Acta vet. scand. 1978, *19*, 192—203.

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A SEROEPIDEMIOLOGICAL SURVEY OF ADENOVIRUS ACTIVITY (TYPES 1—3) AT TWO FINNISH CALF REARING FARMS

By

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SIHVONEN, LIISA and J. TUOMI: *A seroepidemiological survey of adenovirus activity (types 1—3) at two Finnish calf rearing farms.* Acta vet. scand. 1978, *19*, 192—203. — Three series of seroepidemiological studies by microneutralization test on activity by serotypes 1, 2 and 3 of bovine adenovirus were carried out at two calf rearing farms. One farm (A) was operated as a closed, and the other (B) as an open herd. At farm A two serial studies were made, covering periods of six and nine months and including groups of 44 and 41 even-aged bull calves, respectively; at farm B at each bleeding calves of different selected age groups were included, 36 in all.

Maternal antibodies against all three types were common. With few exceptions they had disappeared at the age of five months. At farm B, unexpectedly, in view of the open herd nature, no signs of active infection were revealed during the period of study. At farm A infection by type 3 was judged to be present during both studies, though demonstrably affecting a lesser part of the study population only; infections by types 1 and 2 definitely occurred only during the second study, and then extensively. The titers of type 1 and 2 antibodies were, on the average, higher (with highest titers 1/243 and 1/729, respectively) than those of type 3 (highest titer 1/27). The spread of infection within the groups was relatively slow. A possible causative participation of type 2 virus in a smaller epidemic of mild respiratory and enteric disease at farm A was suggested but not verified by the results. Maternal antibodies did not seem to give effective protection against infection.

seroepidemiology; bovine adenovirus; serotypes; maternal antibodies; microneutralization test; infection versus disease.

Bovine respiratory diseases are world-wide and have been responsible for substantial economic losses. Best documentation concerning their extent and nature exists from Britain, Hungary and the USA. Respiratory diseases constitute a problem especially in beef cattle, in young cattle and in larger herds of cattle in general. The primary role of various viruses in the pathogenesis of these diseases has become increasingly apparent.

Bovine adenoviruses have been isolated from cattle with different diseases: rhinitis, pneumonia, keratoconjunctivitis, pneumoenteritis and diarrhea (*Áldáry et al.* 1965, *Inaba et al.* 1968, *Wilcox* 1969, *Saxegaard & Bratberg* 1971, *Mattson* 1973). Some strains have been isolated from healthy animals (*Klein et al.* 1959, 1960, *Darbyshire et al.* 1965), and some have been discovered as latent viruses in cell cultures (*Ronhuis* 1968, *Bartha & Csontos* 1969). Serologic studies indicate that adenovirus infection is wide-spread. It probably plays an important part in the etiology of bovine respiratory diseases.

Ten serotypes of bovine adenovirus have been reported, separated by neutralization test. They have been divided into two subgroups: types 1 to 3 being referred to group A, and types 4 to 10 to group B. Group A viruses have characteristics similar to those of human adenoviruses and group B viruses, similar to those of avian adenoviruses, which do not share any common CF antigen with human adenoviruses (*Mohanty* 1971, *Adair & McFerran* 1976).

A number of bovine adenovirus isolates originate from calves suffering from respiratory disorders of varying severity, enteritis or a combination of both. Serotypes 1 and 2 (*Darbyshire et al.* 1969), serotype 3 (*Darbyshire et al.* 1966) and serotypes 4 and 5 (*Áldáry et al.* 1965) have been shown to be able to cause respiratory disease in calves by experimental infection.

The present study is a seroepidemiological survey of adenovirus activity (types 1—3) at two farms: one specialized in rearing bull calves for beef, the other for breeding purposes. Outbreaks of respiratory disease and diarrhea were known at the farms. Serial serologic examination was carried out by microneutralization test, which was developed for parainfluenza studies in man by *Schmidt et al.* (1966) and adapted for other respiratory viruses in man by *Rosenbaum et al.* (1970). *Bibrack & McKercher* (1971) have used the same method to demonstrate bovine adenovirus antibody.

MATERIAL AND METHODS

Study population and serum samples

Serum samples were collected at two farms, A and B, both in south-western Finland. Both farms had a history of respiratory disease and diarrhea among calves.

Farm A is specialized in rearing bull calves for beef. All calves move freely in one large barn. Most calves comprised in the study periods were of Ayrshire breed, a few were Finncattle and Charolais calves. Farm A is operated as a closed herd. At the start of the rearing period bull calves arrive to the farm in two-three groups within a two-week period, originating from many herds. They are fattened for about one year. Each time before a new rearing period is started the calf shed is cleaned and disinfected. The group studied in January to June 1972 (designated group 1) consisted of 44 bull calves. Serum samples were collected at dates when the calves were on the average about two, three, five and eight months of age. The range of age differences within the group was about two weeks. Group 2, surveyed in October 1972 to July 1973, consisted of 41 bull calves. The first serum samples were taken upon arrival of all calves of the group. Their age averaged three weeks, ranging from two weeks to one month. The next samples were taken at approx. three, six and nine months of age.

Farm B is a bull breeding station and it is, naturally, operated as an open herd. The inbought bull calves arrive there at ages between one and two months from different parts of Finland. New arrivals are kept in isolation for a few days only. Then each new calf is placed in a pen with four other bull calves. When the calves are five-six months old, they are transferred to a lairage. The pens and lairages are all in one room. The outbreaks of upper respiratory disease and diarrhea in this herd have been concentrated on calves under five months of age. The study period covered the time from September to December 1971. Serum samples were collected at six occasions from a group of 34 bull calves, most of Ayrshire breed, aged from one to five months. At each time of collection samples were taken from calves in different age groups.

All sera were inactivated by heating at 56°C for 30 min.

Cell cultures

Primary bovine embryonic kidney cell cultures (BEK) were prepared by the regular trypsin digestion method from bovine embryonic kidneys received from a slaughter house. The growth medium was Eagle's minimum essential medium (MEM, Orion) with 1 % glutamin (MEM: 100×conc. L-glutamine, Orion), 100 units penicillin per ml (Penicillin, Hoechst 1 mill. i.u.), 100 µg

streptomycin per ml (Streptomycinsulfat, Hoechst) and 10 % bovine fetal serum (Flow laboratories). The maintenance medium was the same except with only 2 % bovine fetal serum.

Viruses

The following viruses were used: Bovine adeno no. 10, serotype 1 (BAV-1); Bovine adeno no. 19 (BAV-2); Bovine adeno WBR, serotype 3 (BAV-3). All viruses were obtained from Dr. P. Rondhuis, Central Veterinary Research Institute, Rotterdam, Netherlands, which is gratefully acknowledged.

Adenovirus grown in BEK cell culture was used as antigen in the neutralization test. A stock preparation of each virus was prepared by harvesting a BEK cell culture with advanced adeno CPE and freezing and thawing it three times. Next, the suspension was centrifuged at 2000 r.p.m. for 15 min. The supernatant fluid was used as antigen and was stored at —70°C. BAV-1 material contained 10^{-4} TCID50/0.025 ml, BAV-2 material 10^{-3} TCID50/0.025 ml and BAV-3 10^{-4} TCID50/0.025 ml (micro virus titration, *Rosenbaum et al.* 1972).

Microneutralization test

In the microneutralization test the following equipment was used: microplate for cell cultures with covers (Greiner laboratories), micropipette, delivery 0.025 ml (Cooke Engineering Co.) and microdiluter, 0.025 ml (Cooke Engineering Co.). The general procedure for the SN test has been described by *Rosenbaum et al.* 1972.

Duplicate wells were used for each dilution of serum. All sera were diluted by threefold steps in growth medium, using the microdiluter. Final reading was made on the seventh day of incubation with an inverted microscope.

Titer 1/9 is judged in this work to indicate the presence of specific antibodies.

RESULTS

Antibodies were found against all three types of adenovirus tested. Their presence tended to be more frequent in the early and late phases of the study periods, with lower frequency in between. The early phase antibodies are called maternal antibodies in the following, and those appearing later are considered to indicate a preceding active infection.

The said general tendencies are demonstrated in Table 1. This table reveals that maternal antibodies were judged to be present against each of the virus types tested in all three groups of calves studied, except for the lack of demonstrable BAV-3 antibodies in group 1 of farm A. The apparent lack probably reflects a generally lower titer of BAV-3 antibodies and thus does not suggest definite absence of maternal BAV-3 antibodies in the calves of the group in question.

Table 1. Occurrence of antibodies to three types of adenovirus in calves during study periods at farms A and B.

	Age at testing, months	Antibody* frequency against serotype		
		BAV-1 posit./negat.	BAV-2 posit./negat.	BAV-3 posit./negat.
Farm A	2	12/28	16/24	0/40
Group 1	3	4/32	3/33	0/40
44 calves**	5	0/38	3/35	2/36
	8	0/26	2/24	6/20
Farm A	3/4	31/9	29/11	6/34
Group 2	3	5/33	15/23	0/38
41 calves	6	6/19	24/1	1/24
	9	13/8	19/2	5/16
Farm B	1	14/2	13/3	3/13
34 calves**	2	26/6	23/9	19/22
	3	15/16	10/21	3/28
	4	5/17	6/16	0/22
	5	1/10	1/10	0/11

* Titers $\geq$ 1/9 were considered positive, $<$ 1/9 negative.
** Serum samples of all individuals of the group were not available on each occasion.

Definite evidence of virus activity was revealed in both study groups (i.e. both study periods) of farm A. At farm B surprisingly no such evidence was found. At farm A there was a clear difference in the respect that in the first group definite evidence of BAV-3 virus activity only was demonstrated, while in the second group activity of all three virus types was demonstrated. Questionable significance attaches to the presence of three late 1/9 titers against BAV-1 in group 1 of farm A. These titers were each preceded by one or two tests with no demonstrable antibodies.

In Table 2 the top (range) and median of the antibody titers

Table 2. The highest and median antibody titers to three types of adenovirus in calves during study periods at farms A and B.

	Antibodies against serotype*									
		BAV-1			BAV-2			BAV-3		
	Age at testing, months	number of positives, titer ≥ 1/9	highest titer	median titer	number of positives, ≥ 1/9	highest titer	median titer	number of positives, ≥ 1/9	highest titer	median titer
Farm A	2	12	27	9	16	81	9	0	—	—
Group 1	3	4	27	9	3	81	27	0	—	—
	5	0	—	—	3	9	9	2	9	9
	8	0	—	—	2	9	9	6	27	9
Farm A	3/4	31	243	27	29	729	27	6	27	9
Group 2	3	5	27	9	15	81	27	0	—	—
	6	6	81	9	24	729	27	1	9	9
	9	13	243	27	19	729	27	5	27	9
Farm B	1	14	243	27	13	243	27	3	9	9
	2	26	729	27	23	243	9	10	27	9
	3	15	729	9	10	81	9	3	27	9
	4	5	243	9	6	27	9	0	—	—
	5	1	27	27	1	9	9	0	—	—

* Titers are the inverse values of the figures shown.

in each specific study are presented. Negative individuals and 1/3 titers are excluded. The results demonstrate the occurrence, as mentioned before, of higher titers for BAV-1 and BAV-2 antibodies than for BAV-3 antibodies.

Tables 1 and 2 give data of a frequent, but not regular, occurrence of maternal antibodies in varying degrees in the calves. As regards the rate of decline of maternal antibodies, a reliable picture, apparently not obscured by simultaneous virus activity, is gained from the calves of farm B, and in reference to BAV-1 antibodies also from group 1 of farm A. The antibodies seem to have virtually disappeared at the age of five months.

In Table 3 full data are given regarding the fate of BAV-1 and BAV-2 antibodies in group 2 of calves at farm A. These data describe the two episodes of virus activity revealed during the present studies, which resulted in the appearance of relatively frequent and high titers of antibodies. The main facts and immediate inferences presented and suggested by the detailed data of the table are: There is an individual variation in titer of maternal antibodies. The rate of disappearance of maternal anti-

Table 3. History of serum antibody titers against BAV-1 and BAV-2 in group 2 of calves at farm A. Grouping of calves by initial and by developing titers.

Calf no.	Antibody titers* against serotype BAV-1 Age of calves				Calf no.	BAV-2 Age of calves			
	3 weeks	3 months	6 months	9 months		3 weeks	3 months	6 months	9 months
114	0+	0	0	0	118	0	0	9	9
206	0	0	—	—	114	0	0+	27	27
210	0	0	—	—	215	0	0+	27	27
218	0	0	3	243	218	0	0	81	729
113	0	0	27	27	113	0	0	243	27
215	0	3+	0	9	117	0	0+	81	—
209	0	3	3	—	101	0	0	—	27
104	3	0	3	27	210	0	0	—	81
201	3	0	27	—	206	0	3	—	—
122	9	0	0+	3	202	0	81	—	—
120	9	0	—	3	201	3	81	243	—
101	9	0	—	9	104	9	0	27	27
105	9	0	—	9	122	9	0+	81	81
203	9	0	9	27	120	9	0	—	0
213	9	0	—	—	102	9	0+	—	—
216	9	0+	—	—	213	9	0	—	—
102	9	0+	—	—	124	9	3+	27	243
217	9	3+	0	9+	207	9	3	—	—
118	27	0	0	3	216	9	3+	—	—
124	27	0+	3	81	217	9	27+	27	27
110	27	0	3	—	208	9	27+	81	243
212	27	0+	—	0	105	9	27	—	—
103	27	0+	—	—	203	9	81	81	81
205	27	3	0	0+	110	27	0	27	—
117	27	3+	0	—	205	27	3	9	9+
208	27	3+	3	27	214	27	3+	729	—
214	27	3+	81	—	212	27	3+	—	—
202	27	3	—	—	111	27	9	—	—
207	27	3	—	—	209	27	27	27	—
107	27	—	0	—	126	27	81+	81	—
111	81	0	—	—	107	27	—	0	—
126	81	3	9	—	211	27	—	27+	—
119	81	3	—	—	123	81	3	27	81
116	81	9+	0+	3	109	81	9+	81+	81
123	81	9	3	27	204	81	9	—	—
211	81	—	9+	—	116	81	81+	27+	27
125	243	9+	0+	27	119	243	9	—	—
204	243	9	—	—	103	243	27	—	—
109	243	27+	3+	3	125	729	27+	27+	81
276	—	0+	3	—	276	—	0+	81	—

* Titers are the inverse values of the figures shown.

\+ Respiratory and/or gastrointestinal symptoms were observed at time closest the bleeding referred to.

— Not done.

bodies is apparently normal or without interference due to active infection in part of the calves only; in part of them it is obscured by such an infection. There is a confinement of the demonstrable infection by BAV-1 to part of the population, while BAV-2 infection covers practically all calves. There is a variation in the time of occurrence of infection and in the strength of provocation of antibodies. Presence of antibodies does not effectively, nor even clearly demonstrably, inhibit the event of active infection.

In addition to occasional cases, a small outbreak of an affection with respiratory symptoms and diarrhea took place during the studies at farm A in group 2, when the calves were three-four months old (Table 3). Sixteen of the calves in this group were reported to be affected, none of them severely. Adenovirus activity by BAV-2 was conspicuous during the said period, but the significance of the relationship between the two events remains conjectural (see Discussion). Some activity by BAV-1 and BAV-3 during the same period is also indicated by the occasional rise of titer, suggestively coincidental.

DISCUSSION

The results demonstrate that infections of all three serotypes tested are common in cattle, at least in that part of Finland where the farms of the present studies are located. The observation agrees in general with earlier reports from other countries (*Bibrack & McKercher* 1971, *Mohanty* 1971, *Schipper et al.* 1972, *Moscari et al.* 1974, *Wizigman* 1974).

As regards the reliability of the present results, it should be kept in mind that according to the literature the neutralization test for adenovirus antibodies has not been found vulnerable to nonspecific inhibitors in sera. The reliability is also clearly indirectly supported by the observed distribution of titers in the present study: the pattern of higher titers (maternal) at the beginning and at the end (infection) of the study period, and lower titers in the middle. That 1/3 titers were not considered positive in arrangement of two generalizing tables is a safety measure based on the findings and comparability argumentation by *Bibrack & McKercher.*

That the antibodies present in the first phase of each study period were maternal, predominantly at least, is clearly suggested by the initial declining pattern of the titers revealed by serial testing. The high frequency of maternal antibodies indicates the

commonness in the study area of the infections concerned. Further proof of prevalence are the episodes of virus activity observed during these studies, which were limited in time and population.

The present studies were primarily designed to study the extent and quality of adenovirus activity in specific types of calf populations, i.e. in larger groups of calves gathered at young age from a large number of small farms in the neighborhood. One of the study populations was an open type and the other a closed type of herd. The initial belief was that adenovirus infection would be more frequent in the former, possibly even constantly present at a low level of virus activity, i.e. patently endemic. However, the negative results, as regards virus activity, from farm B demonstrate that in this type of open herd relatively long periods without patent infection are possible and do occur.

At farm A, operated as a closed herd, adenovirus activity of all three types was observed, but it could also be absent, at least at recognizable level, for periods of months. The difference of activity between the two study periods is conspicuous, as is that between populations.

The negative results of the present study, i.e. the periods without signs of infection, imply either that latent infections are not frequent or that, even if carriers were common among the calves, recognizable activation of infection would not be high in frequency. On the other hand, the epidemics of infection at farm A demonstrate the relative ease with which adenoviruses are introduced from outside in operations of the closed herd type or stay viable on the premises. The carrying of viruses by the recruits themselves is, in the light of the well-known capacity to latent states by adenoviruses in general, the most probable route of introduction into the system, and thus the source of infection within it.

Studies by *Schipper et al.* (1972) and *Mattson* (1973) demonstrated that maternal antibodies against adenoviruses in calves give no effective protection against infection. The present results agree, in principle, with those of *Schipper et al.* and *Mattson* and extend the application to the area of other strains and serotypes. It is seen in Table 3 that several calves experienced a rise of their antibody titer before the maternal antibodies had had time to disappear. Whether maternal antibodies may possess a more or less effective protective value against the disease, even if not against the infection, is difficult to assess in this kind of

study, mainly owning to the evident multicausality, in general, of a bovine disease connected with adenovirus infection.

That infection by bovine adenoviruses far exceeds in frequency the disease initiated by these viruses has been suggested in many studies (*Harbourne* 1966, *Ide et al.* 1969, *Bibrack & McKercher*) and is supported by the main part of the present results. That, on the other hand, BAV-2 infection was probably at least partly responsible for the epidemic characterized by respiratory and/or gastrointestinal symptoms which occurred in group 2 of farm A is suggested, though far from confirmed, by the results. The rise in antibody titers, i.e. the infection, partly at group level and partly at individual level following the epidemic may have been coincidental or it may have been a sequela rather than one of the causes of the affection.

Infectivity of a relatively low degree of the bovine adenoviruses studied is indicated by the time differences observed in the rise of antibodies under conditions favorable for the spread at farm A, where close contact between the group members was allowed. Whether the explanation is to be seen in a relatively high infection dose required in general or in individual variations of resistance, or a combination of both, remains problematic.

The antibody titers against BAV-1 and BAV-2 types of adenoviruses were significantly higher than those against BAV-3 type of virus. That neutralization of BAV-3 test virus was caused by specific antibodies is clearly indicated by the pattern of their occurrence mentioned earlier, and by the lack of correlation in distribution between these titers and those resulting from BAV-1 or BAV-2 infection. Whether the lower level of BAV-3 antibodies observed, in comparison with other types, reflects a real difference in this type of virulence between the virus strains studied or whether the BAV-3 test virus obtained from abroad and the Finnish field virus on which observations were made, though of the same type, differ serologically to the extent of accounting for deceptive appearance of lower titers, cannot be reliably concluded on the basis of the present results. Relatively high antibody titers for BAV-3 virus have been reported by other workers (*Bibrack et al.* 1971, *Mattson* 1973).

ACKNOWLEDGEMENTS

The authors are indebted to Dr. Saara Reinius for a substantial contribution in the arrangements of the field study.

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SAMMANFATTNING

En seroepidemiologisk undersökning av adenovirusinfektion (typer 1—3) vid två finska kalvuppfödningsgårdar.

Tre serier av seroepidemiologiska undersökningar över förekomsten av adenovirusinfektion av typerna 1, 2 och 3 vid två kalvuppfödningstationer utfördes med mikroneutralisationstest. Den ena gården (A) drevs som en sluten, den andra (B) som en öppen besättning. På gård A utfördes successiva undersökningar som varade respektive 6 och 9 månader och omfattade 44 och 41 jämnåriga tjurkalvar; vid provtagningarna inrymdes på gård B kalvar av på förhand bestämda åldersgrupper, inalles 36.

Maternala antikroppar mot alla tre typer av virus var allmänt förekommande. Med få undantag var de försvunna vid 5 månaders ålder. På gård B upptäcktes, trots typen av öppen besättning, inga tecken på aktiv infektion. Vid båda undersökningarna på gård A ansågs infektion av typ 3, som omfattade endast en mindre del av kalvpopulationen, ha förekommit. Infektioner förorsakade av typerna 1 och 2 förekom med säkerhet endast under den senare undersökningsperioden och då vidsträckt. Titrarna av antikroppar mot typ 1 och 2 var i medeltal högre (med respektive 1/243 och 1/729 som högsta) än titrarna mot typ 3 (högsta titern 1/27). Spridningen av infektionen inom grupperna var relativt långsam. Resultaten antydde, men bekräftade inte, att typ 2 virus på gård A kunde ha deltagit i orsakandet av en mindre epidemi som kännetecknades av lindriga respiratoriska och intestinala symptom. Maternala antikroppar såg inte ut att kunna inbringa ett effektivt försvar mot infektionerna i fråga.

(Received November 2, 1977).

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