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EXPERIMENTAL STAPHYLOENTEROTOXICOSIS IN MINK

By

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JUOKSLAHTI, T., A. NISKANEN, S. LINDROTH and T. PEKKANEN: *Experimental staphyloenterotoxicosis in mink.* Acta vet. scand. 1980, *21*, 336—346. — Experimental staphyloenterotoxicosis was produced in minks by oral administration of mink feed containing 5 or 200 μg of purified enterotoxin A per test animal. The animals became very exhausted after the ingestion of toxin. Vomiting was observed in two of seven minks of the lower toxin group with a latent period of 2.5 to 4.0 h. The higher toxin concentration caused vomiting in four of seven test animals with a latent period of 2.0 to 2.5 h. Vomitus was accompanied by strong salivation. Poor appetite was observed in four of seven minks having ingested 5 μg of SEA, and 200 μg caused total loss of appetite in all the test animals. After a test period of 22 h all the animals but one had normal appetite. Diarrhoea was prominent in three of seven minks with the low toxin concentration and in all with the high toxin concentration. Statistically significant haematological changes compared to the control group were an increase in neutrophil count and a decrease in lymphocyte count in the high toxin group. Significant changes in the blood chemical data were an increase in blood urea nitrogen with 200 μg of SEA and a decline in the cholesterol level in both toxin groups.

enterotoxicosis; minks; experimental staphylococcal intoxication; mink feed; bacterial toxins.

Experimental staphyloenterotoxicosis has been reported in various animals and man as reviewed for example by *Bergdoll* (1972). Animals have been found to be much more unsensitive to the effects of orally administered enterotoxin than man on a kilogram basis.

By-products of the food industry are used in the production of mink feed. The bacteriological quality of the raw materials and ready-mixed mink feed varies considerably (*Juokslahti* 1978 and 1979). The raw materials and ready-mixed feed also contain

pathogenic staphylococci capable of producing enterotoxins, the most important reservoir of these staphylococci being slaughterhouse waste and cow udders (*Juokslahti et al.* 1980).

Staphylococcal infections are known to occur in minks (*Head* 1959, *Budd et al.* 1966, *Trautwein & Helmboldt* 1966, *Löliger* 1970, *Crandell et al.* 1971, *Nordstoga* 1979), but staphylococcal enterotoxin intoxication has not previously been observed (personal communication 1979 *M. Hansen,* Denmark, *A. Helgebostad, K. Nordstoga* and *G. Loftsgård,* Norway, *T. Mejerland,* Sweden and *J. Kangas,* Finland). The authors have conducted preliminary experiments during the years 1977 and 1978 in order to evaluate the susceptibility of minks to staphylococcal enterotoxin intoxication. In the first experiment crude enterotoxins A, B, C and D were administered orally to male and female minks. In the second experiment mink feed was seeded with enterotoxin A, B and C producing Staphylococcus aureus cells. After an incubation period of 24 h at outdoor temperature (15—23°C) the feed was orally given to male minks. Because of the marked effect of toxin A shown by the tentative results and wide occurrence of toxin A producing staphylococci (*Niskanen & Koiranen* 1977, *Juokslahti et al.*), enterotoxin A was selected for further experimentation. The aim of the present study was to investigate the effect of orally administered purified staphylococcal enterotoxin A (SEA) on minks.

MATERIALS AND METHODS

Experimental animals. Clinically healthy, adult male Dawn minks, obtained from the Finnish Fur Breeders' Association's experimental ranch, were used in the experiment. The minks had previously been vaccinated against Clostridium botulinum Type C intoxication and Mink Virus Enteritis. The animals weighed between 1110 g and 2100 g and were divided into three experimental groups according to the following mean weights: Group I (controls) 1619 ± 288 g, Group II (5 μg SEA/mink) 1557 ± 226 g and Group III (200 μg SEA/mink) 1504 ± 256 g. The t-test showed no significant difference between the weights of the groups. The animals were kept in cage nettings during the experiment and they were free to drink ad libitum.

Enterotoxin. Staphylococcal enterotoxin A was produced by the strain VTT 530 (*Niskanen* 1977) using fermentor cultivation

in a nutrient medium containing 3 % tryptone, 3 % peptone, 10 mg niacine/l, 0.5 mg thiamine/l, 0.5 % pyruvate and 1.0 % glucose. The crude toxin was purified by the method of *Yamada et al.* (1976). The end product had a purity of 50 % with no haemotoxic activity in a concentration of 500 μg/ml.

Test procedure. The animals were kept without feed 12 h before the beginning of the experiment. Enterotoxin A was dissolved in milk and mixed with the feed. Toxin was administered to the minks in a 15 g feed portion. The animals in Group I (controls) received feed portion without toxin. The animals in Group II received 5 μg SEA per animal and in Group III 200 μg SEA per mink, respectively. During the experiment the animals were under clinical observation. The following symptoms were particularly registered: vomiting, appearance and quantity of faeces, appetite and abnormal behaviour. After the end of the test period lasting 22 h, the minks were narcotized in an ether chamber. A blood sample was aseptically drawn during narcosis by heart puncture with a heparinized 0.90 × 38 mm needle and a syringe. After heart puncture the animals were immediately killed. Routine necropsy procedures with histologic studies of the following organs were performed: ventriculus, duodenum, jejunum, colon, rectum, liver, kidneys and intestinal lymph nodules.

Analysis. Normal haematological analyses and bacterial cultivation of heart puncture blood on blood agar plates with an incubation period of 24 h at 37°C were performed at the Feed Laboratory, Vaasa. Normal plasma analyses were conducted at the Central Laboratory of the College of Veterinary Medicine, Helsinki, using a Gilford automatic serum analyzer.

RESULTS

Clinical symptoms

The vomiting frequency of the minks after ingestion of enterotoxigenic feed is shown in Table 1. A dose of 5 μg caused vomitus in two of the seven minks tested. Both minks vomited during the time period of 3—4 h after the ingestion of the toxin. A dose of 200 μg caused a reaction in four of the seven minks in Group III. The affected minks vomited two to eight times during the time period of 2—5 h after ingestion of the toxin. The vomitus started with nausea, after which the animal vomited in 2 min.

Table 1. Vomiting frequency of minks after ingestion of staphylococcal enterotoxin A (SEA).

Test animal	Reaction
Group I (control)	
mink 4	no reaction
mink 5	no reaction
mink 7	no reaction
mink 15	no reaction
mink 19	no reaction
mink 20	no reaction
mink 22	no reaction
Group II (5 μg SEA)	
mink 1	vomiting once after 3 h 51 min
mink 2	no reaction
mink 6	no reaction
mink 13	no reaction
mink 17	no reaction
mink 18	no reaction
mink 21	vomiting once after 2 h 51 min
Group III (200 μg SEA)	
mink 10	no reaction
mink 11	vomiting five times during 2 h and 4 h 43 min
mink 12	no reaction
mink 14	no reaction
mink 23	vomiting eight times during 2 h 6 min and 2 h 54 min
mink 24	vomiting twice during 2 h 18 min and 2 h 36 min
mink 25	vomiting five times during 2 h 22 min and 3 h 40 min

Vomitus was accompanied by strong salivation. The vomited material was first mainly partly-digested feed turning in the later stage of the experiment to light-yellowish slime.

The appetite of the minks during the experiment is shown in Table 2. Appetite was controlled by giving a small amount of feed (5—10 g) to the minks and observing the consumption of the feed. In the control group one mink had poor appetite between 8 and 11 h after the beginning of the experiment. In Group II, four minks had no appetite between 8 and 11 h after the beginning of the experiment. After 22 h of the ingestion of 5 μg of the toxin all the minks in the group had normal appetite. Two of the minks in Group III had poor appetite after 1.5 h from the beginning of the experiment. Five minks had no appetite

Table 2. Appetite of minks after oral administration of staphylococcal enterotoxin A (SEA).

Test group	Time (hours) after challenge				
	0	1.5	8	11	22
Group I (control)					
mink 4	+	+	—	—	+
mink 5	+	+	+	+	+
mink 7	+	+	+	+	+
mink 15	+	+	+	+	+
mink 19	+	+	+	+	+
mink 20	+	+	+	+	+
mink 22	+	+	+	+	+
Group II (5 μg SEA)					
mink 1	+	+	—	—	+
mink 2	+	+	+	—	+
mink 6	+	+	—	—	+
mink 13	+	+	+	+	+
mink 17	+	+	+	+	+
mink 18	+	+	+	+	+
mink 21	+	+	—	—	+
Group III (200 μg SEA)					
mink 10	+	—	—	—	+
mink 11	+	—	+	—	+
mink 12	+	+	+	—	+
mink 14	+	+	—	—	+
mink 23	+	+	—	—	+
mink 24	+	+	—	—	+
mink 25	+	+	—	—	—

+ = appetite.
— = no appetite.

after 8 h and all the seven minks were without appetite 11 h after the ingestion of 200 μg of the toxin. At the end of the test period, one mink had no appetite, while all the others had normal appetite.

The occurrence of diarrhoea in minks induced by enterotoxin ingestion is presented in Table 3. One mink in the control group had loose stools between 6 and 11.5 h from the beginning of the experiment. Three minks in Group II had diarrhoea 6 h after the ingestion of the toxin. Two of these still had diarrhoea at the end of the test period. Six minks in Group III had diarrhoea 6 h

Table 3. Diarrhoea in minks induced by oral enterotoxin ingestion.

Test group	Time (hours) after challenge			
	0	6	11.5	21
Group I (control)				
mink 4	+	+	+	+
mink 5	+	—	—	+
mink 7	+	+	+	+
mink 15	+	O	+	+
mink 19	+	+	+	+
mink 20	+	+	+	+
mink 22	+	+	+	+
Group II (5 μg SEA)				
mink 1	+	—	—	—
mink 2	+	+	+	+
mink 6	+	—	—	+
mink 13	+	—	O	—
mink 17	+	+	+	+
mink 18	+	+	O	+
mink 21	+	+	+	O
Group III (200 μg SEA)				
mink 10	+	—	—	—
mink 11	+	—	— —	—
mink 12	+	+	—	—
mink 14	+	—	O	—
mink 23	+	— —	—	—
mink 24	+	— —	—	—
mink 25	+	— —	— —	—

O = no defaecation. + = normal defaecation.
— = diarrhoea. — — = diarrhoea with mucosal casts.

following the ingestion of the toxin. During the entire experiment all seven minks had diarrhoea. Four of them had mucosal casts in the diarrhoetic stools. The mucosal casts were initially light-yellowish in colour, mingled with yellowish and greenish slime. They later turned to bloodish and were mingled with clear blood. All the minks receiving 200 μg SEA still had diarrhoea 21 h after the ingestion of the toxin.

Another clinical symptom observed was extreme exhaustion in the minks appearing after the ingestion of toxin-containing feed. The animals lay down on their back or flank.

Table 4. Haematological data[a] on the minks in the experimental enterotoxicosis test.

Parametre	Test group		
	I (control)	II (5 μg SEA)	III (200 μg SEA)
Haemoglobin (g/100 ml)	17.5 ± 2.3	18.3 ± 0.7	17.5 ± 2.8
Haematocrit (ml/100 ml)	52.3 ± 7.5	54.2 ± 1.5	51.0 ± 8.4
Total leucocytes (10^3/ml)	5.8 ± 2.5	6.9 ± 3.6	6.7 ± 1.5
Neutrophils (%)	49.9 ± 6.7	57.4 ± 9.8	65.7 ± 8.8[c]
Eosinophils (%)	2.4 ± 2.8	2.7 ± 2.7	3.0 ± 2.3
Lymphocytes (%)	45.9 ± 6.7	38.6 ± 8.4	30.4 ± 9.3[c]
Monocytes (%)	1.6 ± 2.1	1.3 ± 0.8	0.9 ± 1.2

[a] Blood samples taken after the test period (22 h).
[b] Mean ± s.
[c] $P < 0.01$ between Groups I and III.

Haematological data

Table 4 shows haematological data on the minks in the enterotoxin experiment. The minks receiving toxin had higher leucocyte counts than the minks in the control group, although the differences were not statistically significant. The minks in the toxin groups had higher neutrophil counts and lower lymphocyte counts than in the control group. The difference in neutrophil and lymphocyte counts between Group I and Group III is statistically significant ($P < 0.01$).

Data on chemical analysis of blood

The blood chemical data on the minks are presented in Table 5. Blood urea nitrogen values show a tendency to increase in the toxin groups compared to the controls, the difference being statistically significant ($P < 0.05$) between Groups I and III. The cholesterol level has significantly ($P < 0.01$) declined in both toxin groups compared to the control group. The other parametres investigated — albumin, total protein, phosphorus, creatinine, total bilirubine, α-tocopherol, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, γ-glutamyl transferase and lactate dehydrogenase — showed no statistically significant differences between the toxin and control groups.

Table 5. Blood chemical data on the minks[a] in the staphylococcal enterotoxin A (SEA) intoxication experiment.

Parametre	Group I[b] (control)	Group II (5 μg SEA)	Group III (200 μg SEA)
Albumin (g/l)	38.0 ± 5.7[c]	40.0 ± 3.3	37.0 ± 6.0
Total protein (g/l)	71.0 ± 6.5	67.0 ± 4.7	65.0 ± 12.6
Phosphorus (mmol/l)	3.7 ± 1.1	3.9 ± 1.1	4.1 ± 1.7
Blood urea nitrogen (mmol/l)	7.0 ± 1.0	8.2 ± 1.8	9.9 ± 3.4[d]
Cholesterol (mmol/l)	6.5 ± 0.9	6.1 ± 0.5	5.1 ± 0.7[e]
Creatinine (mmol/l)	71.0 ± 16.9	62.5 ± 10.6	72.9 ± 11.5
Total bilirubine (μmol/l)	11.3 ± 4.4	10.8 ± 2.9	10.2 ± 2.3
α-tocopherol (ng/ml)	22.2 ± 4.9	25.0 ± 16.6	17.8 ± 2.2
Alkaline phosphatase (i.u./l)	106 ± 19	97 ± 54	120 ± 88
Alanine aminotransferase (i.u./l)	283 ± 335	106 ± 18	301 ± 411
Aspartate aminotransferase (i.u./l)	229 ± 46	205 ± 83	244 ± 200
Creatine kinase (i.u./l)	828 ± 231	1012 ± 510	932 ± 868
γ-glutamyl transferase (i.u./l)	16.4 ± 8.8	11.6 ± 5.6	13.0 ± 11.8
Lactate dehydrogenase (i.u./l)	1536 ± 557	1594 ± 727	1701 ± 698

[a] Blood samples taken after the test period (22 h).
[b] Seven minks in each group.
[c] Mean ± s.
[d] $P < 0.05$ between Groups I and III.
[e] $P < 0.01$ between Groups I and II as well as I and III.

Bacterial cultivation of blood

The bacterial cultivations of heart puncture blood were negative.

Post-mortem examination

The picture of the post-mortem examination of the minks in Group I was normal. The only pathological change observed was a slight hyperaemia in the mucous membrane of the jejunum in the minks Nos. 5, 15 and 20. The stomachs of the minks of Group II contained a bit of undigested feed. Their mucous membranes were slightly hyperaemic. The large intestine and the small intestine contained partly-digested grainy feed and bloody mucus, the mucous membrane in this part of the digestive tract being strongly hyperaemic. Several animals had the liver and the kidneys filled with blood. The mucous membrane of the small intestine of the minks in Group III was hyperaemic. The small intestine contained reddish mucus. The intestine gland was enlarged and the cut area was damp. The large intestine

had dilatated parts containing bloody liquid, which in turn contained partly-digested grainy material. Some of the animals had blood congestions in the liver and the kidneys.

DISCUSSION

The raw materials of mink feed often contain enterotoxin producing staphylococci (*Juokslahti et al.* 1980). Moreover suitable conditions for staphylococcal growth (*Chou & Marth* 1969) and production of pathogenic doses of enterotoxin exist during the warm season due to the feeding management, — feed is kept on cage nettings at outdoor temperature for a feeding period of 24 h (*Juokslahti* 1978). The variable bacterial flora in the ready-mixed mink feed can, however, inhibit the outgrowth of staphylococci. On the other hand, the increasing use of bacterial inhibitors, e.g. various acids and antibiotics, in feed may facilitate the outgrowth of pathogenic staphylococci.

Disease outbreaks with symptoms similar to those observed in the present study have been occasionally noticed in commercial mink breeding. A new mink enteritis disease with a so far unconfirmed aetiology has also been reported (*Larsen & Gorham* 1975). Bacterial toxins were considered as a possible causative agent to the disease. The clinical symptoms of the present study closely resemble those of the mink enteritis described by *Larsen & Gorham*.

Wild minks usually eat only food which is captured and killed by the animal itself. They seldom eat cadavers (*Gerell* 1975), contrary to the habits of some other carnivorous animals like foxes, dogs and raccoon dogs. Thus it is unlikely that mink would fylogenetically be accustomed to deal with common food poisoning toxins. This is also ascertained by the high susceptibility of minks to Clostridium botulinum toxin C intoxication. The clinical symptoms (vomiting, diarrhoea, loss of appetite and exhaustion) and changes in haematological and blood chemical data on minks observed in the present study resemble those reported in the literature (review e.g. *Bergdoll* 1970) for enterotoxicosis in other test animals and human beings.

According to the present study it is obvious that the occurrence of enterotoxin producing staphylococci in mink feed can be the causative agent to enteric diseases observed in mink breeding. Since enterotoxin has been shown to be a strong hypotensor both in human beings and test animals and a drop in

blood pressure can lead to foetal deaths and abortions in pregnant animals, the role of enterotoxicosis observed in whelp losses during gestation (*Juokslahti* 1975) deserves closer examination.

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SAMMANFATTNING

Experimentell stafyloenterotoxikos hos mink.

Experimentell stafyloenterotoxikos framkallades på mink genom utfodring med ett minkfoder tillsatt med 5 resp. 200 µg stafylokockenterotoxin A (SEA) per djur. Minkarna visade sig mycket slöa efter toxinupptagandet; efter 2,5—4,0 timmar iakttogs kräkningar hos två av de sju minkar, som fått den lägre toxindosen. Den högre toxindosen gav efter 2,0—2,5 timmar upphov till kräkningar hos fyra av sju försöksdjur i den andra gruppen. Kräkningarna åtföljdes av en stark salivation. Hos fyra av de sju minkar, som fick 5 µg SEA var aptiten nedsatt; 200 µg SEA orsakade fullständig inappetens hos samtliga sju djur i den andra gruppen. Efter 22 timmar hade samtliga djur, så när som på ett, normal aptit. En kraftig diarré förekom hos tre av sju minkar på låg och hos alla på hög toxinkoncentration. En statistisk signifikant ökning av neutrofiler och minskning av lymfocyter förekom i blodet hos gruppen med hög toxindosering. I samma grupp förelåg en ökning av blodets ureakväve medan bägge toxingrupperna visade en sänkning av blodets kolesterolvärden.

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