Acta vet. scand. 1971, *12*, 285—292.

From the State Veterinary Serum Laboratory, Copenhagen, Denmark.

ON PSEUDORABIES IN CARNIVORES IN DENMARK

II. THE BLUE FOX (ALOPEX LAGOPUS)

By

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Since 1958 several outbreaks of pseudorabies or morbus Aujeszkyi (MA) in silver foxes and blue foxes on fur farms have been reported (*Ugorski* 1958, *Lyobashenko et al.* 1958, *Steffen & Szaflarski* 1962, *Hartung & Fritzsch* 1964). The disease caused heavy losses among the foxes, and in almost all cases the infection was attributable to feeding with meat or offal of swine.

In the present article two outbreaks of pseudorabies on fur farms will be described. On both farms mink* and blue foxes (Alopex lagopus) were bred, but only foxes were affected. The outbreaks have been mentioned previously in a brief communication from this institute (*Bitsch et al.* 1969).

MATERIAL AND METHODS

MA was diagnosed in six foxes forwarded to the Laboratory. They were examined virologically, and, except for Nos. 3, 4, and 5, also bacteriologically.

The methods used are given in a previous publication (*Bitsch & Munch* 1971). The present study included virus titrations on positive tissues by the following procedure:

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* MA has not been diagnosed in mink in Denmark. Mink from several farms have been examined because of symptoms, especially convulsions and paralysis, that could have been due to MA, but so far these examinations have given negative results.

Tenfold dilutions of the 10 % tissue suspensions were made. From each dilution 0.1 ml was inoculated into three tissue culture tubes. The titres were calculated according to the Kärber method and referred to as 50 % tissue culture infective doses (TCID50) per 0.01 g of tissue, corresponding to 0.1 ml of a 10 % suspension. The tissue suspensions were stored at — 55 °C.

Examination of serum samples for MA antibodies was made in a few cases. The blood samples were taken from three surviving foxes in Outbreak I about eight weeks after the outbreak. Per sample, three test tubes were each inoculated with 100 TCID50 of MA virus and 0.1 ml of serum. The serum-virus mixture was pre-incubated for 1 hr. at 37 °C.

CASE HISTORIES

Outbreak I. Vendsyssel, Jutland, Foxes 1, 2, 3, 4, and 5

In September 1968 two blue foxes aged about four months, and two ten-day-old piglets from the same farm, were received at the Laboratory. MA virus was isolated from both foxes and piglets.

The course of the disease in the foxes had been characterized by intense pruritus at the mouth, severe slobbering, ataxia followed by apathy, hissing respiration, and death within 12 hrs.

On the farm there were 34 blue foxes in all. Within five days 26 of them, viz., three adults — two males and a female — and 23 pups about four months old, died with the above symptoms. According to the owner's information they had all been scratching their heads. They had been fed with dead piglets from the owner's own herd of swine, where more than 60 piglets died with clinical signs of MA. The owner explained unrequested that the period from exposure to death was one or two days longer in the three adults than in the pups, and that also the actual period of disease was longer in the adults, i.e., about 24 hrs. The incubation period in the pups seemed to be two days.

The 23 pups that died were kept in six cages, three or four in each. Eight foxes, viz., seven adults and one pup, survived without having shown any signs of disease. This pup lived with its mother in a separate cage, and it is probable that those two had not eaten piglets, but at least some of the remaining foxes had had the opportunity to eat infected material.

A further three dead foxes, viz., two pups and one adult (Fox 5), were sent to the Laboratory for virological examination.

Outbreak II. Vendsyssel, Jutland, Fox 6

In October 1968 an adult blue fox and a piglet were received for examination for pseudorabies. In both animals the diagnosis was confirmed by virus isolation. According to statement, the fox had shown marked excitability, convulsions, pruritus on the head, and extensive salivation. Death had occurred about 24 hrs. after the onset of symptoms.

There were eight blue foxes and about 700 mink on the farm. Two days earlier another fox had died with the same symptoms. These two foxes had eaten dead piglets, while no feed of porcine origin had been given to the six other foxes or to the mink. The piglets originated from the same litter as the one examined at the Laboratory.

NECROPSY FINDINGS

At necropsy of the six foxes, signs of pruritus were in all cases noticed on the left or right side of the head or around the mouth. The oral and pharyngeal mucosa was hyperaemic in all foxes, but less distinct in Fox 5. Congestion of the stomach and small intestine was seen, especially in Fox 5 and Fox 6. Lungs were normal except for the fact that small hyperaemic areas were present in the lungs of Fox 2 and Fox 6. Petecchiae were seen in the CNS, most pronounced in the medulla oblongata and cerebellum of Fox 2.

RESULTS OF LABORATORY EXAMINATIONS

Bacteriological examination. No specific pathogenic bacteria were demonstrated.

Virological examination. The virus titres of different organs of the six foxes are recorded in Table 1. The blood was taken from the heart and examined undiluted.

Not the slightest virus-neutralizing effect was observed in serum of the three surviving foxes.

DISCUSSION AND CONCLUSIONS

Course of disease

It is worth noticing that already *Ugorski* (1958), in his report about an outbreak of MA in silver and blue foxes, stated the differences in the course of disease in adult and young foxes. The duration of the disease was found to be 7—14 hrs. in pups and 18—32 hrs. in older foxes, which coincides with the observations in our first outbreak.

Table 1. MA virus titers of organs from six blue foxes. Titers are given as 50 % tissue culture infective doses (TCID50) per 0.01 g of tissue (after Kärber, n = 3).

Tissue	Fox no.					
	1	2	3	4	5	6
nasal mucosa						$10^{1.8}$
tonsil	$10^{0.5}$	$10^{1.8}$	$(10^{-0.2})$	$10^{0.8}$	$10^{0.8}$	0
olfactory bulb	0	0	0	0	0	0
cerebrum mid part	0	0	0	0	0	0
hippocampus	0	0	$(10^{-0.2})$	0	0	0
cerebellum	$(10^{-0.2})$	$10^{2.2}$	$(10^{-0.2})$	0	0	0
pons	$10^{2.8}$	$10^{2.5}$	$10^{3.5}$	$10^{2.8}$	$10^{2.5}$	$10^{1.8}$
medulla oblongata	$10^{1.5}$	$10^{1.5}$	$10^{2.5}$	$10^{3.2}$	$10^{3.5}$	$10^{1.8}$
intumescentia cervicalis	$(10^{-0.2})$	$10^{0.2}$	$(10^{-0.2})$	0	$(10^{-0.2})$	0
intumescentia lumbalis	0	0	0	0	0	0
lung	0	0	0	0	0	0
liver	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidney	0	0	0	0	0	0
small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0

$(10^{-0.2})$ indicates that only one of three tubes showed virus growth after inoculation with 0.1 ml of a 10 % suspension of tissue.
When no result is given, the tissue was not tested.

Internal routes of virus spreading

From Table 1 it is seen that in the six blue foxes the virus must have spread by nervous routes, as neither the cerebrum nor the lungs, liver, spleen, and kidneys seem to have contained virus.

Experimentally, *Sabó et al.* (1968) found that in orally infected cats, the virus was spread from the tonsils and the pharyngeal mucosa along nerves to the brain stem. In experimentally infected pigs, *McFerran & Dow* (1965) found that MA virus reaches the central nervous system by two routes i.e. either by the olfactory nerves to the olfactory bulbs, or by the glossopharyngeal and trigeminal nerves to the medulla oblongata and pons.

In the present material, as well as in two dogs examined at this institute (*Bitsch*, unpublished data) in which the nasal mucosa held virus (as was the case with Fox 6), MA virus was in no case found in the olfactory bulbs. This would seem to indicate that in foxes, and perhaps in other carnivores, too, the olfactory route of infection is not common. That would also be the expected result in cases of alimentary infection.

Including cases where the primary site of infection is in the skin or in aboral parts of the digestive tract, the results so far indicate that in carnivores the virus reaches the brain either by cranical nerves, directly to the medulla oblongata and pons, or by spinal nerves via the spinal cord.

Concerning this second route reference is made to the description given in a previous paper of a red fox with signs of pruritus on its tail (*Bitsch & Munch* 1971). It may also be of interest to refer to a case of MA in a dog with pruritus in the groin, described by *Møller* (1965). Here virus was isolated from the intumescentia lumbalis, but a typical elementary body was demonstrated in a neuron of the medulla oblongata.

Alimentary infection

In Outbreak I 26 foxes died of MA. *Steffen & Szaflarski* (1962) have described an outbreak in silver and blue foxes of which 115 (77 %) died, while two blue foxes with intense pruritus of the skin of the trunk recovered. Furthermore *Ihlenburg & Senf* (1967) have demonstrated MA antibodies in the serum of a dog that had been exposed to natural infection. It may be mentioned here that at this institute antibodies have recently been demonstrated in a dog which under natural conditions had eaten several piglets that had died of MA, without showing any signs of disease (*Bitsch*, unpublished data).

From experimental infection of rabbits we know that a high inoculation dose will give a considerably shorter course of disease than a low one. In natural low-dose infections in foxes it would be quite reasonable to expect to find cases so protracted that the animals will have started antibody production before essential centres of the central nervous system are seriously damaged.

As some of the surviving foxes in Outbreak I might have eaten infected piglets, serum samples of three foxes (viz., two females which each had lived in a cage with a male that died, and a pup)

were tested for antibodies eight weeks after the outbreak, but with a negative result.

From examination of 33 naturally infected piglets, aged one to four weeks, from 11 different outbreaks (*Bitsch & Meyling*, to be published) — an examination in which the virus content in the same organs as listed in Table 1 was determined by titration in tissue cultures — we know that the concentration of virus is very high in many organs. Thence it seems unlikely that our foxes should not have become infected if they had really eaten parts of the dead piglets, in that this would have meant a high-dose infection.

Isolation of virus

The results of the virological examinations would seem to suggest that the medulla oblongata (virus titres ranging from $10^{3.5}$ to $10^{5.5}$ TCID50 per g of tissue) and the pons (virus titres ranging from $10^{3.8}$ to $10^{5.5}$ TCID50 per g of tissue) are the most suitable tissues for routine virological diagnosis of MA. This view, which was already given in a previous article (*Bitsch & Munch*), is further supported by the findings in 11 dogs examined at this laboratory (*Bitsch*, unpublished data). Likewise it is in agreement with the conception that MA virus enters the brain in just those areas.

External spreading of virus

That MA affected foxes may excrete virus was shown already by *Lyobashenko et al.* (1960) who found that one of ten foxes kept together with MA infected foxes died of the disease. *Sabó et al.* have demonstrated the presence of virus in the tonsils and the pharyngeal mucosa and also in the saliva of cats after experimental oral infection. *Trainer & Karstad* (1963) demonstrated the presence of virus in the turbinates of a raccoon kept in contact with orally infected raccoons.

Five of the six blue foxes examined in the present study had virus in their tonsils, the sixth in its nasal mucosa. Six out of eleven naturally infected dogs examined at this laboratory (*Bitsch*, unpublished data) also had virus in their tonsils, and at least two of them had virus in their nasal mucosae, too. Virus was also demonstrated in a pharyngeal swab from one of these dogs. The view that carnivores may transfer MA to other animals under natural conditions is strengthened by these findings.

If virus is excreted by MA infected animals such as foxes, dogs, and cats (and mice or rats?) in fields where animals are grazing, cases of MA in cattle that have been grazing for long periods without being stabled will be easier understandable.

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SUMMARY

In the autumn of 1968 pseudorabies was demonstrated in blue foxes (Alopex lagopus) on two fur farms in Jutland. The foxes had been fed with dead piglets from herds of swine belonging to the two farmers.

At the same time the disease was diagnosed in piglets from these herds. At the two fur farms there were, respectively, 34 and eight foxes in all; respectively 26 and two died with signs of pruritus. Only few of the surviving foxes had had the opportunity to eat piglets. Eight weeks after the period of disease no antibodies against psedorabies virus could be demonstrated in serum of three surviving foxes.

The virus content in various organs of six foxes submitted to the Laboratory was determined by titration in tissue cultures (Table 1).

The results indicate that the spreading of virus from the port of entry of the virus to the brain must have occurred by nervous routes.

The presence of virus in the tonsils of five of the six foxes and in the nasal mucosa of the sixth one, together with similar results from examination of dogs at this institute would seem to suggest that a transmission of the disease from these carnivores to other animals is possible.

Based upon the high virus titers in the medulla oblongata and pons, and with reference to similar results of examination of dogs and to a previous publication on pseudorabies in Danish red foxes, it is concluded that these tissues must be the material of choice for demonstration of pseudorabies virus in these carnivores.

SAMMENDRAG

Aujeszky's sygdom hos kødædere i Danmark.

II. Blåræve (Alopex lagopus).

I efteråret 1968 diagnosticeredes Aujeszky's sygdom hos blåræve (Alopex lagopus) på 2 pelsdyrfarme i Jylland. Rævene var blevet fodret med selvdøde pattegrise fra ejernes svinebesætninger, hvor sygdommen samtidig blev påvist ved isolation af virus. I de 2 besætninger med 34 og 8 ræve døde henholdsvis 26 og 2 ræve, alle med tegn på kløe. Kun få af de overlevende ræve havde haft mulighed for at æde de døde grise. Hos 3 overlevende ræve kunne ikke påvises Aujeszky-antistoffer 8 uger efter infektionen.

Ved titrering på vævskulturer måltes virusindholdet i forskellige organer hos 6 indsendte ræve (Tabel 1). På basis af de fundne resultater fastslås, at spredningen af virus fra det primære infektionssted må være foregået langs nervebanerne.

Påvisning af virus i tonsillerne hos de 5 af rævene og i næseslimhinden hos den 6., sammenholdt med lignende resultater fra egne undersøgelser af hunde, antyder muligheden for overførsel af smitte fra sådanne angrebne dyr til andre.

På grund af de høje virus-titre i medulla oblongata og pons hos alle 6 ræve, og under henvisning til tilsvarende resultater fra egne undersøgelser af 11 hunde og en tidligere publikation vedrørende vilde danske ræve, fremhæves det, at disse hjerneafsnit må være det bedste udgangspunkt for diagnosticeringen af Aujeszky's sygdom hos disse kødædere.

(Received April 14, 1970).