From the State Veterinary Serum Laboratory, Copenhagen, Denmark.

A STUDY OF OUTBREAKS OF AUJESZKY'S DISEASE IN CATTLE

II. FURTHER INVESTIGATIONS ON THE ROUTES OF INFECTION

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BITSCH, V.: A study of outbreaks of Aujeszky's disease in cattle. II. Further investigations on the routes of infection. Acta vet. scand. 1975, 16, 434—448. — The probable routes of infection in 29 outbreaks of Aujeszky's disease in cattle are discussed in the light of virological and epidemiological data, given in a preceding paper, and of histories of the individual outbreaks given in the present paper. The view was substantiated that anterior pruritus is mostly associated with infection by the respiratory route. In outbreaks with posterior pruritus the route of infection appeared to be a question of a more complex nature. With reference to cases examined later, it is concluded that perineal pruritus is often associated with vaginal infection. In a few cases there was evidence of virus transmission by man. Three cases with pruritus on a hind leg were suggestive of an alimentary infection. In many outbreaks with posterior pruritus, the route of infection remained obscure.

cattle; Aujeszky's disease; pseudorabies; epidemiology; infection routes.

A previous paper (Bitsch 1975 a) has dealt with the virological and main epidemiological findings related to 29 outbreaks of Aujeszky's disease (Auj.D.) in cattle examined from December 1971 to December 1973. The fact that the outbreaks fell into a group with anterior (Group I) and one with posterior (Group II) localization of pruritus was suggestive of infection by different routes, and this was supported by the virological findings, which indicated airborne infection in some Group I outbreaks and a primary vaginal infection in two Group II outbreaks.

Tables 2 and 3 of the preceding paper give information about the individual outbreaks as to the sex and age of the affected animals and the site of pruritus if present. The present paper gives additional information about these same outbreaks, which is, or could be, of importance for judging about the mode of infection. Practitioners and farmers were questioned about the stalling and handling of the animals, feeding and watering procedures, movement of animals, and other things, such as vaginal or rectal explorations, which might have caused or contributed to a transmission of virus from swine to cattle. Cases are referred to by the serial numbers used in the above-mentioned Tables 2 and 3, and the information given in these tables will be used without further reference in the discussion of routes of infection.

HISTORY OF OUTBREAKS WITH DISCUSSION OF THE MODE OF INFECTION

Outbreaks with pruritus on the anterior part of the body

- 4-1 3 5. See Fig. 1. A severe outbreak of Auj. D. had occurred in this herd one year before, in March 1971, when 14 head of cattle (abt. 50 %) and over 100 piglets died (Bitsch 1971). Since then there had been no contact with other herds except for the buying of a boar from an élite herd controlled for Auj. D. No piglets died during the 1972 outbreak, but fattening pigs showed signs of disease, and a nasal swab from one of these contained virus (Pen I). As during the first outbreak, the house was overcrowded and badly ventilated with one simple air duct in the ceiling. The distance from affected cattle to pigs, the bad ventilation, and especially the presence of virus in the nasopharyngeal cavity of Animal 1 support the idea of a respiratory infection.
- 5-136. See Fig. 2. Piglets in two litters had died from Auj. D. a few days before the death of the cows. The house was low and aired through one ventilator in the ceiling just behind the two affected cows. The findings strongly indicate a respiratory infection. Owing to the position of the ventilator, the affected cows were just the ones that most probably would acquire a respiratory infection from the swine.
- 8-139. See Fig. 3. Of abt 30 cows, heifers, and calves, the affected heifer had the closest contact with swine, in that she was stalled next to a pen housing a sow. This sow had been mated five to six days earlier with a centre boar which had been brought into the herd a few days before that. Most probably the infection was introduced by the boar eight to ten days before it struck the heifer. As the heifer had no possibility of an oral uptake of

virus shed by the infected sow or boar, the infection is most likely to have occurred by the respiratory route.

- 11-142. See Fig. 4. Pigs for fattening were bought at regular intervals, last 15 days before the outbreak. Ordinarily, the house was sufficiently aired through two ventilators in the ceiling, but one of these (V_1) had been out of operation for some time. While Animals 1 and 3 were housed day and night, Animals 2 and 4 were on pasture except for a few hours during milking. The virological findings in Animal 1 suggest a respiratory infection. Considering the position of the ventilators there should be little risk of airborne infection of cattle in this house with the ventilators working satisfactorily. But ventilator V_1 was not working, which explains at least Cases 1 and 3. Animals 2 and 4 may have caught the infection when passing through the house at milking time.
- 13-144. See Fig. 5. The cows were on pasture during the day and housed overnight. The house was old and badly ventilated. The insufficient ventilation will have favoured the formation of a virus aerosol, and the presence of virus at a relatively high titer in a sample of the nasal mucosa indicates infection by the respiratory route.
- 16-147. See Fig. 6. The disease appeared at the same time in piglets and cattle. All of the six affected cows were in late pregnancy. The swine had had no recent contact with swine from other herds, but a boar which had previously functioned in a boar centre had been added to the herd about a year ago. The ventilation of the house was not good.—The insufficient ventilation and the demonstration of virus in a nasal swab and in samples of the oral (tonsillar) and pharyngeal mucosa of the cow examined are suggestive of an airborne infection.
- 17-148. See Fig. 7. There were abt 5 m from the affected cow to the swine, but two piglets could, and did occasionally, run out of their pen. The ventilation was very bad with no air duct or ventilator. The fairly long distance from swine held together with the insufficient airing and the finding of virus in a swab and a tissue sample from the pharynx speak in favour of an airborne infection of the cow.
- 19-150. See Fig. 8. Sows were in a separate house. A litter had died in early January, but Auj. D. had not been suspected. No pigs had been bought for many years, except boars from herds controlled for Auj. D. Occasionally, however, sows from a neigh-

bouring farm were brought in for mating. This neighbour's dog died of Auj. D. a few days later (diagnosed by virus isolation).

— Poor ventilation, and the distance from the swine to the affected cow speak in favour of infection by the respiratory route.

- 22-153. See Fig. 9. There were 25 fattening pigs in the house, five of which had been bought two weeks before. The house was aired through windows only, and was often warm and humid. The insufficient ventilation and the virological findings give strong evidence of a respiratory infection.
- 24-155. In each of two pens with fattenings pigs, respectively one and two calves were confined in a corner. Two or three days before the disease, the affected calf was removed to a stall. The area of pruritus involved the left eye. No definite conclusions can be drawn concerning the route of infection.
- 26-157. See Fig. 10. The affected cow, which was in late pregnancy, was housed only during milking time. Even if virus was not demonstrated in the oral or pharyngeal mucosa the infection may well have entered by the respiratory route. Alimentary infection cannot be excluded, while there is nothing to suggest a cutaneous infection.
- 29-160. See Fig. 11. In the house there were 130 fattening pigs and 52 bull calves and young bulls. Pigs had been brought in three weeks before the outbreak. The two affected animals were placed side by side. There was a ventilator in the ceiling just behind one of them. The position of the ventilator gave a strong reason to suspect infection by the respiratory route. Given the possibility of airborne infection, the animals most at risk would seem to be the three calves placed between the ventilator and the pigs. In fact two of these three animals did become infected.

Outbreaks with pruritus on the posterior part of the body

1-132. Besides the affected heifer there were three calves, a sow, and a few fattening pigs in the house; but the calves were not particularly close to the swine. Ten cows were in a separate house. Later the veterinary surgeon explained that in several outbreaks affected cows and heifers had recently shown oestrus. In the present case the heifer had been in heat a few days before the appearance of symptoms, and on that occasion both he and the owner had inspected the vaginal mucosa. — Instead of being associated with heat, the changes in the vaginal mucosa might have been the manifestation of a vaginitis resulting from infection with

- Auj. D. virus, which might even have been transferred by the owner during a previous inspection; or the heifer was in fact in heat, in which case the infection may have been brought into the vagina by the veterinarian and the owner on their examination.
- 2-133. The affected cow was placed in the middle of one of three rows with 75 cows in all. Young cattle, breeding sows, and fattening pigs were in separate buildings, and the pigs had never had access to the cow-house. Nine days before the disease the cow had calved, assisted by the veterinarian. Both the site of pruritus and the virus titers of the spinal cord indicate a more cranial site of primary virus growth than in cases with pruritus in the perineal region. Most probably, the uterus was infected at calving with virus transferred by the veterinarian, or perhaps the owner, who also assisted.
- 3-134. The affected calf was in a pen with two other calves; in a neighbouring pen there was a sow with piglets, but the calves had no possibility of direct contact with the pigs. The feeding trough of the calves was separated from that of the sow, but both were swept with the same broom. Nothing particular can be concluded.
- 6-137. Besides 26 cows, heifers, and calves, there were a few weaned pigs in the cow-house. The two affected heifers, both in late pregnancy, were placed side by side at the end of a row with young animals, but far from the pigs. Occasionally, however, pigs being moved from one section to another were carried along the feeding alley. The possibility of an alimentary infection must be considered.
- 7-138. The affected cow was in the middle of a row of cattle. Behind there were pens with pigs. The cow had not been examined for heat or pregnancy. No certain conclusions can be drawn.
- 9-140. The affected cow, in late pregnancy, was in the middle of a row of 10 cows and heifers, with no feeding alley, and far from the pigs housed in the same premises. No certain conclusions can be drawn.
- 10-141. The affected cow had calved one month earlier, and about two weeks after that she had been attended by the veterinary surgeon because of unspecific signs of disease. There were swine in the same house, but far from the cow. No certain conclusions can be drawn.
- 12-143. Eleven calves were stalled in a row. There were two mangers, one shared by the younger calves, the other by the

older calves. Calves 2, 3, and 4 died. Calves 1 and 5 were males. Four days before appearance of symptoms two sows had broken out of their pen and eaten from the manger of Calves 1—5. Two days before, the sows had returned from a stay of several days at a boar centre. — The circumstances are suggestive of an alimentary infection, although the fact that only the three females became infected might also give reason to suspect vaginal infection.

- 14-145. About five days before it fell ill, the calf had been taken in from pasture for the winter. Earlier that day the owner had brought a sow to a boar centre for service. The fact that virus was demonstrated in the vagina and not in several samples from the intestines indicates vaginal infection. In pulling or pushing the calf in order to stall it the owner may have transferred virus to its tail root or perineum.
- 15-146. The cow-house and the piggery were connected through an open doorway. The affected heifer was inseminated four days before showing symptoms. No certain conclusions can be drawn concerning the mode of infection. However, since the heifer was in heat at the probable time of infection, it could be imagined that virus had been introduced into her vagina by the hands of a person.
- 18-149. The only cattle in the house were the two affected heifers. They were stalled next to two sows with a common feeding alley and trough. Five and three days before the onset of disease in Heifer 1, a boar from a centre had served one of the sows just behind the heifers after having stayed in the herd for some days, and three days before (28/12) Heifer 2 had been inseminated and Heifer 1 examined for pregnancy. The sows and heifers were watered from the common manger. Both an alimentary and a vaginal infection would seem to be possible.
- 20-151. In one and the same house there were cows, four calves, a sow, and, in two pens, fattening pigs. Three of the calves were in a pen next to the sow, but without possibility of direct contact with her. The affected bull calf was at large in the house and used to eat from the trough of the sow through a hole in the trough panel. About nine days before the calf fell ill the sow had been served by a boar from a centre. Inevitably, virus excreted by the sow will have been taken up orally by the calf, and since a respiratory infection would hardly result in posterior pruritus, it is reasonable to assume an alimentary infection.

- 21-152. The affected heifer had been housed with two sows for several weeks. Eight days before the appearance of disease in the heifer one of the sows was mated with a boar from a centre. The heifer was inseminated a few months earlier. It was possible for her to eat from the trough of the sow, but it is uncertain if she did. No definite conclusions can be drawn concerning the route of infection.
- 23-154. Fourteen heifers and calves were housed together with six sows, all in a row with a common feeding alley. The affected heifer was the one closest to the sows. There were separate feeding troughs for the heifers and the sows, but the affected heifer used to eat from that of the neighbouring sow. She had been doing so as late as the evening before she fell ill. Over a period of four days, ending two days before the heifer fell ill, a boar from a centre had been present for mating with this particular sow. The heifer had been inseminated two months previously and not been examined after that. Presuming that the sow in question was infected, the heifer is likely to have taken up virus orally, which renders an alimentary infection probable. A cutaneous infection is a less obvious possibility.
- 25-156. Eight calves were stalled far from the pigs present in the same house. According to a report from the local veterinary officer, the owner had explained that the calves had been fed with remains from the troughs of the pigs. Later, however, the owner denied to have done so. No certain conclusions can be made in this case.
- 27-158. The only cattle in the pig house was the affected cow. She was in late pregnancy and had for at least a week been stalled next to a boar. The two animals were fed and watered from the same trough. The boars in the herd were frequently used for service in other herds. Presuming the boar was infected, and that he was excreting virus by the nose and mouth, the cow will have taken up virus orally with feed or water, which makes an alimentary infection conceivable. Yet, a cutaneous infection cannot be definitely excluded.
- 28-159. The affected calves were side by side in the middle of a row with 20 heifers and calves. Behind them there were pens and stalls with sows. Auj. D. had been diagnosed in pigs five years before. The demonstration of virus in the vagina of both calves speaks in favour of a primary vaginal infection, but the mode of virus transmission is obscure.

Figures 1 to 11. Plans of houses related to 11 outbreaks of Aujeszky's disease with anterior pruritus.

Symbols used in figures:

C: cow(s); Ca: calf (calves); S: sow(s); SP: sow(s) with sucklings;

FP: fattening pigs.

FA: feeding alley; AD: air duct; V: ventilator.

x: location of affected cattle, identified by their serial numbers.

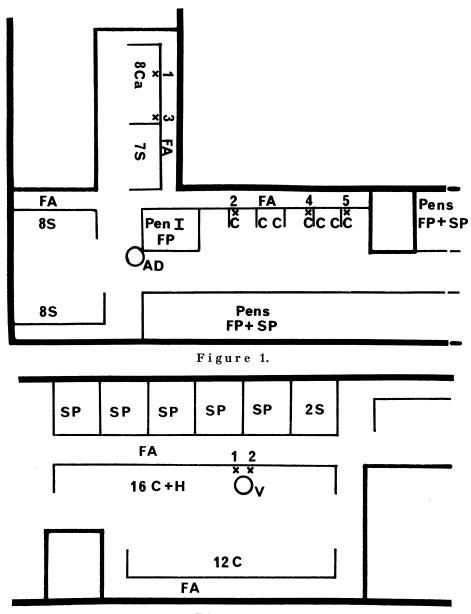


Figure 2.

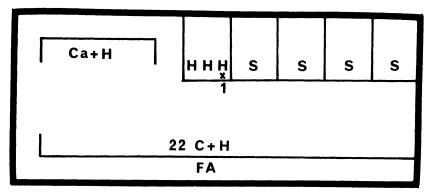


Figure 3.

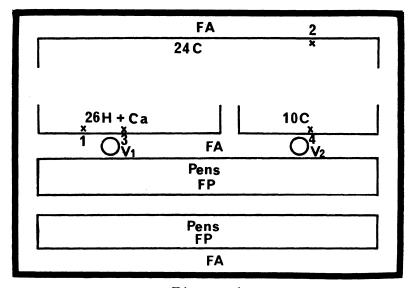


Figure 4.

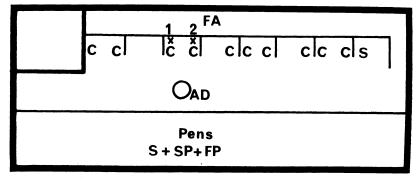


Figure 5.

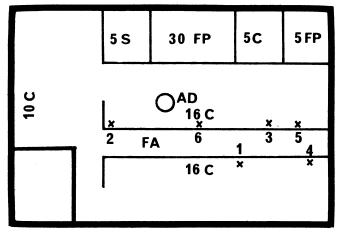


Figure 6.

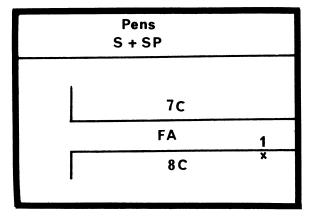


Figure 7.

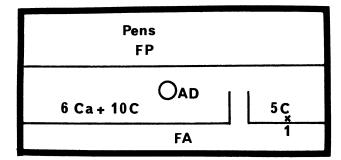


Figure 8.

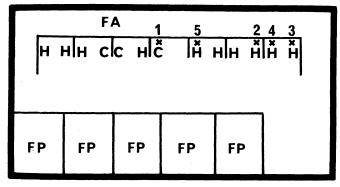


Figure 9.

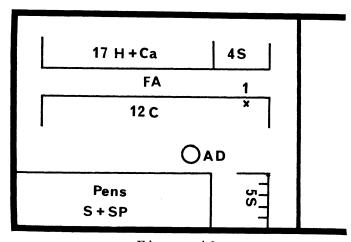


Figure 10.

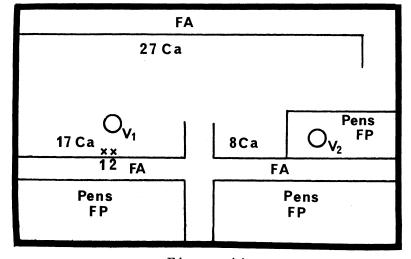


Figure 11.

DISCUSSION AND CONCLUSIONS

In cattle, owing to its neural spread, Auj. D. virus must presumably be present in greatest amount in the part of the CNS innervating the area of primary virus growth. Considering the virological findings, primary virus growth should therefore not be expected to have taken place in the vagina or intestines of animals in Group I outbreaks, while respiratory infection would be improbable in Group II outbreaks.

Outbreaks with pruritus on the anterior part of the body

The epidemiological findings in Part I of the present study indicated different routes of infection for Group I and Group II outbreaks, and the virological findings directly suggested respiratory infection in six of 12 Group I outbreaks.

It is generally accepted that insufficient ventilation and high humidity will increase the tendency to airborne infection with a virus such as the Auj. D. virus. The Group I material comprises both well and badly ventilated houses with infection patterns varying accordingly. In two houses with ventilators (5-136, 29-160), four head of cattle placed just between the ventilators and the pigs were the only ones affected. Nine outbreaks occurred in houses that were unsatisfactorily aired through simple air ducts and/or doors and windows. In three of these outbreaks the affected animals were very close (8-139, 24-155) or relatively close (26-157) to swine, but in six outbreaks the affected animals, or most of them, were several meters removed from the pigs. In accordance with the view that simple air ducts cannot determine the movements of a virus aerosol as effectively as ventilators, cases occurred as desultorily in houses with air ducts as in houses with no ventilation appliances.

The additional information about the Group I outbreaks has substantiated the notion of respiratory infection in six outbreaks (4-135, 11-142, 13-144, 16-147, 17-148, 22-153) and given strong evidence of this same route of infection in a further two outbreaks (5-136, 29-160). In three outbreaks (8-139, 19-150, 26-157), though, other routes of infection can be imagined, the respiratory route seems most probable, since all that distinguishes these outbreaks from the above-mentioned six outbreaks is the fact that nasal and pharyngeal samples were either not examined or not found virus-positive. In only one outbreak (24-155) was the

evidence not strongest for airborne infection, in that also oral or conjunctival infection was conceivable.

It should be noted that in all seven cases with pruritus on the chest there was strong evidence of respiratory infection.

The demonstration of virus on the surfaces of mucous membranes indicates that virus may have been excreted, but no findings have given strict evidence of a spread of virus among the cattle.

No findings were particularly suggestive of cutaneous infection.

Outbreaks with pruritus on the posterior part of the body (Group II)

Obviously, the outbreaks with posterior pruritus form a less homogeneous group than those with anterior pruritus. Both the female genital tract and the alimentary tract would seem to be suitable sites of virus entry.

Genital infection

Auj. D. virus was demonstrated in vaginal samples from three animals with perineal pruritus (14-145, 28-159) and after 1973 virus has been isolated from the vagina of seven such animals from seven outbreaks. Thus, in animals with pruritus involving the perineal region the presence of virus in the vagina seems to be the rule rather than the exception.

Deductively, it would be natural to consider the vagina to be the port of virus entry in such cases. Yet, the act of virus transmission is not easily understandable. Outbreaks 1-132, 14-145, 15-146, and 18-149 may have been due to transfer of virus by the hands of human beings, and Outbreak 2-133, i.e., the only outbreak where cattle and swine were not housed together, lends strong support to the notion of an unintentional transmission by man, as do also cases described in a subsequent paper (*Bitsch* 1975 b). In other cases, however, the mode of infection remains obscure, as f. ex. in Outbreak 28-159.

Future studies will have to clarify if a transmission by flies is possible, and if the presence of virus in the vagina may be the result of metastasis, for instance by centrifugal spread from the CNS.

Alimentary infection

Even if virus was in no case demonstrated in the intestines, certain cases gave such circumstantial evidence of an alimentary infection that a discussion of this route of infection appears to be justified.

If pruritus occurs after vaginal infection, the perineal region is most likely to be involved. Theoretically, growth of virus in the terminal part of the rectum may also give perineal pruritus (sacral nerves)*, while a more anterior localization in the large intestine could give rise to pruritus of the hind limbs (lumbar or sacral nerves), or colic.

Although Auj. D. virus is relatively acid-sensitive (Benndorf & Hantschel 1963), the pH in the abomasum (1.5 to 3.5) does not necessarily exclude the possibility that virus particles may pass the stomachs of ruminants without being inactivated. In Auj. D. vaccination trials with sheep, A. Bartha, Hungary (pers. comm.) found, after challenge infection by the nasal route, that although the major part of the animals affected showed pruritus in accordance with the pattern observed here in the Group I outbreaks, there were some animals which developed pruritus on the hind legs. These cases may perhaps be taken as evidence of a passage of virus through the stomachs with subsequent growth in the intestines.

Of the Group II outbreaks in which the affected animals did not show perineal pruritus, two might well be related to genital infection (2-133, 15-146), while in others there were no particular clues (7-138, 10-141). The remaining three outbreaks (20-151, 23-154, 27-158), however, were characterized by two things, namely, that the affected animal had been eating of the same trough as a pig and that pruritus occurred in the hock-knee region.

It is highly probable that the three pigs in question were infected, and virus shed by them will inevitably have been taken up orally by the affected animals. A respiratory infection being incompatible with the location of pruritus and a cutaneous infection just a remote possibility, in particular in Outbreaks 20-151 and 23-154, an alimentary infection seems probable in these three cases.

^{*} Rabbits inoculated per rectum developed pruritus in the perineal region (author's unpubl. data).

No other cases showed evidence of an alimentary infection route, and no findings supported the idea of a cutaneous infection.

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SAMMENDRAG

Undersøgelser af udbrud af Aujeszky's sygdom hos kvæg. II. Yderligere undersøgelser vedrørende infektionsvejene.

De mulige og sandsynlige smitteveje i forbindelse med 29 udbrud af Aujeszky's sygdom hos kvæg diskuteres på baggrund af virologiske og epidemiologiske data publiceret i en foregående artikel og beskrivelserne af de enkelte udbrud i nærværende artikel. Der blev fundet vægtig dokumentation for, at kløe på forparten regelmæssigt er forbundet med luftbåren infektion. I tilfælde med kløe på bagpart forekom spørgsmålet om infektionsvejen at være mere kompliceret. Med henvisning til senere undersøgte udbrud konkluderes det, at kløe i perinealregionen ofte vil være forbundet med vaginal infektion. I enkelte tilfælde fandtes sandsynlighed for, at virus var blevet overført af mennesker. I tre tilfælde med kløe på et bagben forekom alimentær infektion mest sandsynlig. I mange tilfælde med bagpartskløe forblev infektionsmåden et uafklaret spørgsmål.

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