

From the State Veterinary Serum Laboratory, Copenhagen, Denmark.

## A STUDY OF OUTBREAKS OF AUJESZKY'S DISEASE IN CATTLE

### I. VIROLOGICAL AND EPIDEMIOLOGICAL FINDINGS

By  
*V. Bitsch*

BITSCH, V.: *A study of outbreaks of Aujeszky's disease in cattle. I. Virological and epidemiological findings.* Acta vet. scand. 1975, 16, 420—433. — Twenty-nine outbreaks of Aujeszky's disease in cattle, involving 54 animals, were studied virologically and epidemiologically. The outbreaks could be divided into two distinct groups, viz., such with pruritus on the anterior (Group I) and such with pruritus on the posterior part of the body (Group II). Though low, the average number of affected animals per outbreak was twice as high in Group I as in Group II. Besides in the tissue of the central nervous system, virus was demonstrated in the oral, pharyngeal, and nasal mucous membranes of eight out of 12 animals from Group I outbreaks, which was thought to be indicative of respiratory infection, and in the vagina of the three animals with perineal pruritus from which such material was examined. It is concluded that the site of pruritus need not be identical with the site of virus entry. The probable routes of infection in the outbreaks examined will be discussed in detail in a subsequent paper.

cattle; Aujeszky's disease; pseudorabies;  
epidemiology.

In 1902 *Aujeszky* described a previously unrecognized disease of domestic animals, reporting natural cases in a steer, a dog, and a cat. The infectious nature of the disease was demonstrated in transmission experiments. *Köves & Hirt* (1934) found that the infection was prevalent among pigs. *Shope* (1935) furthermore claimed that swine were the natural host of the aetiological agent. In Denmark the infection was observed for the first time in 1931 in cattle (*Bang* 1932).

The routes of infection in cattle have always been puzzling. *Aujeszky* was able to reproduce the disease by inoculation of

infectious material through the skin or nasal mucosa, and he postulated that natural infection took place through the injured skin or mucous membrane. *Shope* favoured the idea of an infection penetrating the skin, and this has since been widely accepted, mainly because of the fact that after experimental infection of cattle, rabbits, and dogs, pruritus resembling that occurring in natural cases is regularly seen at the site of inoculation.

The possibility of alternative modes of infection had to be taken into consideration when *Dow & McFerran* (1962) and *McFerran & Dow* (1964) succeeded in infecting calves orally and nasally with high doses of virus; but six natural cases reported were apparently all considered to have resulted from cutaneous infection. The same authors maintained that virus was not excreted from infected cattle.

The aim of the present investigation of outbreaks of Aujeszky's disease (Auj. D.) in cattle was to gain more knowledge of the epidemiology of that disease. The main results of these studies have previously been discussed briefly in review articles (*Bitsch* 1974a, 1974b).

## MATERIAL AND METHODS

All of 29 outbreaks of Aujeszky's disease in cattle, recorded at the State Veterinary Serum Laboratory during the two-year period from December 1971 to December 1973, are included in the present study (Table 1).

### *Samples*

Material for confirmation of the clinical diagnosis was forwarded to the laboratory as follows: the head (brain) in cases of pruritus on the head, the head and thoracic part of the spinal cord in cases of pruritus on the shoulder and/or chest, the lumbar part of the spinal cord in cases of pruritus on the posterior part of the body, and all three sections if no signs of pruritus had been observed.

The material examined, i.e. nervous tissues, mucous membranes, and swabs from such, will appear from Tables 2 and 3. From the intestines several samples were collected in order to have large areas represented.

Ten or 20 % suspensions of tissue samples were made with

phosphate-buffered saline or Hanks balanced salt solution after grinding in a mortar with sterile sand. Swabs were shaken thoroughly with 2—5 ml of the same solutions with 2 % SPF calf serum added.

### *Tissue cultures*

Primary or secondary kidney cell cultures deriving from SPF pigs were used. Maintenance medium was Earle's solution with 0.5 % lactalbumin hydrolysate, 0.01 % yeast extract, 100 i.u./ml of penicillin, 0.1 mg/ml of streptomycin, and 2 % SPF calf serum.

### *Virological examination*

After centrifugation of the suspensions at about 1500 g for 10 min., 0.2 to 0.5 ml of each was inoculated into each of the three tissue culture tubes. After an incubation period from one to several hrs. at 37°C fresh medium was added to the cultures. Sample suspensions were stored at —55°C. When virus was demonstrated, a titration of the original suspension was performed. Ten-fold serial dilutions were made and two or three tubes were each inoculated with 0.1 ml of each dilution. Titers were expressed as 50 % endpoints.

The appearance in inoculated cultures of a reproduceable cytopathic effect identical with the characteristic changes induced in pig kidney cells by Aujeszky type virus was taken as proof of the presence of Aujeszky virus.

Some isolates from extra-neural sites, but none from nervous tissues, were identified in a neutralization test with rabbit immune serum. Only one isolate from each animal was tested.

The rabbit immune serum was prepared against the Danish laboratory strain DaS67.

## RESULTS

The outbreaks could be divided into two distinct groups according to pruritus occurring on the anterior (Group I) or posterior (Group II) part of the body. The number of outbreaks examined and the number of affected animals appear from Table 1.

Table 1. Outbreaks of Aujeszky's disease in cattle during the two-year observation period, with regard to site of pruritus.

	Number of outbreaks	Number of affected animals	Affected animals per outbreak
With pruritus on the anterior part of the body	12	31	2.6
With pruritus on the posterior part of the body	17	23	1.4
Total	29	54	1.9

Pruritus on the head was typically one-sided: on the nose, over or around the eye, or below the ear, but also in some cases on the lower jaw. Pruritus on the chest was invariably seen dorsolaterally behind the scapula, in one animal extending down to the sternum. In cases with a posterior localization, pruritus was most often seen in the perineal region, to a varying extent involving the back of the thighs; occasionally it was one-sided in the hock-knee region.

The results of the virological examinations and relevant information about individual cases are given in Tables 2 and 3. As regards extra-neural localizations of virus, isolations from samples taken from the oral, pharyngeal and/or nasal cavities were successful in eight of the 12 animals with virus in the medulla oblongata. These eight positive animals, together with two of the negative ones, originated from six outbreaks. Virus was demonstrated in the vagina of all three animals from which material from that organ was examined.

All isolates tested were significantly neutralized by rabbit immune serum.

The duration of illness is shown in Table 4.

Table 5 is a survey of Group I and II outbreaks with more than one animal involved, giving the number of affected animals, and Table 6 illustrates the course of these outbreaks.

Table 2. Virological examination of cases from outbreaks with pruritus on the anterior part of the body.

Outbreak no.	Animal no.	Sex	Age (m: months y: years)	Date of appearance of symptoms	Date of death	Received for examination	Site of pruritus
4—135	1	♂	1 y.	26/1	26/1	28/1	head
	2	♀	> 3 y.	25/1	26/1	31/1	no pruritus
	3	♀	1 y.	25/1	27/1	3/2	no pruritus
	4	♀	> 3 y.	27/1	28/1 <sup>4</sup>	3/2	no pruritus
	5	♀	> 3 y.	29/1	29/1	4/2	head
5—136	1	♀	> 3 y.	1/3	1/3		head
	2	♀	> 3 y.	2/3	2/3	4/3	head
8—139	1	♀	18 m.	18/3	18/3 <sup>4</sup>	22/3	head
11—142	1	♀	8 m.	17/8	17/8	20/8	head
	2	♀	3 y.	17/8	18/8	20/8	head
	3	♂	7 m.	20/8	21/8 <sup>4</sup>		chest
	4	♀	30 m.	20/8	21/8 <sup>4</sup>		head
13—144	1	♀	> 3 y.	12/10	13/10		head
	2	♀	> 3 y.	13/10	14/10 <sup>4</sup>	17/10	head
16—147	1	♀	> 3 y.	15/12	16/12	18/10	head
	2	♀	> 3 y.	17/12	17/12		head
	3	♀	> 3 y.	18/12	18/12 <sup>4</sup>		head
	4	♀	> 3 y.	19/12	21/12 <sup>4</sup>		chest
	5	♀	> 3 y.	20/12	22/12 <sup>4</sup>		chest
	6	♀	> 3 y.	21/12	23/12 <sup>4</sup>		chest
17—148	1	♀	> 3 y.	22/12	23/12	29/12	head
19—150	1	♀	> 3 y.	26/1	27/1	31/1 <sup>7</sup>	head
22—153	1	♀	> 3 y.	20/3	21/3	24/3	no pruritus
	2	♀	2 y.	20/3	21/3		no pruritus
	3	♀	2 y.	22/3	23/3	27/3	head
	4	♀	2 y.	23/3	24/3	27/3	head
	5	♀	2 y.	22/3	24/3	28/3 <sup>8</sup>	chest
24—155	1	♂	½ m.	10/7	10/7	11/7	head
26—157	1	♀	3 y.	11/9	11/9	13/9	head
29—160	1	♂	9 m.	14/11	16/11		chest
	2	♂	9 m.	17/11	19/11	22/11 <sup>8</sup>	chest

<sup>1</sup> TCID<sub>50</sub> per 0.1 ml of suspension. Symbol —: no virus demonstrated.

<sup>2</sup> Middle part. <sup>3</sup> Mainly tonsillar region. <sup>4</sup> The animal was killed.

<sup>5</sup> Confirmed by virological examination. Date refers to the death of the animal examined. <sup>6</sup> Virus isolated from nasal swab taken 26/1

from fattening pig. <sup>7</sup> Only brain received. <sup>8</sup> Medulla thoracalis not received.



Table 3. Virological examination of cases from outbreaks with pruritus on the posterior part of the body.

Outbreak no.	Animal no.	Sex	Age (m: months y: years)	Date of appearance of symptoms	Date of death	Received for examination
1—132	1	♀	10 m.	10/12	11/12	15/12
2—133	1	♀	> 3 y.	2/1	4/1	8/1
3—134	1	♀	3 m.	16/1	17/1	21/1
6—137	1	♀	30 m.	4/3	5/3	
	2	♀	30 m.	5/3	7/3	10/3
7—138	1	♀	> 3 y.	15/3	15/3	17/3
9—140	1	♀	> 3 y.	2/5	4/5	6/5
10—141	1	♀	> 3 y.	6/5	8/5 <sup>3</sup>	13/5
12—143	1	♀	7 m.	1/9	2/9	5/9
	2	♀	5 m.	1/9	2/9	
	3	♀	5 m.	1/9	2/9	
14—145	1	♀	6 m.	25/10	27/10 <sup>3</sup>	31/10
15—146	1	♀	2 y.	1/12	2/12 <sup>3</sup>	5/12
18—149	1	♀	2 y.	30/12	31/12 <sup>3</sup>	
	2	♀	2 y.	1/1	2/1 <sup>3</sup>	5/1 <sup>4</sup>
20—151	1	♂	9 m.	9/2	10/2	13/2
21—152	1	♀	2 y.	13/3	15/3	16/3
23—154	1	♀	2 y.	26/3	28/3	2/4
25—156	1	♀	7 m.	23/8	24/8	30/8 <sup>5</sup>
	2	♀	7 m.	26/8	26/8	30/8
27—158	1	♀	> 3 y.	3/11	4/11 <sup>3</sup>	7/11 <sup>4</sup>
28—159	1	♀	10 m.	14/11	15/11	22/11
	2	♀	10 m.	14/11	15/11	22/11

<sup>1</sup> TCID50 per 0.1 ml of suspension. Symbol —: no virus demonstrated.

<sup>2</sup> Several samples from various sections. <sup>3</sup> The animal was killed.

<sup>4</sup> Medulla lumbalis not received. <sup>5</sup> Advanced autolysis.

Table 3 (continued).

Site of pruritus	Demonstration of virus <sup>1</sup>							Other species affected
	medulla oblongata	medulla thoracalis	medulla lumbalis	intestinal mucosa <sup>2</sup>	swabs, intestines <sup>2</sup>	vagina	swabs, vagina	
perineal reg.	—	—	10 <sup>2.2</sup>	—	—	—	—	no
left flank	—	10 <sup>2</sup>	10 <sup>0.5</sup>	—	—	—	—	no
perineal reg.	—	—	10 <sup>2</sup>	—	—	—	—	no
perineal reg.	—	—	10 <sup>1.2</sup>	—	—	—	—	no
both thighs	—	—	10 <sup>2.2</sup>	—	—	—	—	no
perineal reg.	—	—	10 <sup>3.2</sup>	—	—	—	—	no
"posterior part"	—	—	10 <sup>3.2</sup>	—	—	—	—	no
perineal reg.	—	—	10 <sup>0.5</sup>	—	—	—	—	no
perineal reg.	—	—	—	—	—	—	—	no
perineal reg.	—	—	≥ 10 <sup>0.5</sup>	—	—	≥ 10 <sup>0.5</sup>	10 <sup>-0.2</sup>	no
left thigh	—	—	10 <sup>1.5</sup>	—	—	—	—	no
perineal reg.	—	—	—	—	—	—	—	no
and thighs	—	—	—	—	—	—	—	no
left thigh	—	—	≥ 10 <sup>0.5</sup>	—	—	—	—	no
and hock	—	—	—	—	—	—	—	no
perineal reg.	—	—	≥ 10 <sup>0.5</sup>	—	—	—	—	no
right knee	—	—	≥ 10 <sup>0.5</sup>	—	—	—	—	no
perineal reg.	—	—	—	—	—	—	—	no
perineal reg.	—	—	10 <sup>1</sup>	—	—	—	—	no
left knee	—	10 <sup>-0.2</sup>	—	—	—	—	—	no
perineal reg.	—	—	10 <sup>3.2</sup>	—	—	10 <sup>0</sup>	—	no
and thighs	—	—	10 <sup>2.5</sup>	—	—	10 <sup>0</sup>	10 <sup>0.2</sup>	no



Table 4. The duration of clinical disease.

	Total number of animals	Number of animals that died on			
		first day of disease	second day	third day	
Outbreaks with pruritus on the anterior part of the body	on the head	19 (15)	10 (8)	9 (7)	0
	on the chest or without pruritus	12 (7)	0	5 (3)	7 (4)
Outbreaks with pruritus on the posterior part of the body	23 (17)	2 (2)	14 (10)	7 (5)	

Figures in brackets give number of animals minus animals killed.

Table 5. Outbreaks with more than one affected animal.

	Number of outbreaks	Number of affected animals	Animals per outbreak
With pruritus on the anterior part of the body	7	26	3.7
With pruritus on the posterior part of the body	5	11	2.2

Table 6. The course of outbreaks with more than one affected animal.

	Outbreak no.	Number of affected animals	Interval (days) between first and last case		Death of subsequent cases recorded on day									
			at onset of symptoms	at death										
					1	2	3	4	5	6	7	8		
With pruritus on the anterior part of the body	4—135	5	3	3	1	1	1	1 <sup>1</sup>						
	5—136	2	1	1		1								
	11—142	4	3	4		1			2					
	13—144	2	1	1		1								
	16—147	6	6	7		1	1			1	1	1		
	22—153	5	3	3		1		1 <sup>2</sup>	2					
29—160	2	3	3				1							
					(Total: 2	5	3	4	2	1	1	1)		
With pruritus on the posterior part of the body	6—137	2	1	2			1							
	12—143	3	0	0		2								
	18—149	2	2	2				1						
	25—156	2	3	2				1						
	28—159	2	0	0		1								
					(Total: 3	0	3)							

<sup>1</sup> Remaining part of the herd, 10 animals, were slaughtered on day 4.

<sup>2</sup> " " " " " , 8 " , " " " " 3.

## DISCUSSION AND CONCLUSIONS

*The virological diagnosis*

The neural spread of Auj. D. virus in cattle was stated by *Hurst* (1933) and has been well documented by later authors. Consequently, for the virological diagnosis virus should be sought in the part of the CNS related to the area of pruritus (see the following section).

The virus isolations from the CNS achieved in the present study show that the instructions given for the selection of material (see Material and Methods) have been satisfactory (Tables 2 and 3). In three cases only could virus not be demonstrated, and that was obviously due to advanced autolysis (25—156) or to the fact that the material received was not relevant (18—149, 22—153). In a further two cases of the latter category, virus was demonstrated in very low titers in adjacent parts of the CNS (27—158, 29—160).

Four cases without pruritus were examined. In one case virus was found in the medulla oblongata (22—153), in the others in the thoracic part of the spinal cord. This, however, does not mean that examining the lumbar part would have been superfluous. In a recent case with no pruritus, but with severe symptoms of colic, virus was found in that part of the CNS (author's unpubl. data).

In the 14 cases with pruritus on the head, examination of the medulla oblongata proved to be essential, and sufficient, for the virological diagnosis.

*The site of pruritus in relation to the site of infection*

The demonstration of virus in mucous membranes, be it in swabs or in tissue samples, is important.

It has been claimed that Auj. D. virus is strictly neurotropic and without ability to multiply outside the nervous system (*McFerran & Dow* 1964). If the site of pruritus was also the site of entry of the virus, this postulate would be reasonable, because virus has never been demonstrated at the site of pruritus in natural cases. With the present findings, however, this view can no longer be maintained in that the obvious explanation of the presence of virus in, or on the surface of, mucous membranes is that these are the sites of entry and multiplication of the virus.

Furthermore these findings would seem to indicate that virus may occasionally be excreted.

The development of pruritus in cases of infection of the mucous membranes will be understandable if the pruritus results from a central nervous involvement or stimulus as thought by *Hurst*. In that case the area of pruritus needs not necessarily be the site of infection, but these two areas will have to be related to a common sensory ganglion or part of the CNS. The possibility of such a relationship has been postulated in a previous paper on Auj. D. in carnivores (*Bitsch & Munch* 1971).

#### *Virological findings in non-neural samples*

Only in connection with a few outbreaks was the Laboratory approached other than by forwarding of post-mortem material, and in most cases material additional to what was necessary for a diagnosis was not available.

The head was received in most cases of Group I outbreaks, while in cases with posterior pruritus, mucous membranes were usually not included in the material. The vagina and intestines were available in only three cases from two outbreaks.

Virus was demonstrated in non-neural material in connection with six Group I outbreaks, though not in animals with a thoracic cord involvement. But three such cases with no pruritus and five unexamined cases with pruritus on the chest occurred in herds where other animals showed virus in nasal and pharyngeal samples. This would seem to indicate that all of these animals had become infected by the respiratory route.

In the rest of Group I outbreaks the virological findings give no indication of any route of infection. It is worth noting that virus in mucous membranes was often present in modest amounts suggesting that negative isolation attempts might be due to an inadequate sampling technique.

In two Group II outbreaks, virus was demonstrated in vaginal samples from three animals, indicating a vaginal route of infection. In the remaining 15 outbreaks nothing can be concluded about the mode of infection.

The probable routes of infection will be discussed in a subsequent paper on the basis of epidemiological analysis of the individual outbreaks. The respiratory, alimentary, and vaginal routes will receive consideration (*Bitsch* 1975).

*Epidemiological features*

The duration of illness is obviously related to the site of CNS involvement (Table 4). Animals with pruritus on the head showed a significantly shorter course of disease than such with posterior or no pruritus. The latter two groups were alike in that respect. It should be noted that in one case with no pruritus virus was found in the medulla oblongata, which relates it to the group with pruritus on the head, while in three other such cases virus was found in the thoracic cord. As a rule, cases with involvement of the medulla and the spinal cord died, respectively, 10—36 hrs. and 24—60 hrs. after appearance of the disease.

Regarding morbidity, Group I and Group II outbreaks differed considerably, with approx. twice as many affected animals per outbreak in the former group as in the latter. In Group II herds there was generally only a single affected animal.

Regarding outbreaks with more than one affected animal (Tables 5 and 6), it appears that, presuming an incubation period of four to maximally nine days, the affected animals may have been exposed to infection over a period of up to one week or all have become infected on one and the same occasion. The latter situation seems more likely to have existed in Group II than in Group I outbreaks, which would be consistent with the view that the routes of infection are different in the two groups. With a predominantly airborne infection, as of Group I animals, the risk of infection is likely to have existed for some time, since virus excretion from pigs will usually be high for several days. With other routes of infection, such as the alimentary and vaginal routes, there must be higher tendency to only a single animal becoming infected, or to simultaneous infection of a number of animals, because exposure will be provided by close contact with pigs or by direct transmission of virus. Finally, it should be mentioned that also the idea of cutaneous infection fits into this picture.

That two thirds of both Group I and Group II outbreaks occurred during the months November-April, when practically all cattle are housed, is in accordance with the view that the virus reservoir is in swine.

Disease in pigs was seen in connection with very few outbreaks. This is not surprising, since distinct clinical signs have been seen in only a minority of the herds of swine in which the infection has been demonstrated by serological tests.

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## SAMMENDRAG

*Undersøgelser af udbrud af Aujeszký's sygdom hos kvæg.**I. Virologiske og epidemiologiske fund.*

Niogtyve udbrud af Aujeszký's sygdom hos kvæg med 54 angrebne dyr undersøgtes virologisk og epidemiologisk. Udbruddene kunne inddeles i to grupper, nemlig udbrud med kløe på forparten (Gruppe I) og udbrud med kløe på bagparten (Gruppe II). Antallet af angrebne dyr pr. udbrud var lavt, men gennemsnitligt dobbelt så højt i Gruppe I som i Gruppe II. Virus påvist i prøver fra mund-, næse- og svælgslimhinden hos otte af 12 dyr fra Gruppe I udbrud, hvilket antydede en respiratorisk infektion i disse tilfælde. Endvidere påvist virus

i vagina hos tre dyr med perineal kløe. Det konkluderedes, at kløestedet ikke nødvendigvis er identisk med det primære infektionssted. De sandsynlige smitteveje behandles særskilt i en efterfølgende artikel.

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Reprints may be requested from: V. Bitsch, The State Veterinary Serum Laboratory, Bülowsvej 27, DK-1870 Copenhagen V, Denmark.