CLINICAL OBSERVATION OF EXPERIMENTAL PSEUDORABIES IN MINK AND FERRETS

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Clinical and virological studies of experimental pseudorabies (Aujeszky's disease) in domestic animals have been reported by many workers\textsuperscript{1,2,8,9}. While pseudorabies is apparently common on mink farms in Europe\textsuperscript{6,10}, the naturally-occurring disease has not been reported on North American or Japanese mink farms. Previously, we showed that mink and ferrets are highly susceptible to this virus\textsuperscript{4}. The present report gives clinical observations of experimental pseudorabies in mink and ferrets. The rabbit is also included for comparative purposes because of its well-known susceptibility\textsuperscript{3}.

MATERIALS AND METHODS

Viruses

The Shope and Buk strains\textsuperscript{4} of pseudorabies virus were employed at a high passage level (>100) in chick embryo fibroblast cell culture. These strains were obtained from Dr. Dieter Burger, of the Department of Veterinary Microbiology, Washington State University. The Shope strain had a titer of about $10^8$ plaque-forming units (PFU) per ml and the Buk strain, approximately $10^8$ PFU per ml.

Cell culture

Trypsinized cells from 9- or 10-day-old chick embryos were suspended in medium 199 containing 8% heated calf serum. Five milliliters of the medium containing $2 \times 10^6$ cells/ml were seeded onto a 60×15 mm plastic plate. Antibiotics were added to give a final concentration of 200 units of penicillin G potassium, 100 μg of dihydrostreptomycin sulfate, and 100 μg of neomycin sulfate per ml of medium.

Plaque assay

After incubation in a humidified 5% CO\textsubscript{2} incubator at 37°C for 2 or 3 days, the cell sheet was complete. Then the medium was replaced with 5 ml of Earle's solution containing 0.5% lactalbumin hydrolysate. At this time, the plates were inoculated with 0.2 ml of tenfold serial dilutions of virus inoculum, 3 to 5 plates being used per dilution. After a 2-hour adsorption period under the same conditions as described previously, the fluid was removed and the cell sheets were overlaid with 10 ml of methocel overlay medium\textsuperscript{5}.

For plaque counting, the cells in each plate were fixed with 3 ml of fixative consisting of 6 parts of 95% ethanol, 2 parts of glacial acetic acid, and 1 part of neutralized formalin. They were allowed to stand for 15 minutes, or until all parts of the cell layer turned yellow. After the fixative and overlay medium were removed with slowly
running water, the cells were stained with 1% crystal violet solution for 30 minutes. The plates were washed again with tap water and dried in an inverted position for counting in a Quebec colony counter. The virus titer was expressed as PFU per ml of virus fluid.

Mink, ferrets, and rabbits

The animals used in the present study were standard dark and mutation mink, black-footed and albino ferrets, and white rabbits and included both males and females. They were all adult and were fed a mixture of horse-meat, fish, and cooked cereals. Each animal was housed in an individual pen.

EXPERIMENTS AND RESULTS

Subcutaneous inoculation

A total of 25 mink, 19 ferrets, and 26 rabbits were inoculated subcutaneously with 1 ml of inoculum containing varying numbers of PFU (0.3~320 PFU) of the Shope or Buk strain. The animals were observed for 3 weeks following injection, and clinical signs were recorded (Table 1).

<table>
<thead>
<tr>
<th>Animals (No.)</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Mink (25)</td>
<td>13</td>
</tr>
<tr>
<td>Ferrets (19)</td>
<td>16</td>
</tr>
<tr>
<td>Rabbits (26)</td>
<td>16</td>
</tr>
<tr>
<td><strong>Total (70)</strong></td>
<td><strong>45</strong></td>
</tr>
</tbody>
</table>

No clinical signs were observed in 25 of the 70 animals during the observation period. Thirteen mink, 16 ferrets, and 16 rabbits had clinical signs of illness. The ferrets generally showed mild signs, as compared with the mink and rabbits. When necropsied, however, a number of animals, including mink and rabbits, had evidence of itching and scratching at the site of injection (Figs. 1 and 2), and bleeding from the mouth by tongue-biting (Fig. 3). The first clinical sign in the ferrets was lack of appetite. Subsequently, the majority of the ferrets showed depression, became reluctant to move, lost consciousness, and died. Nervous signs and spasms were observed in only 3 ferrets.

In addition to the signs described above for the ferrets, the mink and rabbits exhibited nervous signs. These signs included spasms of the body, opisthotonos, and extension of the legs. In a few mink, the eyes were narrowed prior to the onset of the signs described above.

The course of infection observed in the inoculated animals is shown in Chart 1. The incubation period ranged from 2 to 8 days after inoculation of the virus. Twenty mink, 11 ferrets, and 18 rabbits (70% of the 70 animals) showed clinical signs or died 3 to 4 days after inoculation. The duration of illness was less than 24 hours. Of all the animals, 92% (22 mink, 17 ferrets, and 26 rabbits) died either without manifesting any apparent sign or 1 day after the onset of illness. The earliest death occurred as soon as 3 days after the inoculation of the virus. About a half of the animals (13 mink, 12 ferrets, and 18 rabbits) died on day 4 or 5. The infection developed rapidly in the
mink, and in the rabbits as well.

There were no marked differences in signs or course of infection between the animals inoculated with the Shope strain and those inoculated with the Buk strain. As shown in Chart 2, however, the incubation period and the time of death varied from 2 to 8 days according to the number of PFU from larger to smaller.

Aerosol exposure

Eleven ferrets were exposed to the Shope strain of virus in a form of aerosol for varying lengths of time (1/2, 1, 2, and 4 minutes). Two ferrets were placed in a closed wooden box (14×10×12 inches) at a time. The orifice of the nebulizer was fitted into
a hole on one side of the box. The aerosol had been produced by a DeVilbiss type 40 nebulizer (the DeVilbiss Company, Somerset, Pennsylvania) operated by a Cenco vacuum pressure pump at 5 pounds of air pressure. After inhalation of the virus, the ferrets were placed in individual wire cages and checked for clinical signs and body temperatures over a period of 5 weeks.

Death was observed in 6 of the 11 ferrets. The length of time of exposure to the virus was not related to the length of the course of infection or to the severity of clinical signs; i.e., 2 ferrets which had been exposed to the virus for 1/2 and 4 minutes, respectively, died 5 days after inhalation, whereas another 2 ferrets, exposed to the virus for 2 and 4 minutes, respectively, died 6 days. The other two ferrets, exposed to the virus for 4 and 2 minutes, died 7 and 10 days, respectively. The first clinical signs were observed in the ferrets 3 days after inhalation of the virus. Depression and hard breathing were found at the beginning, as well as the nervous signs described above for the subcutaneous inoculation. Some ferrets died with a bloody, frothy discharge from their nostrils. No definite evidence of scratching was observed on the bodies of the animals.

Thirty-eight days after inhalation of virus, each surviving ferret was given a subcutaneous challenge inoculation of 1 ml of 100 FLD_{50} (1,580 PFU) of the Shope strain to determine whether immune status was present against the virus. However, all 5 ferrets given the challenge inoculation developed clinical signs rapidly and died on the 3rd to the 5th day after the challenge inoculation.

In ferrets exposed to, or given a challenge inoculation of, the Shope virus, body temperature became rather low after the 2nd or 3rd day following inoculation. Body temperature ranged from 37.8 to 40.0°C in these ferrets during the observation period, and was 36.0°C in some of them on the day before death.

DISCUSSION

The clinical pictures presented by the mink, ferrets, and rabbits used in this experiment were essentially the same as those by experimentally infected sheep, swine, and calves (Table 2). The course of infection in those animals was also similar to that in sheep and calves. It was reported that death had occurred to piglets 2 days after infection. However, among the animals used here, there was no such early death. Clinical signs which extended over a long period of time (7 to 16 weeks in some pigs) were manifested by none of these animals. The manifestation of signs of long-term illness in those pigs supports the fact that the pig has been recognized as a possible reservoir of pseudorabies virus. Although mink and ferrets have a high susceptibility to pseudorabies virus, the course of infection demonstrated in this investigation indicates that these animals are not important as carrier hosts of the virus.

It can be seen in Chart 2 that the incubation period and the time of death are inversely related to the dose of inoculation of virus. The same finding was described previously on the incubation period of pseudorabies virus infection in rabbits and sheep. The duration of illness, however, was not correlated with the dose of inoculation of virus in the mink, ferrets, and rabbits used in the present experiment. This fact might be due to the short duration of illness in experimental pseudorabies of these animals.
Table 2. Summary of Clinical Findings in Experimental Pseudorabies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Exp. animal (age)</th>
<th>Dose of virus inoculated (virus titer)</th>
<th>Route of inoculation</th>
<th>Incubation period [days]</th>
<th>Duration of illness [days]</th>
<th>Main signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dow and McFerran (1966)</td>
<td>Sheep (3 1/2 years)</td>
<td>0.5 ml (10^6.9-7.0/0.5 ml)</td>
<td>Subcut.</td>
<td>4~7</td>
<td>0~2</td>
<td>Pruritus Spasms Nibbling at site of injection</td>
</tr>
<tr>
<td>Olander et al. (1966)</td>
<td>Pigs (6 weeks)</td>
<td>0.5 ml (10^6/ml)</td>
<td>Intramuscul. or Intranasal</td>
<td>2</td>
<td>4 (7~16 weeks)</td>
<td>4~9</td>
</tr>
<tr>
<td>Corner (1965)</td>
<td>Piglets (1 day~3 weeks)</td>
<td>0.5 ml (10^5~10^9/ml)</td>
<td>Intramuscul. or Intranasal</td>
<td>3</td>
<td>4</td>
<td>1~3</td>
</tr>
<tr>
<td>McFerran and Dow (1964)</td>
<td>Calves (2<del>4 mo., 8</del>9 mo.)</td>
<td>0.5~5 ml (10^6.4/ml)</td>
<td>Intramuscul. or Subcut. Intranasal Oral</td>
<td>4~7</td>
<td>0~3</td>
<td>Pruritus Depression Circling</td>
</tr>
<tr>
<td>Goto and Gorham</td>
<td>Mink and ferrets (12 mo.)</td>
<td>1.0 ml (0.3~320 PFU)</td>
<td>Subcut. or Intranasal</td>
<td>3~8</td>
<td>0~3</td>
<td>Depression Pruritus Spasms Bleeding from mouth or nostrils</td>
</tr>
</tbody>
</table>

**SUMMARY**

Mink, ferrets, and rabbits were inoculated with pseudorabies virus by subcutaneous injection or aerosol exposure. They were then observed for clinical signs and course of infection. The clinical signs developed by the inoculated mink and rabbits were severe, but those by the inoculated ferrets were generally mild. In the animals subcutaneously inoculated, the dosage of virus inoculated was inversely related to the length of incubation period and the time of death. The duration of illness, however, showed no correlation to the dose of inoculated virus.

**REFERENCES**

ミンクおよびフェレットにおける実験的
Pseudorabies の臨床的観察

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Pseudorabies virus は Herpes Group に属し、
そのウイルス学的性状は、Herpes virus と同様
に、詳細に検討されている。そしてまた、各種動
物における Pseudorabies の自然発生例や実験的
感染試験誇、多くの研究者によって報告されている。
しかし、ミンクやフェレットにおける本症の
試験研究に関する報告は、国内はもとより、国外
においてもはたは少ないので、最近われわれは、チ
ェコスロバキアで牛用のワクチン・ウイルスとし
て報告された弱毒 Buk 株を入手する機会を得
た。そこで、この株と、米国で広く本症の研究に
供されている Shope 株とを使使用し、ミンクやフ
ェレットおよび本症に対してもっとも感受性が高い
動物とされている家兎について、感染試験を行
なった。本報では、それら感染動物の臨床的観察
について記述した。

ミンク、フェレットおよび家兎の計70匹が、
Shope 株または Buk 株の種々のウイルス量を皮
下接種された。それぞれ動物で観察されたおもな臨
床症状は、羊・豚・牛にみられると同様に、接種
症（いわゆる“Med itch”）にともなう接種部位
の腫脹、口からの流血、神経症状として四肢または
全身の発症である。また重篤なものでは、死の
直前に反応緊張がみられた。これらの症状は、フ
ェレットでは一般に軽く、ミンクでは、家兎と同
様に、重度のもののが多かった。感染動物の潜伏期
は、2日から8日にわたっていた。症状の持続期
間は短く、1日から3日で全例発症した。この潜
伏期間およびウイルス接種から死までの期間と、
接種ウイルス量との間には逆相関が見られた。

吸入感染をうけたフェレットでは、発病症状によ
る腫脹はみられず、多くのもので、鼻孔からの出
血がみられた。これら感染動物の体温は、急激な
上昇もし、むしろ下降を示し、とくに死の前日
にその傾向が著しかった。

なお、これらの感染動物でみられた臨床症状、
潜伏期間および症状持続期間においては、弱毒
Buk 株と Shope 株との間に、著明な差は認めら
れたかった。
EXPLANATION OF PLATE

Fig. 1. Evidence of scratching at the site of injection with the Shope strain of pseudorabies virus in a ferret.

Fig. 2. Involved area in the same ferret as shown in Fig. 1.

Fig. 3. Bleeding from the mouth that was caused by lacerations of the tongue in a ferret inoculated subcutaneously with the Shope strain of pseudorabies virus.