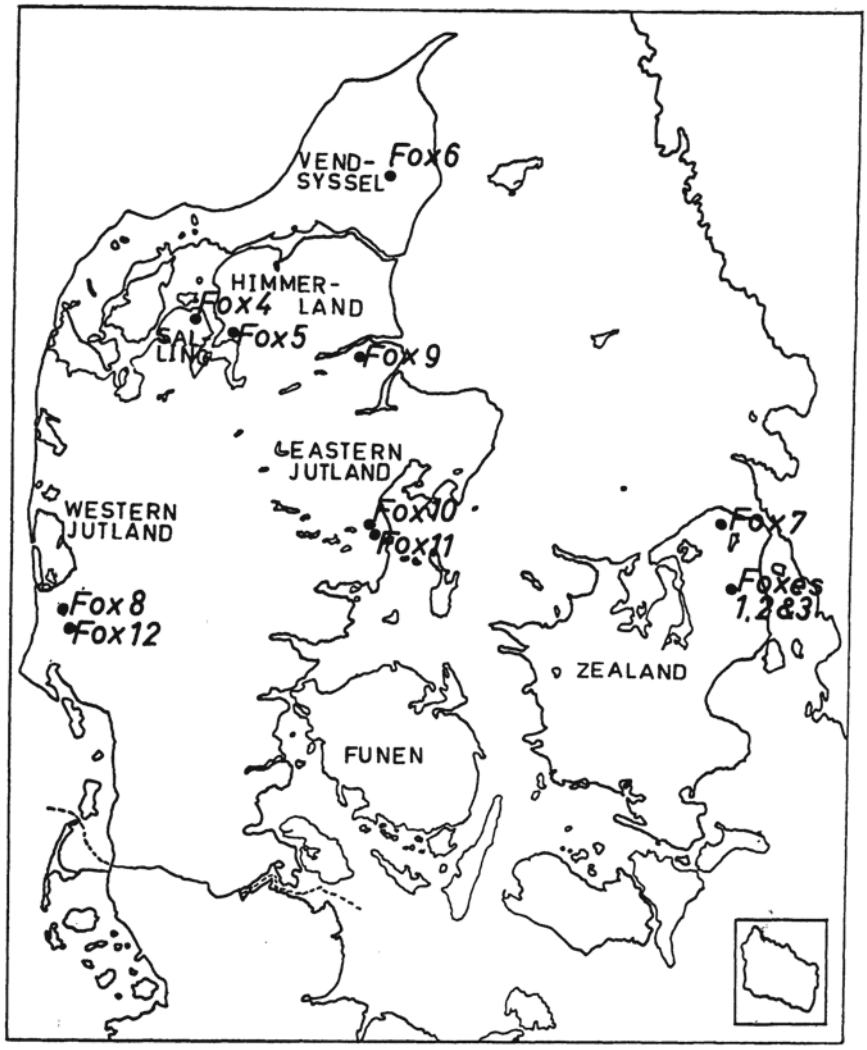

Figure 1. Geographical distribution of cases of pseudorabies in red foxes in Denmark up to April 1970.

in the barn of the farm referred to above. Post-mortem findings included hairlessness and laceration of the skin of the lower jaw.

Fox 3. In late February another red fox was found dead near a fox hole a few hundred m from the same farm. About 10 cm from the root of the tail a hairless, excoriated area, 2 by 2 cm in size, was found. In the oral cavity and pharynx as well as in the stomach fox hairs were found.

II. Salling, Jutland

Fox 4. In late January 1968 two red foxes were received at the Laboratory with the information that during the last month three more foxes and seven dogs had died in the environs. The cause of death was thought to be poisoning. Of the two foxes forwarded one had been found dead (Fox 4), while the other one had been shot because of apathy and salivation. The brains of the two foxes were temporarily stored at —20°C and examined virologically for MA one month later. From one of them (Fox 4) MA virus was isolated, while attempts to isolate virus from the other one was unsuccessful, even by rabbit inoculation. On the right side of the head of Fox 4 the skin was hairless, and at the right labial commissure there were lacerated lesions. In the other fox no skin lesions were found.

Until 1968, Central Jutland including Salling and Himmerland, (see Fox 5), was thought to be free of MA. In February 1968, however, several outbreaks occurred in herds of swine in Himmerland, and the source of infection seemed to be a certain farm in Salling, situated in the region where the above mentioned dogs and foxes had died. Three of the dogs belonged to the owner of this farm. The disease in these dogs was characterized by acute illness, increase of temperature, and signs of severe colic, while pruritus and convulsions were absent. One dog had been killed and necropsied at the Laboratory, and a haemorrhagic gastro-enteritis was the only specific finding (*Knox*, personal communication). No animals from this farm were obtainable for virus isolation, but serological investigations (*Bitsch*, not published) proved that an MA infection was present among the swine in the period in question.

III. Himmerland, Jutland

Fox 5. This fox was shot in mid-December because of abnormal behaviour. No signs of pruritus were found. As the post-mortem findings did not exclude the possibility of poisoning, the brain was stored at —20°C for an eventual toxicological examina-

tion. Two months later, when MA had been diagnosed in Himmerland, MA virus was isolated from this brain.

IV. Vendsyssel, Jutland

Fox 6. This fox was found dead in a court-yard one morning in October 1968. The presence of blood stains on a nearby brick-wall and lacerated wounds on the forehead and nose of the fox indicated that it had been rubbing its head against the wall during the night. In the small intestine a few pig bristles were found. Unfortunately the head of this fox was destroyed immediately after material had been collected for rabies examination, but the trunk was left, and MA virus could be isolated from the anterior part of the cervical cord. There was no reason to suspect MA to be present in pigs on the farm in question, since no piglets had shown signs of disease during the last weeks, but a fortnight earlier MA was diagnosed in pigs on a farm 3.5 km away.

V. Northern Zealand

Fox 7. In March 1969 MA was diagnosed in a fox which had been found dead about 1 km from a farm where MA was diagnosed in pigs seven weeks earlier, the only outbreak in that area diagnosed for months. Here a fox with abnormal behaviour had been noticed a few days before. Some still-born piglets had been buried in the dunghill. In the left buccal region the skin was hairless and excoriated, and several lacerated wounds were seen on the lips. In the stomach and intestine pig bristles were found in addition to digital bones and hoofs of piglet origin.

VI. Western Jutland

Fox 8. This fox was killed in a woodshed in April 1969. Trails of blood in the snow led to the assumption that the fox had been tossing about in a wounded condition. At the left labial commissure the skin was hairless and lacerated. MA is known to be widespread among pigs in Western Jutland, but the case of this fox could not be related to a particular outbreak.

VII. Eastern Jutland

Fox 9. This fox was found dead in a field in November 1969. Nearby another fox had been found dead about one month earlier, but it had not been examined. No skin lesions were present.

VIII. Eastern Jutland

Fox 10. This fox, showing paralysis of the hindquarters and a "curious shaking of the head", was killed in a farm-yard in February 1970. No skin lesions were found.

IX. Eastern Jutland

Fox 11. In February 1970, a few days after Fox 10 and not far from where it had been found, another fox was encountered in a farm-yard, presenting staggering of the hindquarters. It took cover in an outhouse, which was blockaded, and 6 hrs. later it was found dead. On its left shoulder an excoriated, almost hairless area, about 6 by 10 cm in size, was seen.

MA is widespread in Eastern Jutland. During 1969 the disease was diagnosed in 170 herds of swine in Jutland and Zealand, one fourth of which were in Eastern Jutland. No relationship could be detected between any of the three foxes 9 to 11 and a particular outbreak in pigs, nor could it be established whether the source of infection was the same for Foxes 10 and 11 which had died almost at the same time in places within a few km of each other.

X. Western Jutland

Fox 12. In February 1970 the head of this fox was received for rabies examination. The fox had been observed near a farm, showing marked incoordination, and was found dead there a couple of hours later. In the left buccal, labial, and nasal regions the skin was excoriated and almost hairless, and lacerated wounds were seen in the upper lip and in the gingiva. This case could not be related to any outbreak in pigs diagnosed at this laboratory.

RESULTS OF VIROLOGICAL EXAMINATIONS

The foxes submitted for rabies examination were found to be free from this disease (*Brest Nielsen*, personal communication).

The results of inoculation of pig kidney cell cultures with suspensions of tissues from the 12 red foxes are recorded in Table 1.

Table 1. Isolation of MA virus from 12 red foxes by inoculation of primary pig kidney cell cultures.

Tissue	Fox no.											
	1	2	3	4	5	6	7	8	9	10	11	12
tonsil	— — —						— — —	— — —			— — —	— — —
medulla oblongata and pons		+ + +	+ + +	+ + +	+ + +		+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
cerebrum	— — —*	— — —	+ + —,	+ + —	— — —		— — —		+ — —	— — —	+ — —	— — —
cerebellum			+ + +	+ + +			— — —		+ + +	+ + +	+ + +	+ + +
anterior part of cervical cord						+ + +	+ + —	+ + +				
intumescentia cervicalis							— — —	+ + +				+ + +
intumescentia lumbalis			+ — —				— — —	— — —				

+ : Virus growth in a tissue culture tube.
— : No virus growth.
Where no results are given, the tissue was not tested.

* 1.5 ml of the suspension inoculated intramuscularly into each of two rabbits caused pruritus and death within six days.

DISCUSSION AND CONCLUSIONS

Pruritus

Experimental infection with MA in red foxes and natural cases in farm foxes have shown that foxes may die of MA without itching (*Trainer & Karstad* 1963, *Steffen & Szaflarski* 1962). All of the 12 foxes but three (Foxes 5, 9, and 10) showed signs of having had pruritus. Foxes 5 and 10 were killed and it is possibly because of this that they showed no such signs; but still remains one fox that died of MA after a natural course of disease without signs of itching.

Seven of the foxes had signs of pruritus on their heads, one (Fox 3) on its tail, and one (Fox 11) on its left shoulder. From experimental infection of different animals by intramuscular and subcutaneous injection of high doses of MA virus it is known that pruritus, when occurring, always occurs at the site of injection. So, in Fox 3 and Fox 11 the virus may have penetrated the skin.

But still the possibility of an alimentary infection in those two foxes must be considered. Observations from outbreaks of MA in farm foxes, reported by *Ugorski* (1958) and *Steffen & Szaflarski* give reason to believe that in cases of virus entering the body through the alimentary tract, pruritus may occur elsewhere in the area innervated by the part of the CNS involved.

Source of infection

The major source of infection seems to have been farms with MA among the swine. Fox 1 was fed with piglets that had died of MA, and Foxes 2 and 3 lived near the farm from which those piglets originated. Also Fox 4 lived near a farm with MA among the swine. Regarding Fox 5 nothing particular is known. Fox 6 had pig bristles in its intestine, so it must have eaten pigs. Fox 7 was found near a farm where pigs had died of MA some weeks earlier and had remains of a piglet in its intestine. Concerning the remaining five foxes there is no other indication of infection from pigs than that they occurred in areas where the disease is known to be widespread in the swine population (*Borgen et al.* 1969).

Foxes 2, 3, and 7 died four to seven weeks after piglets on a nearby farm had died of MA. As, however, MA virus will be well preserved in carcasses in winter time, it is still possible that these

foxes died from eating just those piglets, which may very well merely have been thrown out upon the dunghill. This bad habit has caused the death of many dogs in Denmark (*Bitsch*, unpublished), and it should therefore be impressed upon farmers that dead piglets, aborted foetuses, and placentas must always be buried or incinerated.

In reports from other countries the role of rats in the transmission of MA to carnivores has been emphasized (*Cassels & Lamont* 1942, *Lamont* 1947, *Nicitin* 1960). This source of infection seems unimportant in Denmark. MA virus has been demonstrated in just one rat (*Bendixen & Borgen* 1966), and one case of MA in a dog is the only one assumed to have been caused by the eating of an MA infected rat (*Bitsch*, unpublished).

Demonstration of virus

MA virus was easily isolated from the medulla oblongata and pons of the 10 foxes from which those tissues (in a mixed suspension) were examined, even from Fox 3, which had signs of pruritus on its tail.

The concentration of virus in positive tissues was determined only in Foxes 9 to 12. The highest titres were found in the medulla oblongata and pons, where they varied from $10^{2.2}$ to $10^{2.5}$ TCID50 per 0.01 g of tissue (after Kärber, n = 3). The fact that these tissues have proved to be the most suitable material for virus isolation will be further discussed in a subsequent paper (*Bitsch & Knox* 1971).

On necropsy of foxes found dead, it will be difficult to make even a provisional diagnosis of MA if characteristic "mad-itch" lesions are absent, unless signs of central nervous disturbances have been observed. It is advisable, however, to make a tissue culture inoculation in all cases where MA cannot be excluded.

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SUMMARY

From January 1968 through February 1970 pseudorabies was demonstrated in 12 red foxes (Vulpes vulpes) from different parts of Denmark. Eleven of them were free-living, while one had been kept in captivity.

In nine foxes signs of intense local pruritus were found. Two of the remaining three foxes, which did not show such signs, had been killed in an acute phase of disease, while the last one had been found dead.

The finding of remains of pigs in the digestive tract in some of the foxes, and the demonstration of pseudorabies in piglets on a nearby farm in several cases, indicated that the foxes had caught the infection by eating dead piglets. The fox that had been kept in captivity had been fed with dead piglets which had shown signs of pseudorabies.

The medulla oblongata and pons of these foxes were found to be the most suitable material for demonstration of psudorabies virus.

SAMMENDRAG

Aujeszky's sygdom hos kødædere i Danmark.

I. Røde ræve (Vulpes vulpes).

Fra januar 1968 til marts 1970 er Aujeszkys sygdom ved virusisolation blevet påvist hos 12 røde ræve (Vulpes vulpes) fra forskellige egne af Danmark. Af disse var 11 vildt-levende, medens een levede i fangenskab.

Hos ni ræve fandtes tegn på intens lokal hudkløe. Af de resterende tre, som ikke viste tegn på kløe, var de to aflivede, medens een var fundet som død.

Fund af ufordøjede rester af grise i tarmkanalen hos flere af rævene og i flere tilfælde dødsfald hos smågrise på grund af Aujeszkys sygdom på en nærved liggende ejendom antyder, at rævene er blevet inficerede ved at æde sådanne døde grise. Ræven i fangenskab var blevet fodret direkte med døde pattegrise, som havde udvist symptomer på sygdommen.

Det er fundet, at medulla oblongata og pons hos disse ræve var det bedste materiale til diagnostisk påvisning af Aujeszky-virus.

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