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GOAT POX IN IRAN
SERIAL PASSAGE IN GOATS AND THE DEVELOPING EGG,
AND RELATIONSHIP WITH SHEEP POX

By

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INTRODUCTION

Goat pox, first described by Hansen in Norway in 1879, is a common disease among goats in the Middle-Eastern countries. Although the disease is usually comparatively benign, it is sometimes characterized by severe symptoms and constitutional disturbances which may result in death. The virus can be transmitted to the goat by a variety of routes.

Thus Melanidi and Tzortzaki (1937) found that the inoculation of goat virus by the intradermal, subcutaneous, intravenous and intraperitoneal routes caused generalized lesions. Intratracheal inoculation induced a rise in temperature followed by generalization, while intracerebral injection caused an encephalitis and death. Kerato-conjunctivitis followed the inoculation of the virus into the cornea. Goats were not susceptible by the mouth.

A number of workers have studied the relationship between sheep pox and goat pox viruses in an attempt to produce a single vaccine which would protect sheep and goats against both diseases. Grimpret (1938), Kolayli, Marvidis and Ilhami (1933), and Slagsvold (1942) claim that they infected sheep with goat pox virus. In addition, some of these workers state that they could immunize sheep by using a vaccine prepared with the goat pox virus. Bennett, Horgan and Mansur (1944) and Lall, Singh and Singh (1947) failed, however, to confirm these findings.

During the past two years we have undertaken an investigation on the transmission and cultivation of the virus, and on cross-immunity experiments with sheep pox and goat pox viruses respectively.

NATURAL DISEASE

The number of infected animals in a flock may vary from 5 to 100 per cent. In the acute stage, the lesions may appear over the whole of the body but particularly around the mouth and eyes, on the udder and teats, on the scrotum and on the inner side of the thighs. The disease follows the same stages as other members of the pox group. After a period of incubation of 14 to 17 days (Kolayli, *at al.*, 1933) the temperature rises to 40 to 41°C and the animal suffers from anorexia. A few days later, small greyish-red papules are formed which are later converted into nodules about the size of a lentil; these nodules, which contain a little clear lymph, form the vesicles. The content of the vesicles becomes turbid, the surface becomes necrotic, and a yellowish purulent liquid which exudes dries into scabs and crusts. Recovery takes place in 25 to 30 days. In severe cases common symptoms are respiratory tract disturbances, enlargement of the udder and abortion. The horse, donkey, calf, sheep, pig, dog, rabbit and birds never contract the disease and the authors have failed to infect sheep by co-habitation with diseased goats. In experimental infection of goats the incubation period is reduced to 3 to 5 days.

In our experience, sheep pox virus rarely causes a typical local reaction in the goat.

EXPERIMENTAL DISEASE

Production of Vaccine in Goats

The method described in this paper follows closely that employed for the production of sheep pox and is based on the classical method of Borrel. A field virus from Iranian goats was inoculated into goats by the intradermal and subcutaneous routes. After four serial passages, the virus was propagated satisfactorily in the subcutaneous tissues producing a widespread oedematous reaction similar to that described by Borrel for sheep pox.

For the preparation of the vaccine, goats weighing 30 to 35 kg. are injected on both sides of the thorax. Temperatures are recorded

daily. A rise in temperature occurs between the third and fourth day and lesions develop on the skin after 5 to 6 days. Goats showing fever and a local reaction are killed between the seventh and ninth days.

The hair on the underside of the trunk is clipped and the borders of each side cauterized. The whole surface is then disinfected with tincture of iodine and an incision is made through the cauterized parts. The skin together with adjacent tissues is lifted and held by 4 sterile hooks. The exudate and the oedematous tissue are harvested and ground in a Latapie blender.

Each goat yields approximately 800 g. of infected tissues and 100 ml. of liquid material. After checking for sterility the material is stored at -30°C until used. The virus does not lose its activity under these conditions. The local reaction to vaccination is not severe and goats receiving the material are not capable of infecting healthy goats placed in contact with them.

Culture of the Virus in the Developing Egg

White Leghorn eggs incubated for 12 days are drilled according to the standard technique for chorio-allantoic inoculation. The inoculum (0.2 ml.) treated with 400 i. u. penicillin and 2 mg. streptomycin is deposited on the chorio-allantoic membrane and the hole in the shell sealed with Scotch tape. Ten eggs are used for each passage. After 3 to 4 days eggs with live embryos are put at -20°C for 20 minutes. The chorio-allantoic membrane is harvested aseptically and checked for opaque spots. These lesions were detected in all passage material from the second to the twelfth. Infected membranes are ground in a sterile mortar and divided into two parts. One part is used for the next passage and the other lyophilized in an Edwards's apparatus and kept at low temperature.

The fourth and eighth egg passages were inoculated subcutaneously into two goats (70, and 79) respectively. Both animals showed a slight local reaction about the size of a hazel-nut.

Immunity Induced by Vaccination

Both sheep and goats have been used in these trials. The preparation of the goat vaccine has been described above. Some batches were added to aluminium gel and others were not. The sheep pox vaccine was absorbed on aluminium hydroxide and its preparation has been described elsewhere (Rafyi and Mir-Chamsy, 1956). For challenging the immunity, the virulent sheep pox virus was the 3rd passage of the Delbosc strain with a titre of 1/1,000,000.

The goat pox was the 6th passage of an Iranian virus (Gorgan strain) with a titre of about 1/200,000. Vaccinated animals were challenged intradermally with 0.5 ml. of undiluted virus and controls with the same dose of a 1/200 dilution.

As there is a difference in susceptibility of some of the Iranian breeds to the pox viruses, four types of experiment were carried out. The technique was similar, but the breed of animals and the batch of vaccine were varied because of differences in the degree of susceptibility (Delpy and Rafyi, 1951). In these experiments 100 goats and sheep were divided into two groups. The first group was vaccinated subcutaneously with 0.5 ml. of goat pox vaccine and the second group with 0.5 ml. of the sheep pox vaccine by the same route.

Vaccination of Goats. Ten goats were vaccinated with absorbed goat pox vaccine and ten with sheep pox vaccine. Six controls were not vaccinated. Fifteen days later all the animals were challenged with goat virus with the results shown in Table 1. The animals protected with goat vaccine were completely immune but those which had received sheep vaccine showed a rise in temperature and a typical local reaction. All the controls reacted. It would appear, therefore, that goats vaccinated with the sheep pox vaccine are not protected against a challenge dose of the goat pox virus and that sheep pox vaccine cannot be used in the control of goat pox. The experiment was repeated on 25 goats and 4 sheep, but on this occasion the challenge dose was sheep pox virus. The results are also set out in Table 1.

In these experiments with goat pox vaccine no symptoms or lesions were observed in the ten vaccinated and three control goats; the four control sheep showed a typical pox reaction. Fifteen days later, the 13 goats were tested with goat pox virus; the ten vaccinated goats did not react, whereas the controls showed a local reaction and hyperthermia.

In the case of the group vaccinated with sheep pox all the vaccinated and control goats failed to react, but the control sheep showed a mild but typical response. The ten vaccinated and two control goats were rechallenged with the goat pox virus and all 12 animals showed a local reaction and a rise in temperature.

TABLE 1
VACCINATION EXPERIMENTS IN GOATS

| Vaccine | No. of animals | 1st challenge | | 2nd challenge | |
|-------------------------|----------------|---------------|--------|---------------|--------|
| | | Material | Result | Material | Result |
| Goat pox + gel .. | 10 G | GPV und. | 0/10 | | |
| Sheep pox + gel .. | 10 G | GPV und. | 10/10 | | |
| — | 6 G | GPV 1/200 | 6/6 | | |
| Goat pox + gel .. | 10 G | SPV und. | 0/10 | GPV und. | 0/10 |
| Sheep pox + gel .. | 10 G | SPV und. | 0/10 | GPV und. | 10/10 |
| — | 5 G | SPV 1/200 | 0/5 | GPV 1/200 | 5/5 |
| — | 4 S | SPV 1/200 | 4/4 | — | — |
| Goat pox without gel .. | 10 G | SPV und. | 0/10 | GPV und. | 0/10 |
| — | 2 G | — | | GPV 1/200 | 2/2 |
| — | 2 S | SPV 1/200 | 2/2 | — | |

G-goat S-sheep und.-undiluted
 GPV-goat pox virus SPV-sheep pox virus

In addition to goat vaccine with aluminium gel a test was made with vaccine without gel. Ten goats were injected with vaccine. They were tested with sheep pox virus together with 2 control goats and 2 control sheep. The sheep reacted normally but all the goats remained negative. Fourteen days later all the goats were reinoculated with goat virus. All the vaccinated animals were immune: the two controls reacted.

Vaccination of Sheep. Ten sheep were vaccinated with goat pox vaccine and ten with sheep pox vaccine. Fifteen days later they were challenged with goat pox virus. In the first group, there was no response in the vaccinated animals, but two control goats reacted. The vaccinated sheep with two control sheep were subsequently challenged with sheep pox virus: only the controls reacted.

TABLE 2
VACCINATION EXPERIMENTS IN SHEEP

| Vaccine | No. of animals | 1st challenge | | 2nd challenge | |
|-------------------------|----------------|---------------|--------|---------------|--------|
| | | Material | Result | Material | Result |
| Goat pox + gel .. | 10 S | GPV und. | 0/10 | SPV und. | 0/10 |
| Sheep pox + gel .. | 10 S | GPV und. | 0/10 | SPV und. | 0/10 |
| — | 4 G | GPV 1/200 | 4/4 | — | |
| — | 4 S | — | | SPV 1/200 | 4/4 |
| Goat pox + gel .. | 10 S | SPV und. | 0/10 | | |
| Sheep pox + gel .. | 10 S | SPV und. | 0/10 | | |
| — | 5 S | SPV 1/200 | 4/5 | | |
| Goat pox without gel .. | 10 S | SPV und. | 0/10 | | |
| — | 3 S | SPV 1/200 | 3/3 | | |

G = goat S = sheep und. - undiluted
 GPV = goat pox virus SPV = sheep Pox virus

TABLE 3
VACCINATION EXPERIMENT EGG VIRUS IN GOATS

| No. of animal | Vaccination | | | Challenge | | |
|---------------|-------------|-------------|----------------|-----------|------------------------|----------------|
| | Date | Material | Local reaction | Date | Material | Local reaction |
| 70 | 30/3/57 | 4th passage | + | 14/4/57 | } Goat virus undiluted | 0 |
| 79 | 13/6/57 | 8th passage | + | 28/6/57 | | 0 |
| 72 | | — | | 14/4/57 | } Goat virus 1/200 | ++ |
| 80 | | — | | 28/6/57 | | ++ |

0 = no reaction + = reaction size of hazel nut
 ++ = reaction size of walnut with scabs

In the second group, there was no reaction in the vaccinated sheep to goat pox whereas two control goats reacted. After a second challenge with sheep pox the vaccinated animals were unaffected but two control sheep reacted (Table 2). These experiments would seem to establish the ability of goat pox vaccine to induce a solid immunity in sheep.

In the next trials 10 goats were vaccinated with goat pox and 10 with sheep pox. They were then tested for their resistance to sheep pox virus. All 20 sheep were immune whereas 4 out of 5 controls reacted (Table 2). Goat pox vaccine without aluminium gel gave complete protection to 10 sheep against sheep pox virus (Table 2).

Vaccination with egg-adapted virus. Two goats which had been inoculated, one with 4th passage egg virus and one with virus in the 8th passage gave slight local reactions. When tested 15 days later, they showed no reaction, whereas two controls developed a rise in temperature and local reactions (Table 3).

DISCUSSION

Sheep pox and goat pox are two of the important virus diseases in Iran.

Various strains of sheep pox virus have been investigated for vaccine production in Iran since 1936. Freeze-dried virus adsorbed on anthrax spores (Delpy, Rafyi and Mir-Chamsy, 1951) was formerly used for mass immunization of sheep in this Institute. It was not possible, however, to produce a special vaccine for the control of goat pox until 1957 although veterinarians and farmers have sometimes used the combined vaccine in goats to protect them against anthrax without having any intention of immunizing them against goat pox. There has been a considerable difference of opinion about the efficacy of this vaccine in protecting goats against goat pox.

During the past two years large amounts of goat pox vaccine have been prepared and fairly extensive experiments have been completed. According to our findings, although goat pox virus does not produce a large reaction in sheep, it immunizes them against sheep pox. The duration of the immunity is at present unknown. On the other hand goats injected with virulent sheep pox virus or with sheep pox vaccine fail to react and are subsequently fully susceptible to goat pox.

The advantages of goat pox vaccine as produced by the

methods outlined lies in its value as an efficient prophylactic not only against goat pox but also to protect sheep against sheep pox. It is emphasised that in order to immunise goats this special goat pox vaccine must be used.

CONCLUSIONS

Goat pox virus can be easily isolated from naturally infected animals. It maintains its virulence after successive passages in susceptible goats.

The virus can be propagated in the developing hen's egg.

Sheep pox vaccine does not protect goats against goat pox: goat pox vaccine, on the other hand, affords a solid protection against both goat pox and sheep pox.

Egg-adapted goat pox virus can be used as a prophylactic.

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