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SEVEN YEARS' CONTROL OF SHEEP POX IN IRAN WITH AN ADSORBED TISSUE VACCINE ON ALUMINIUM GEL

By

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In previous notes (Delpy and Rafyi, 1947; Delpy and Mir Chamsy, 1947; Delpy, Rafyi and Mir Chamsy, 1951a) we have described, in collaboration with L. P. Delpy, the evolution of sheep pox disease control in Iran, together with different kinds of vaccines produced at the State Razi Institute and used largely by the veterinary department in this country. We decided finally on a tissue vaccine adsorbed on aluminium hydroxide: some 20 million sheep have been vaccinated during the last seven years without any ill-effects, immunity being established within two weeks and persisting for 9 to 12 months.

This article deals with some technical details concerning the production of this vaccine, and its tests in sheep. Without entering into the history of this subject, a brief account of sheep pox control is included.

CLAVELISATION

While variolisation was a common pratice in Europe and some countries of Asia, in the 18th century, clavelisation was the method for protection against sheep pox used largely in Europe (Rafyi, 1935). This method, used at first by shepherds and modified later by some experimentalists, has the object of producing a limited and local reaction which is considered necessary for the production of im-

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munity. The results of this method were not always satisfactory; in fact, generalisation often occurred and deaths took place in some sensitive breeds following the treatment. Ewes in an advanced stage of pregnancy aborted. In addition, the local reaction with the formation of pustules after clavelisation constituted a grave danger of spreading the infection.

EFFECTS OF ANTISERA

The discovery of the curative and preventive effect of antisera by Duclert (1896) and a method of obtaining large quantities of the virus by Borrell (1903) resulted in improvement in the method of protective inoculation. Borrel introduced serum inoculation into enzootic areas. Later, Chefik, Kolayli and Nicolaki Mavrides (1933) used, with encouraging results, a mixture of serum and sheep pox virus. In Turkey, in 1952, Bacharan, *et al.*, published some details of the method of production of dried sensitised virus.

Meanwhile, others attempted to attenuate the virus by physical, chemical or biological means; Duclert and Conte (1899) tried heat and desiccation, Ducloux and Cordier (1926) used formaldehyde, ethanol and butanol without result; Ramazotti (1933) worked with saponin, and Nello-Mori (1933) made use of ethylic ether. These methods were not satisfactory and the virus, treated by any of these means, proved to be of such low antigenic value that it failed to produce a clear local reaction or a substantial immunity.

Bridré and Boquet (1912, 1913a, 1913b, 1914 and 1933) introduced the sensitised virus, a process which surmounted some of the difficulties of clavelisation. At first, this process appeared to be satisfactory but later on, many disadvantages were revealed. The attenuated virus was obtained by successive passages on sheep and contamination of the virus by different micro-organisms, mainly agalactia, occurred (Bridré and Donatien, 1925; Bridré and Boquet, 1933). After a succession of passages, the virulence of the virus became so reduced that, when sensitised by serum, it no longer induced any immunity. These workers also found that sensitised virus could cause abortion when used on pregnant sheep. It is recognised that all strains of sheep pox virus do not respond to sensitisation. Virus may be passaged under the skin of sheep and according to Bridré, the transformation can be speeded up by using a very active virus on sheep already immunised against some other pox virus (neurolapine). These and other considerations complicated the use of sensitised virus.

Since 1932, Iranian sheep flocks have been systematically vaccinated against sheep pox; the strains utilised in the production of sheep pox vaccine have the following properties:

(*a*) The diffusion potency must be sufficient to produce some large Borrel pustules in the hypodermis of Merinocs-Iranian cross-bred sheep (Delpy and Rafyi, 1938).

(*b*) The virulence of the virus must be such that the inoculation of the suitably diluted virus into the skin of the Iranian sheep gives a strong immunity and that the most severe experimental inoculation does not lead to generalisation.

In common with other workers (Delpy and Rafyi, 1947; Sabban, 1955), we have demonstrated that there is no immunological difference between the virus of various origins. The difference in strains is due to variation in diffusibility and virulence. In Iran, there are two distinct breeds of sheep. One is theSyrian breed with a big tail which constitutes the majority of our sheep flocks. The other, called Mazanderany, is found in the area of the Caspian Sea and is characterised by a tail resembling that of European sheep. From the point of view of sheep pox virus inoculation, the distinction between these two breeds is important. The Mazanderany is very sensitive; the inoculation of virulent sheep pox virus into, or under the skin produces a severe local reaction and fever and often a generalised infection identical with the natural disease occurs. The Syrian sheep, on the contrary, show a different susceptibility: although highly sensitive to natural infection, which sometimes causes a mortality of 40 per cent in a flock, the response to intra-cutaneous inoculation of the virus induces a local reaction and a short hyperthermia only.

A combined vaccine against anthrax and sheep pox has been used on Syrian sheep (Delpy and Mir Chamsy, 1947; Delpy, Rafyi and Mir Chamsy, 1951a). The Roumanian strain of sheep pox virus received from Algeria, which produced exceptionally strong Borrel pustules in the native breeds, is used as the sheep pox antigen. The anthrax antigen is a merthiolated suspension of anthrax spores (Delpy and Mir Chamsy, 1947). The virus adsorbed on the spores is lyophilised. This mixed and dried vaccine gives an immunity against both diseases, certainly for a period of more than one year. From the immunological standpoint it is interesting to note the triple rôle of the anthrax spores; specific antigenic rôle, rôle of support for the sheep pox virus and the adjuvant rôle which produces the initial inflammatory reaction and permits fixation and multiplication of virus. Notwithstanding the indisputable advantage of the mixed vaccine, it causes, sometimes in Mazanderany sheep, large pustules with various

complications, and it may also happen that the disease becomes generalised.

On considering the publication of Balozet (1938) who adsorbed the sheep pox virus (S.P.V.) on aluminium hydroxide, and of Nelis and Lafontaine (1948)who showed that the vaccine virus can be attenuated by the action of 0.01 percent formaldehyde at 20° C. for 2 to 6 days without destroying its immunising properties, an attempt was made to find if this method would have a similar effect on the S.P.V. already adsorbed on aluminium hydroxide. In this connection, Manninger (1948) uses the «disinfection action of formaldehyde on the virus adsorbed by aluminium hydroxide» but no details are given of the technique of production and particularly of the quantity of virus contained in an immunising dose, and the rate of formolisation.

Since 1948 the vaccine adsorbed on aluminium gel and inactivated by 0.01 per cent formaldehyde has been distributed in Iran. Later, it was found that the addition of formaldehyde is not necessary and the adsorbate of S.P.V. on aluminium gel with 0.01 per cent thiomerthiolate, added as a preservative, is a potent antigen for sheep pox control.

MATERIALS AND METHODS

Production of Virus. At the present time S.P.V. is produced by the routine procedure of Borrel. The Merino-Iranian crass-bred sheep are injected with the Roumanian strain of S.P.V. On harvesting, an abundant amount of lymph is present. After killing the sheep, while the fever is still high, this lymph, as well as the œdematous and infiltrated muscles are harvested asepticaly and placed in a sterile jar which is covered with some layers of gauze and cotton-wool. It has been shown that the S.P.V. may be cultivated *in vitro* on living cells. Bridré (1935), for example, obtained a culture on the living cells of sheep testes suspended in sheep serum and Drew solution. In some work not yet published, we have found that it is possible to cultivate the virus on the whole tissue of sheep or guinea pigs by adding some salts, vitamins growth factors, fresh sheep serum and antibiotics. In the first passage, the multiplication of virus is normal and it is antigenically strong, but it fails to produce local reactions as the number of subcultures is increased; the seed must, therefore, be taken regularly from the original material.

The lymph and gelatinous tissues of the Borrel pustules are collected, passed through an electric grinder and mixed with three

times their weight of saline. One ml. is taken for titration of the virus. The whole ground-up material is then stored at — 20° C.

Titration of Virus.

Twenty-four hours before titration, the skin of both sides of four cross-bred Merino-Iranian sheep is shaved and cleansed. One ml. of the above material is dissolved in 250 ml. of saline to make a dilution of 1/1,000. From this dilution the following dilutions are made: 1/10,000, 1/20,000, 1/50,000, 1/100,000, 1/200,000, 1/500,000, and 1/1,000,000. From each dilution one ml. is injected intradermally at each of four

Table 1 illustrates the result from a routine test.

TABLE 1 - *Titration of vaccine batch 34/2*

Dilution of Virus

Sheep	10. -4	0.5 x10. -4	0.2 x10. -4	10. -5	0.5 x10. -5	0.2 x10-5	10. -6
34/107	+++	+++	+++	+++	++	++	+
34/108	+++	+++	+++	++	++	+	+
34/109	+++	+++	+++	++	+	+	+
34/110	+++	+++	+++	++	+	+	+

+ = one reaction dose

spots on a transversal line in each sheep; thus, on each side there are twelve injections comprising three dilutions. The reactions are recorded from the 5th day to the 11th day. The reaction dose (R.D.) is the dilution which gives a specific reaction of 15 mm. size in 50 per cent of the total injections.

Relation between the Reaction Dose and the Immunising Dose of Adsorbed Virus.

When a dilution of virus injected intradermally into the sheep results in a specific local reaction and a rise in temperature, the animal is shown to have a solid immunity against sheep pox; thus the R.D. and the immunising dose (I. D.) are the same. But when the virus is adsorbed on aluminium gel, this relationship does not apply because the *elution* of adsorbed virus by different procedures is impossible. The inoculation of such a virus, on the other hand, is followed by the slow release of the antigen and this prevents the occurrence of specific local reaction. It is, therefore, necessary to deter mine, experimentally, the relation between the R.D. and I.D. By repeated experiments it has been found that the I.D. must contain 100

R.D. in order to produce a satisfactory immunity, lasting for one year. This relationship is shown in Table II.

TABLE 2 - *Relation between the Reaction and Immunising Doses of sheep pox virus adsorbed on aluminium gel.*

Sheep	Vaccinal dose : Number of R.D. adsorbed on aluminium hydroxide	Result of challenge with 25,000 R.D., 3 weeks after vaccination.	
		Local reaction	Rise of Temperature
141. 142	0.5	+	+
143. 144	1	+	+
145. 146	5	+	+
147. 148	10	+	+
151. 152	25	+	+
153. 154	50	+	+
155. 156	100	−	−
157. 158	200	−	−
159. 160	300	−	−
149. 150	controls 10 R.D.	+	+

Final Production of Vaccine.

When the R.D. is determined the vaccine sould be made rapidly. The aluminium gel is placed under sterile conditione into a container having an electric stirrer. The virus is then added slowly, the mixture being continually stirred. The amount of virus te be added is calculated so that one ml. of the final product contains 200 R.D. (the vaccinal dose for sheep being 0.5 ml. of this product). Thiomerthiolate is then added to an amount of 0.01 per cent. The compound gel-virus is then distributed in small vials, sealed and stored in the cold room until used. The aluminium gel is prepared in our chemical department, following the Willastater technique, as follows:

Aluminium Gel Production.

For the preparation of 90 litres of aluminium hydroxide the following reagents are necessary :

Ammonium sulphate cryst. $SO_4(NH_4)2$ . . . . . 2,200 gr.
Aluminium ammonium sulphate $Al_2(SO_4)3(NH_4)_2SO_4, 24H_2O$ . . . 7,670 gr.
Ammonium hydroxide NH_4OH 10 per cent . . . 10,000 ml.
The method of preparation is as follows :

Dissolve the ammonium sulphate in 60,000 ml. of tap water and heat to 63° C. Dissolve also the aluminium ammonium sulphate in 25,000 ml. of water and heat to 58° C.

Put the first solution into a stainless container having an electric stirrer; add 10,000 ml. of 10 per cent ammonium hydroxide, stirring constantly and then slowly add the aluminium ammonium sulphate solution.

Stir for a few minutes and wait several hours until the gel is precipitated. Siphon off the supernatant fluid and replace it by distilled water; repeat the washing of the gel several times by the same method until the excess of ammonium hydroxide is removed. Bring the volume of the gel up to 90,000 ml. with distilled water, distribute it in jars and sterilise by autoclaving. The adsorption value of the gel is controlled by the use of Congo red as follows :

To 70 ml. of a solution containing 7 mg. of 7 per cent Congo red add 4ml. of gel, mix well and allow the mixture to precipitate.

The adsorbing value of the gel is satisfactory when all the colour is adsorbed on the aluminium gel after 30 minutes.

Duration of Immunity.

The immunity established in 12 to 15 days by a single dose of this vaccine containing 0.01 per cent formaldehyde remains effective at least for one year; and even after 12 months, exposed sheep resist natural infection, although immunity to experimental challenge fades after that period. In the case of compound gel-virus vaccine without formaldehyde our field and laboratory records are limited to the results obtained during the last nine months. By using this vaccine as a prophylactic or in actual outbreaks of sheep pox, our veterinary colleagues in most parts of Iran are very satisfied with results. Table III gives data concerning a batch of the vaccine.

TABLE 3 - *Test for duration of immunity of batch 34/1*

Sheep	Vaccinal doses	Results of challenge with 25000 R.D. afters		
		3 monlhs	6 months	9 months
174, 181, 185, 175 and 176	100 R.D.	−		
182 control	10 R.D.	+		
177, 178, 179, 180 and 183	100 R.D.		−	
169 control	10 R.D.		+	
184, 187, 191, 192 and 193	100 R.D.			−
195 control	10 R.D.			+

− = immune : + = reacted.

Field Application

During the last seven years more than 20 million doses of adsorbed aluminium gel sheep pox virus with 0.01 per cent formaldehyde were used in Iran. During the last year, four million doses of the vaccine without formaldehyde have been distributed in the country. The results are so good that the State veterinarians of different areas of Iran refuse to accept the old living virus vaccine which sometimes causes unhappy results.

SUMMARY

Sheep pox has been a serious disease in Iranian flocks, especially among lambs. Since 1930 the disease has been controlled first by the use of living vaccine and later by an aluminium gel adsorbate or virulent sheep pox virus.

A short history of the use of killed vaccines and a neutralised mixture of virus and serum is described. The disadvantage of living virus vaccines, especially the permanent danger of transmission of the disease from vaccinated animals to unvaccinated susceptible subjects, is discussed. Serious reactions following the use of virulent living vaccines in highly susceptible flocks, abortion in pregnant ewes and the short period of valency pointed out by the manufacturers when the living vaccine is to be kept in the field are main objections to using living virus, dry or in liquid state.

The adsorbate virus is, on the other hand, a safe and effective vaccine which has been used widely in Iran during the past seven years without any ill after-effects. More than 24 million doses of the vaccine have been used as a prophylactic or in actual outbreaks of the disease.

The method of Production and titration of Vaccine is described.

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