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ACTIVE IMMUNIZATION OF CATTLE WITH KILLED VACCINES PREPARED FROM CELL-CULTURED RINDERPEST VIRUS*

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SUMMARY. Cell cultured rinderpest virus inactivated by treatment with 0.01% *merthiolate or* 0.013% *formaldehyde or by exposure to ultraviolet light induced the production of transient neutralizing antibodies in cattle. The addition of aluminium hydroxide or incomplete Freund's adjuvant significantly enhanced neutralizing antibody titres whereas the addition of saponin dramatically depressed the titres. The dose of adjuvant inactivated vaccine was critical.*

Although inactivated rinderpest vaccines have the great virtue of safety, they have been universally replaced by attenuated live virus vaccines because of their high cost of production and the short duration of immunity they induce. Thus, in recent years, the attenuated cell-cultured rinderpest viruses developed independently by Plowright & Ferris (1962) and De Boer (1962) have supplemented other rinderpest vaccines, both attenuated and inactivated. There are, however, serious objections to the use of live vaccine in countries free of rinderpest and, moreover, there is the fear of importing rinderpest virus in meat from cattle immunized with live vaccine. The development of a potent inactivated rinderpest vaccine is therefore justified.

Scott & Witcomb (1958) compared vaccines produced by inactivation of a suspension of rinderpest-infected bovine spleen or fluid from rinderpest infected cultures of bovine kidney cells, using either 1% formalin or ultraviolet light (uv). The vaccines contained adjuvant, either aluminium hydroxide or mineral oils. The vaccine to which there were fewest objections was an aluminium hydroxide adsorbed preparation but multiple doses were required to obtain a satisfactory antibody response. Provost *et al.* (1969) tested cell cultured rinderpest virus inactivated with ß-propiolactone and incorporated in oil adjuvant. The vaccine failed to protect susceptible calves.

Complete sero-conversion has been attained in children using measles virus inactivated with sodium ethylenmercurithiosalicylate (merthiolate) and the antibodies persisted for more than 3.5 years (Masuno *et al* 1968). Since

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the viruses of measles and rinderpest have similar properties we decided to compare a merthiolate inactivated rinderpest vaccine with attenuated live virus vaccine and with vaccines prepared from virus inactivated by formaldehyde or uv. The value of various adjuvants was also investigated.

MATERIALS AND METHODS

Virus Strains

Two strains of rinderpest virus grown in primary calf kidney cells were used in the preparation of inactivated vaccines; the virulent RP2 strain and the cell culture attenuated Kabete O (KOA) strain. The former was isolated in Vero cell cultures in this laboratory during the recent rinderpest epidemic in Iran (Mirchamsy *et al.*, 1970). The KOA strain had undergone 100 passages in primary calf kidney cells (Plowright & Ferris, 1962).

The attenuated live virus vaccine was a batch of KOA produced at the Razi Institute and kindly supplied by Dr H. Ramyar. Each calf received 100 TCID50.

The challenge virus was the virulent RP2 strain, the challenge dose containing 1000 LD100 (6 challenge control calves which received 1 LD100 developed clinical rinderpest and died in 7 to 9 days).

Inactivation

Merthiolate. One per cent merthiolate was added to virus suspensions containing 10^5 to $10^{5.5}$ TCID50 per ml to give a final concentration of 0.01%. The pH was adjusted to 7.1 and the mixture held in a water bath at 37°C, being shaken several times a day. A like volume of the virus suspension without merthiolate was handled similarly.

Formaldehyde. Forty per cent formaldehyde solution was added to virus suspensions to give a final concentration of 0.013% and the PH adjusted to 7.1. The mixture was shaken vigorously and held in a water bath at 37°C. Samples were neutralized with 12% sodium disulfate and when tested for residual virus were first dialysed overnight against distilled water in the cold.

Ultraviolet Light. Two ml volumes of virus suspension in 9 cm petri dishes were inactivated with continuous shaking at a distance of 9 cm from a 30 W germicidal lamp.*

Safety Tests

All inactivated virus preparations were checked for residual virus and innocuity. The former was carried out in Vero cell cultures and innocuity in 2

* Philips, Holland.

susceptible calves, one receiving 25 ml subcutaneously and the other 25 ml intramuscularly. The inoculated calves were observed closely for 4 weeks.

Adjuvants

Aluminium Hydroxide. Aluminium gel was prepared by the technique described by Rafyi & Mirchamsy (1956). The final product was adjusted to pH 6.6 and autoclaved for 45 min at 121°C. An equal volume of inactivated virus suspension was added slowly to the aluminium gel which was continuously stirred.

Freund's Incomplete Adjuvant. A mixture containing 8.5 parts of mineral oil and 1.5 parts of mannide mono-oleate (Arlacel A) was sterilized at 110°C. for 20 min and emulsified with inactivated virus suspension to give a final concentration of 10%. The emulsion was shaken vigorously before use.

Saponin. A solution containing 56 mg of purified saponin (BDH) per ml of distilled water was prepared and the pH adjusted to 7.5. The solution was clarified by Seitz filtration and autoclaved at 108°C for 45 min. It was mixed with inactivated virus suspension to give a final concentration of 0.25 mg per ml.

Cattle

Indigenous cattle were purchased in a mountainous area in north Iran where rinderpest has not been known for 35 years. They ranged in age from 1.5 to 3 years. They were screened for rinderpest neutralizing antibodies and all were negative.

Virus Neutralization Test

The detection of neutralizing antibodies in sera was carried out in Vero cell cultures using the serum dilution–virus constant method described by Yamanouchi *et al.* (1969). The antigen used was strain KOA. Neutralizing antibody (NA) titres were expressed as the log 10 of the reciprocal of the serum dilution which protected 50% of the Vero cell cultures.

RESULTS

Inactivation of Virus

Heat. The rate of inactivation of heat-inactivated virus was I log per day. No residual virus was detected after 5 days exposure to 37°C.

Merthiolate. Samples of merthiolate-treated virus suspensions were

still virulent for calves after 5 days exposure to 37°C. Samples held at 37°C for 8 days were, however, inert.

Formaldehyde. No residual virus was detected in samples treated with 0.013% formaldehyde and held for 48 h at 37°C.

Ultraviolet Light. Virus was completely inactivated after 90 sec exposure to uv.

Vaccine Trials

Attenuated Live Virus Vaccine. The mean antibody titre of 12 cattle inoculated with the live KOA strain declined from a titre of 2.47 at one month after vaccination to a titre of 1.35 twenty-four months later. The line of best fit was linear and significant ($Y = 2.50–0.04\,X$; $r = 0.979^{**}$). All 12 cattle withstood challenge 24 months after vaccination.

Merthiolate- Treated Vaccine. All preparations of merthiolate-treated virus induced antibodies in cattle within one month of vaccination except that incorporated with saponin. The highest titres were induced by merthiolate-treated RP2 combined with Freund's incomplete adjuvant and lowest titres by merthiolate-treated RP2 virus without adjuvant (Fig. 1). The antibody decay slopes in cattle vaccinated with preparations incorporating adjuvants other than saponin were linear and similar to that induced by the live KOA strain although their position differed significantly (Table 1). The rate of antibody decay in cattle vaccinated with merthiolate-treated RP2 virus without adjuvants was significantly faster and no antibody was detected 12 months after vaccination.

Local reactions were limited to swellings at the sites of vaccination which persisted for longer in animals receiving preparations containing Freund's incomplete adjuvant. No purulent lesions were found when the cattle were slaughtered after challenge.

Half the cattle were challenged 2 years after vaccination. The animals vaccinated with preparations incorporating adjuvants other than saponin were found to be immune. Cattle receiving the saponin-containing preparation or merthiolate-treated virus without adjuvants developed clinical rinderpest and rinderpest virus was recovered from their lymph nodes.

Formaldehyde-Treated Vaccine. Preparations of formaldehyde-treated virus induced antibodies in cattle within one month of vaccination except that incorporated with saponin (Fig. 2). The antibody decay slope induced by formaldehyde-treated virus combined with aluminium hydroxide was linear and similar to that induced by live KOA but the position was lower (Table 1). The rate of antibody decay in cattle vaccinated with formaldehyde-treated virus without adjuvants was significantly faster and no antibody was detected 18 months after vaccination.

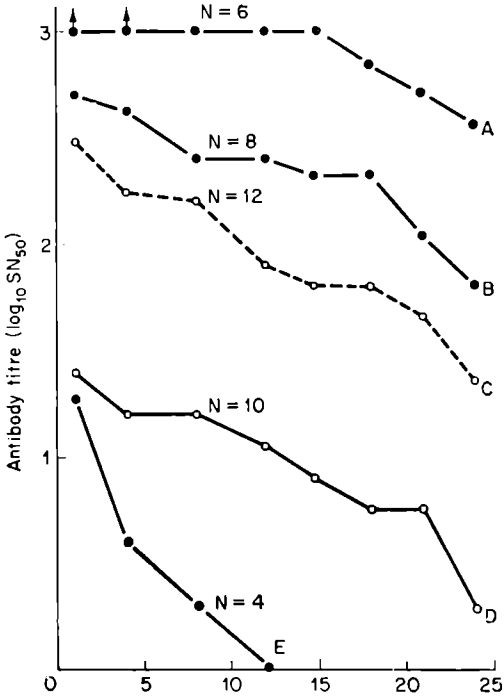

Fig. I. Mean antibody titres of cattle inoculated with merthiolate-treated virus preparation.
A. Merthiolate-treated RP2 virus with Freund's incomplete adjuvant
B. Merthiolate-treated RP2 virus with aluminium hydroxide
C. Untreated live KOA virus
D. Merthiolate-treated KOA virus with aluminium hydroxide
E. Merthiolate-treated RP2 virus without adjuvants.

TABLE I

REGRESSION EQUATIONS FOR MEAN NEUTRALIZING ANTIBODY TITRES ON MONTHS AFTER VACCINATION IN CATTLE IMMUNIZED WITH INACTIVATED VIRUS COMPARED WITH THE REGRESSION EQUATION FOR ANTIBODIES IN CATTLE VACCINATED WITH ATTENUATED VIRUS

Source strain	Inactivating agent	Adjuvant	Variance ratios Between slopes	Between constants
RP2	Merthiolate	Nil	21·54**	—
RP2	,,	Incomplete Freund's	5·08	591·31**
RP2	,,	Aluminium hydroxide	3·33	75·43**
RP2	,,	Saponin	—	—
KOA	,,	Aluminium hydroxide	2·07	649·14**
RP2	Formaldehyde	Nil	31·89**	—
RP2	,,	Saponin	—	—
KOA	,,	Aluminium hydroxide	2·04	30·00**
RP2	UV light	Nil	25·76**	—
RP2	,,	Saponin	—	—
KOA	,,	Aluminium hydroxide	4·20	83·40**

** $P < 0.01$

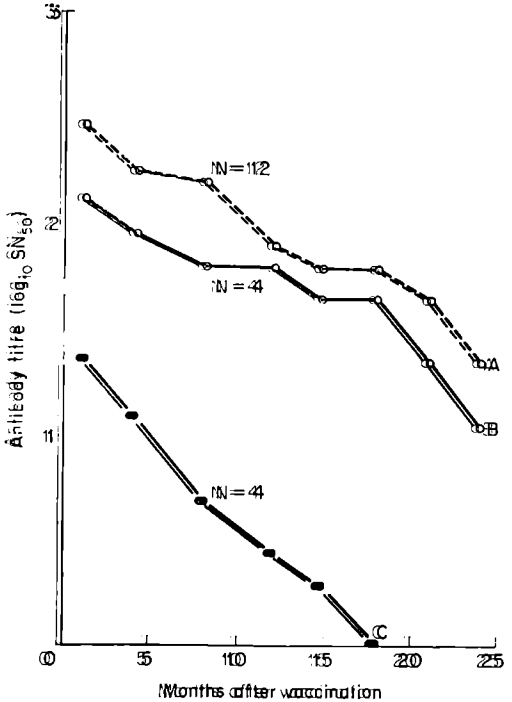

Fig. 2. Mean antibody titres of cattle inoculated with formaldehyde-treated virus preparations.
A. Untreated live KOA virus
B. Formaldehyde-treated KOA virus with aluminium hydroxide
C. Formaldehyde-treated RP2 virus without adjuvants.

When challenged 2 years after vaccination, the cattle inoculated with formaldehyde-treated virus combined with aluminium hydroxide proved to be immune. The cattle given formaldehyde-treated virus without adjuvants developed mild pyrexias after challenge and recovered. The animals that were injected with saponin mixtures developed clinical rinderpest and virus was recovered from their lymph nodes.

UV-Treated Vaccine. Preparations of uv-treated virus incorporated with saponin failed to stimulate the production of antibodies in cattle;; the cattle proved to be susceptible to rinderpest when challenged 2 years later. The other preparations induced the formation of antibodies ((Fig. 3)) and the cattle were found to be immune when challenged 2 years later.

The antibody decay slope induced by uv-treated virus combined with aluminium hydroxide was linear and similar to that induced by live KOA but the

position was higher (Table 1). The rate of antibody decay in cattle vaccinated with uv-treated virus without adjuvants was significantly faster and no antibody was detected 24 months after vaccination. The cattle, nevertheless, resisted challenge.

Dose Response

Doses of merthiolate-treated RP2 virus with aluminium hydroxide ranging from 1.0 ml to 10.0 ml were injected into groups of 3 to 12 cattles. All animals developed high antibody titres within one month of vaccination and there was no relationship between dose and mean antibody titre. The rates of antibody decay in cattle vaccinated with 2.5, 5.0 and 10.0 ml doses were similar and were significantly slower than the rate of antibody decay in cattle receiving a dose of 1.0 ml (Fig. 4).

Storage Life

Merthiolate-inactivated RP2 virus adsorbed on aluminium hydroxide

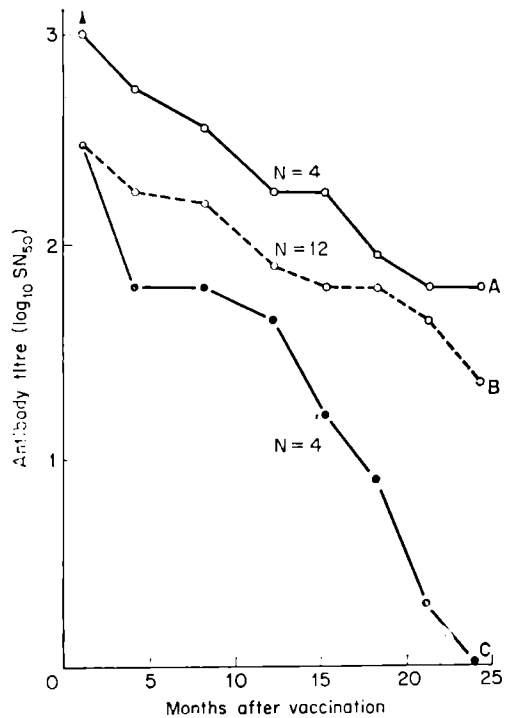

Fig. 3. Mean antibody titres of cattle inoculated with UV-treated virus preparation.
A. UV-treated KOA virus with aluminium hydroxide
B. Untreated live KOA virus
C. UV-treated RP2 virus without adjuvants.

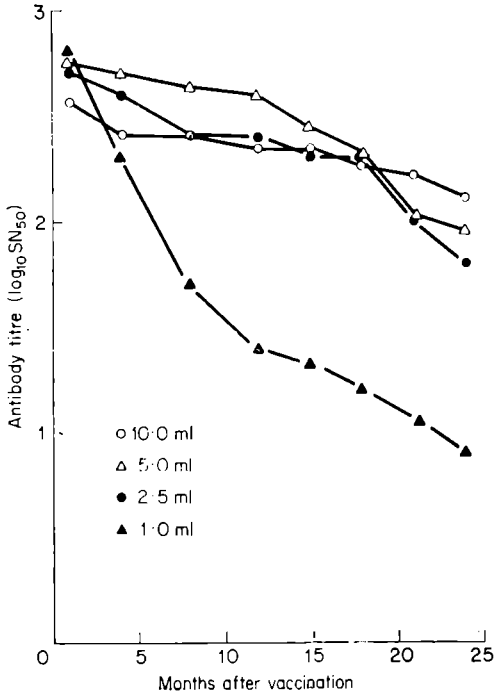

Fig. 4. Mean antibody titres of cattle inoculated with different doses of merthiolate-treated virus incorporated with aluminium hydroxide.

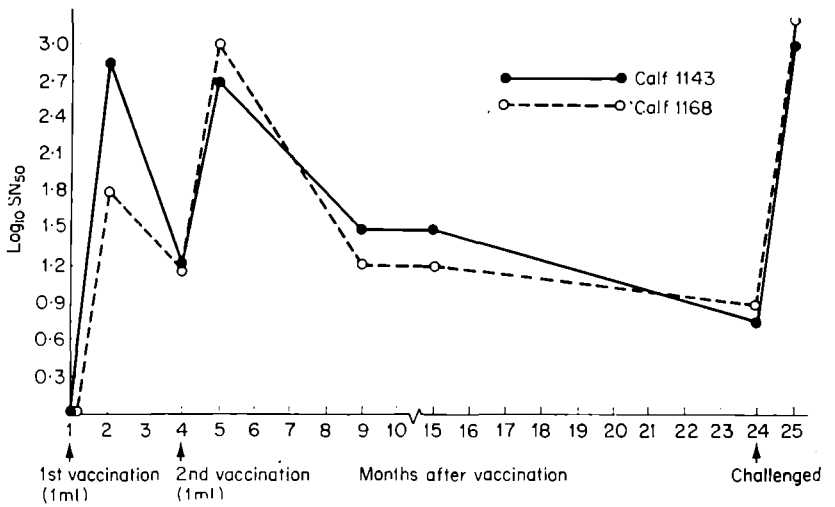

Fig. 5. The protective value of merthiolate inactivated rinderpest vaccine after exposure to 30°C for one month.

was stored for 30 days at ambient temperature (30°C). Two cattles were injected with I ml of the stored vaccine and both developed antibody within one month of vaccination (Fig. 5). A booster dose of I ml of the same vaccine was injected 3 months later and induced an anamnestic antibody response. The antibodies declined slowly and were still present 24 months after primary vaccination. When the cattle were challenged they again responded with an anamnestic rise of antibodies but they did not exhibit any clinical signs of rinderpest.

DISCUSSION

Inactivated rinderpest virus combined with adjuvants other than saponin induced active immunity in rinderpest susceptible cattle which was durable for at least 24 months. Saponin apparently destroyed the antigenicity of inactivated virus preparations. The virulence of the virus used in the preparation of inactivated vaccine was not apparently relevant. Differences were, however, associated with the method of inactivation; uv-treated virus adsorbed onto aluminium hydroxide induced higher antibody levels than similar preparations inactivated with merthiolate or formaldehyde. The differences were even more marked when adjuvants were not used, antibodies induced by uv-treated virus persisting for 21 months whereas antibodies induced by formaldehyde-treated virus lasted for 15 months and those induced by merthiolate-treated virus for 8 months.

Differences were also attributable to the type of adjuvant; inactivated preparations incorporating Freund's incomplete adjuvant induced the highest levels of neutralizing antibodies, significantly higher than those induced by attenuated live virus.

The dose of inactivated vaccine was only critical when the dose was small.

Merthiolate-inactivated virus adsorbed onto aluminium hydroxide was remarkably stable at ambient temperatures and, in this respect, it is superior to attenuated live virus vaccines which have to be stored and transported in the cold (Johnson & Smith, 1962). Finally, rinderpest virus was not recovered from the lymph nodes of cattle challenged 2 years after vaccination with inactivated virus preparations containing adjuvants other than saponin.

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