STUDIES ON LIVE AND INACTIVATED SHEEP POX VACCINES

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Sheep pox causes considerable economic loss and it is essential to have a safe and efficient vaccine for its control. Reports of different workers to evolve a better vaccine have been reviewed by Uppal et al (1967). The present paper records observations on extensive laboratory and field trials on live and inactivated sheep pox vaccines.

MATERIALS AND METHODS

Sheep Pox Vaccine was prepared by Borrel's technique as described by Rafi and Mirchamsey, (1956) using an unattenuated local strain of virus. The lyophilised vaccine contained $1 \times 10^5$ reacting doses of virus per ml. The same strain was used for challenge.

A. Live adjuvant vaccines:

i. Aluminium hydroxide gel adsorbed sheep pox vaccine:

Aluminium hydroxide gel was prepared on the lines of Willastater as detailed by Rafi and Mirchamsey (loc. cit.).

Freeze dried sheep pox vaccine was reconstituted in required quantity of gel, so as to get different reacting doses (R. Ds.) i.e. 50, 75, 100, 200 and 500 R. Ds. of virus per 0.5 ml. dose, stirred for 30 minutes in a magnetic stirrer and kept overnight at 4°C before use.

ii. Sodium alginate (BDH) adjuvant vaccine:

One percent Sodium alginate in distilled water was used as the diluent for the vaccine which contained 75 or 100 R. Ds. of virus in 0.5 ml. dose.

iii. Incomplete Freund's adjuvant vaccine:

Incomplete Freund's adjuvant vaccine was prepared by mixing equal quantities of virus suspension in buffer (800 R. Ds/ml.) and

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Bayol F and Arlacel 'A' Oil Phase (9:1) as recommended by Cunliffe and Graves (1963).

Immunogenicity trials:

The live gel adsorbed vaccine containing various R. Ds. of virus were injected, subcutaneously on the external aspect of the ear in different batches of sheep. Three weeks later the vaccinated sheep were challenged with 10,000 or 25,000 R. Ds. of the virus subcutaneously in the caudal fold. Similar trials were carried out with sodium alginate and oil adjuvant vaccines.

Studies on the onset, duration of immunity and keeping quality of the gel adsorbed vaccine:

Based on the encouraging results with gel adsorbed vaccine detailed studies were made with this vaccine containing 200 R. Ds. of virus per 0.5 ml. on the onset and duration of immunity produced by this vaccine and its keeping quality.

Batches of vaccinated sheep were challenged on sixth, ninth, twelfth and fifteenth day after vaccination, with 10,000 R. Ds. of virus to find out the earliest period by which immunity is set up.

Sheep vaccinated at the laboratory and field were tested at different intervals to assess the duration of immunity produced by this vaccine.

The vaccine stored at room temperature (28–30°C) and at cold room temperature (4°C) was used at periodic intervals for studies on keeping quality. Vaccinated sheep were challenged 3–4 weeks later with 5,000 or 10,000 R. Ds. of virus.

Field trials with live gel vaccine:

Field trials were carried out in Mecheri, Mandya, Tiruchi black breeds and Red hairy type animals both in infected and disease free flocks. Vaccinated sheep were observed for local and general reactions for a month.

B. Inactivated vaccines:

Formalin, Betapropiolactone (BPL) and Acetylaiziridine, a chemical closely related to Acetylstyreneimine were used as inactivating agents.
i. Inactivation with Formalin:

Viral concentrations of 2,000, 5,000 and 10,000 R. Ds./ml. were treated with 0.05%, 0.01% and 0.005% final concentration of formalin in Phosphate buffer (pH 7.4) and the mixtures were kept at 4°C and 28-30°C for 48 hours. Tests for viable virus were carried out by intradermal injection of 0.1 ml of treated samples in sheep.

ii. Inactivation with BPL

Following the method of Logrippo and Hartman (1955) a 10% w/v solution was prepared in chilled distilled water, keeping both the diluent and BPL in an ice bath. Different concentrations of BPL were mixed with equal volumes of virus in phosphate buffer (pH 8) so as to get final concentrations of 0.1%, 0.05%, 0.01% and 0.005% of the chemical and 10,000 R. Ds. of the virus per ml. and the mixtures were kept at 37°C for 2 hours.

iii. Inactivation with Acetylasizidine:

Double concentrations of Acetylasizidine in phosphate buffer (pH 7.4) were mixed with equal volumes of virus suspension, so as to get 0.1%, 0.05%, 0.025%, and 0.01%, final concentration of the chemical and 10,000 R. Ds. of the virus per ml. and kept at 28°C. Samples removed at the end of 8, 16, 24, 48 and 72 hours were tested for residual virus.

Based on preliminary trials, batches of inactivated vaccine were prepared using the virus inactivated with 0.01% Formalin at 28-30°C for 48 hours; 0.05% BPL at 37°C for 2 hours and 0.025% Acetylasizidine at 28°C for 24 hours separately. Final concentration of the virus was adjusted to 1,000 R. Ds. in 0.5 ml buffer or aluminium hydroxide gel. Inactivated Oil adjuvant vaccines were prepared as in the case of live vaccine.

Immunogenicity trials:

Sheep were vaccinated with 0.5 ml of inactivated vaccine, with and without adjuvants, subcutaneously on the external aspect of the ear. Three to four weeks later, animals were challenged with 200 R.Ds. of virus in the other ear (the dose used in the live vaccine) or 5,000 or 10,000 R.Ds. of virus subcutaneously in the tail.
Onset, duration of immunity and keeping quality studies:
Studies on onset, duration of immunity and keeping quality of formalinised adjuvant vaccines were done as in the case of live gel vaccine.

Field trials:
Field trials of formalinised gel vaccine were carried out in red hairy type animals both in infected and disease free flocks. About 42% of the animals that received formalinised gel vaccine in the field were subsequently given 200 R.Ds. of live gel vaccine as booster dose. The reaction to primary and secondary vaccinations was recorded. In one village apart from those which received the two vaccines, 48 sheep were given live gel vaccine alone for comparative study.

RESULTS

A. Live adjuvant vaccines:

(i) Aluminium hydroxide gel adsorbed sheep pox vaccine:
A persistent nodular thickening developed at the site of vaccination in 88% of the vaccinated animals (Fig. 1). Ulceration and generalisation were noticed in 9% and 5% respectively of the vaccinated animals. The results of laboratory trials are given in Table-1.

TABLE—1

<table>
<thead>
<tr>
<th>The vaccinal dose</th>
<th>No. of sheep vaccinated</th>
<th>Reaction to vaccination No. which developed</th>
<th>Result of challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nodular thickening</td>
<td>Severe Ulceration</td>
</tr>
<tr>
<td>50 R.Ds.</td>
<td>26</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>75 R.Ds.</td>
<td>60</td>
<td>53</td>
<td>2</td>
</tr>
<tr>
<td>100 R.Ds.</td>
<td>24</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>200 R.Ds.</td>
<td>12</td>
<td>11</td>
<td>—</td>
</tr>
</tbody>
</table>

Numerator : Number reacted
Denominator : Number tested.
(iii) Sodium alginate adjuvant vaccine:

Out of 37 sheep given the vaccine 27 developed severe ulceration at the site (Fig. 2) and two developed generalised pox. All the 33 sheep available for challenge withstood the same while all the 8 controls reacted.

(iii) Incomplete Freund's adjuvant vaccine:

A batch of eight sheep which received oil vaccine developed diffuse hard thickening at the site and withstood a subsequent challenge while the four controls reacted.

Onset, duration of immunity and keeping quality of live gel adsorbed vaccine:

Live gel adsorbed vaccine set up immunity on the twelfth day after vaccination.

Sheep vaccinated with 75-reacting doses withstood challenge after 3 months but not after 4 months. Different batches of sheep vaccinated with 200 reacting doses of virus withstood challenge tests when tested at fourth, seventh, fourteenth, eighteenth and twenty-third months (maximum period tested).

The potency tests with the vaccine stored for 15, 21 and 30 days at 28–36°C showed that keeping quality of the vaccine was satisfactory up to 21 days. The tests with vaccine stored for 75, 120 and 180 days at 4°C revealed that the same was potent up to 75 days.

Field trials with live gel vaccine:

Totally 9467 sheep were vaccinated and the observations are tabulated. (Table II)

TABLE—II

<table>
<thead>
<tr>
<th>Area in which vaccination was carried</th>
<th>No. of sheep vaccinated</th>
<th>No. available for subsequent examination</th>
<th>Reaction to vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nodular thickening</td>
<td>Severe ulceration</td>
</tr>
<tr>
<td>Free area</td>
<td>2547</td>
<td>2286</td>
<td>2250 (98.43%)</td>
</tr>
<tr>
<td>Outbreak area</td>
<td>6920</td>
<td>6188</td>
<td>5874 (94.93%)</td>
</tr>
</tbody>
</table>
B. Inactivated Vaccines:

Inactivation of virus with formalin:

A final concentration of 0.01% formalin inactivated the virus suspension containing 10,000 R.D.s./ml. in 48 hours at 23-30°C; whereas a higher concentration of 0.05% failed to inactivate when the treatment was done at 4°C (Table—III)

<table>
<thead>
<tr>
<th>Concentration of formalin</th>
<th>0.05%</th>
<th>0.01%</th>
<th>0.005%</th>
<th>Untreated virus control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of virus R.D.s./ml,</td>
<td>2,000</td>
<td>5,000</td>
<td>2,000</td>
<td>5,000</td>
</tr>
<tr>
<td>Kept at 4°C</td>
<td>1/3</td>
<td>1/3</td>
<td>1/3</td>
<td>1/3</td>
</tr>
<tr>
<td>Kept at 28-30°C</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

N: No. of spots showing reaction.
D: No. of spots injected with 0.1 ml of treated and untreated virus.

Inactivation with BPL:

BPL inactivated the virus (10,000 R.D.s./ml.) in 2 hours at 37°C, in a final concentration of 0.05% as evidenced by the results given below: (Table IV)

<table>
<thead>
<tr>
<th>Concentration of BPL</th>
<th>0.5%</th>
<th>0.4%</th>
<th>0.3%</th>
<th>0.2%</th>
<th>0.1%</th>
<th>0.05%</th>
<th>0.0%</th>
<th>0.005%</th>
<th>untreated virus control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>9/9</td>
<td></td>
</tr>
<tr>
<td>Experiment II</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0/6</td>
<td>0/6</td>
<td>5/6</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td>Experiment III</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>6/6</td>
<td></td>
</tr>
</tbody>
</table>

N: No. of spots showing reaction.
D: No. of spots injected with 0.1 ml of treated and untreated virus.
Inactivation with Acetylarizidine:

Acetylarizidine, in a final concentration of 0.05% inactivated the virus in 16 hours, while there was still active virus at the end of 8 hours. In 0.025% concentration, inactivation was complete in 24 hours (see Table V).

**TABLE—V**

<table>
<thead>
<tr>
<th>Sl.No. of experiment</th>
<th>Concentration of acetylarizidine</th>
<th>Period of inactivation in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>I</td>
<td>0.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.025%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Untreated virus control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.025%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Untreated virus control</td>
<td></td>
</tr>
</tbody>
</table>

N. No. of spots showing reaction
D. No. of spots injected with 0.1 ml. of treated and untreated virus.

**Immunogenicity trials:**

No local reaction was observed in the sheep vaccinated with inactivated sheep pox vaccines without adjuvants while only nodular local thickening was observed in all the sheep that received the above inactivated vaccines with adjuvants. The results of challenge tests are given in the Table VI.
### TABLE—VI

| Inactivated vaccine used | No. of sheep vaccinated | Challenge Dose | Result of challenge | | |
|--------------------------|-------------------------|----------------|---------------------|----------------|
| Formalinised Vaccine     | 12                      | 200 R.Ds.      | 11/12               | 8/10           |
| Formalinised Gel Vaccine | 23                      | 200 R.Ds.      | 2/23*               | 6/10           |
|                          | 10                      | 5,000 R.Ds.    | 3/10                | 9/10           |
|                          | 21                      | 10,000 R.Ds.   | 2/21                | 3/7            |
| Formalinised Oil Adjvant Vaccine | 5   | 10,000 R.Ds.   | 0/5                 | 1/3            |
| BPL Inactivated Vaccine  | 17                      | 200 R.Ds.      | 6/17                | 4/4            |
| BPL Inactivated Gel Vaccine | 5   | 200 R.Ds.      | 2/5                 | 1/6            |
| BPL Inactivated oil adjuvant vaccine | 16  | 5,000 R.Ds.    | 6/16                | 11/12          |
|                          | 6                       | 10,000 R.Ds.   | 0/6                 | 2/2            |
| Acetylaziridine inactivated vaccine | 5  | 5,000 R.Ds.    | 5/5                 | 5/6            |
| Acetylaziridine inactivated gel Vaccine | 9  | 3,000 R.Ds.    | 6/9                 |               |
| Acetylaziridine inactivated oil adjuvant vaccine | 6  | 10,000 R.Ds.   | 2/6                 |               |
|                          | 6                       | 10,000 R.Ds.   | 1/6                 | 1/3            |

* Four sheep developed slight thickening but recovered completely.
N. No. reacted
D. No. challenged.

**Onset, duration of immunity and keeping quality of formalinised Adjvant vaccines:**

Formalinised gel vaccine set up immunity by ninth day after vaccination.

Batches of sheep vaccinated with formalinised gel and oil vaccines withstood the infection, when challenged four months later (maximum period tested.)
Formalised gel and oil vaccines, stored at 28-30°C for 15, 30, and 45 days, were tested for potency and found to confer satisfactory immunity up to one month storage. The vaccines, tested after a maximum storage of 120 days at 4°C were also found potent.

Field trials:

Totally 4,434 sheep of red hairy type in infected and disease free flocks were vaccinated with formalised gel vaccine. Of these 1,862 animals received a second vaccination with live gel vaccine. Except nodular thickening at the site, no other reaction was observed after primary vaccination. Only seven out of 1,862 sheep that received the second injection of live vaccine showed ulceration at the site. Among 48 sheep that received the live gel vaccine alone as controls, severe reaction at the site was observed in six and one developed generalised pox. (Table-VII)

TABLE VII

<table>
<thead>
<tr>
<th>Area in which sheeps were vaccinated</th>
<th>No. available for subsequent examination</th>
<th>Reaction</th>
<th>No. which available for 2nd sub-vaccination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free area</td>
<td>1310 1200 1200</td>
<td>— —</td>
<td>652 652 652</td>
<td>— —</td>
</tr>
<tr>
<td>Affected area</td>
<td>3124 2472 2472</td>
<td>— —</td>
<td>1210 1210 1203</td>
<td>7 —</td>
</tr>
<tr>
<td>area</td>
<td></td>
<td>48*</td>
<td>48 41 6 1</td>
<td></td>
</tr>
</tbody>
</table>

*Received live gel vaccine alone.

DISCUSSION

Sheep Pox virus used for protecting sheep in its free form produces severe local reaction and generalisation in some cases. Studies on adjuvant vaccines have been undertaken to improve the safety, potency and keeping quality of such vaccines. Of the three
adjuvants used for live vaccine, the severity of the local and general reactions observed with sodium alginate adjuvant vaccine discouraged further studies on this adjuvant. Secondary pox lesions in a small number of animals vaccinated with live sodium alginate adjuvant vaccine was also observed by Uppal et al (loc. cit). The limited studies made with virus in combination with incomplete Freund's adjuvant gave satisfactory results. But this type of vaccine is not bereft of adverse reactions according to Nilakantan, (1967). Following large scale use of Gel adsorbed sheep Pox vaccine in Iran by Rafyi and Mirchamay (loc. cit), Ramani (1961) and Abdullakhan (1961) tried this vaccine in India in Madras and Mysore states respectively. In the present study, the aluminium hydroxide gel adsorbed vaccine was found to confer satisfactory immunity in 75 R. Ds, but the immunity lasted only for three months. When the viral content of the vaccine was increased to 200 R. Ds, it gave satisfactory immunity up to a period of 93 months, the maximum period tested. This vaccine kept well at room temperature up to 21 days and at 4°C for 75 days. Detailed laboratory and field trials of the vaccine indicated that the adsorbed virus is still capable of setting up generalised form of disease in 0.39% of vaccinated animals in free areas and 1.66% in outbreak areas. Similar observations were made by Obhaklovaskii (1941), Manninger (1948) and Abdullakhan (loc. cit.)

To overcome the hazard, the virulent virus has been inactivated with various chemicals and used as vaccine. Formalin BPL and Acetyldihydropyridine (AEI) have been used extensively for vaccine production against New-castle disease, Foot and Mouth Disease, Rabies, Pox Disease etc. (Rweryemam, 1970). In the present studies, Formalin, BPL and also Acetazolamide a chemical closely related to AEI were used.

Formalin inactivated vaccine without any adjuvant was found to be inferior to that incorporating aluminium gel or oil as adjuvants. The latter gave consistent and satisfactory results. Voronovich et al (1963) also reported good results with formalinised gel adsorbed vaccine. The failure to get satisfactory results in the experiments conducted earlier in this Institute, (Annual reports 1956-57 and 1957-58) may be due to the use of formalised vaccine alone without any adjuvant. Again the unfavourable results with formalised adjuvant vaccines observed by Nilakantan (1957) and Uppal et al (loc. cit) may be due to the high concentration of the chemical (0.1%) used for virus inactivation.
Results of BPL inactivated adjuvant vaccines were satisfactory but more studies have to be carried out to compare it with formalised adjuvant vaccines. Uppal et al (loc. cit) reported satisfactory results with adjuvant vaccines, prepared after 15 minutes inactivation with 0.05% concentration BPL.

Acetylationidine inactivated vaccine did not confer good immunity. Ramana Rao (1969) working with vaccinia virus found AEI to be inferior to formalin, though Brown et al (1969) found AEI inactivated foot and mouth disease vaccine to give satisfactory results.

Formalinised gel adsorbed vaccine sets up immunity by about ninth day and the same lasted for a period of four months, the period tested in our studies. Raji and Mirchamay (loc. cit) reported that this vaccine established immunity in 12-15 days and maintained the same upto 12 months. Formalinised gel vaccine has better keeping quality (30 days at room temperature and 120 days at 4°C) than that of live gel vaccine (21 days at room temperature and 75 days at 4°C). According to Celiker and Arik (1969) the aluminium hydroxide gel adsorbed sheep pox vaccine containing 0.01% formaldehyde retained its immunising value for 7 months when stored in dark, at room temperature.

Results of field trials with formalinised gel vaccine followed by live gel vaccine are very encouraging. Even the small percentage of generalisation and severe ulceration at the site as a result of vaccination with live gel vaccine has been overcome by the primary injection of the inactivated vaccine. However elaborate studies on the duration of immunity of inactivated vaccines and the exact time at which the second vaccination has to be carried out to give maximum booster effect, have to be made before they are introduced for large scale use in field.

SUMMARY

Live gel adsorbed sheep pox vaccine containing 200 reacting doses of virus in 0.5 ml dose, conferred satisfactory immunity upto a period of 23 months (the maximum period tested). The vaccine however sets up generalisation in 0.39% of sheep vaccinated in free flocks.

Immunogenicity trials were carried with sheep pox virus inactivated with formalin, BPL and Acetylationidine. Use of the
adjuvants like alumina gel and oil considerably enhanced the efficacy of the inactivated vaccines.

Formalinised gel vaccine conferred satisfactory immunity for 4 months (the maximum period tested).

Formalinised adjuvant vaccine had better keeping quality both at room temperature and at 4°C, than live gel vaccine.

Formalinised vaccine when given prior to live gel vaccine wards off the severe reaction caused by the latter.

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REFERENCES


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STUDIES ON LIVE AND INACTIVATED SHEEP POX VACCINE

Fig. 1.
Sheep vaccinated with aluminium hydroxide gel adsorbed sheep pox vaccine showing nodular thickening at the site of vaccination.

Fig. 2.
Sheep vaccinated with sheep pox vaccine containing sodium alginate adjuvant showing severe ulceration at the site of vaccination.