STUDIES ON ANTIGENIC IMPROVEMENT OF THE EXISTING F.M.D.CELL CULTURE GEL VACCINE.

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CERTIFICATE

Certified that the research work embodied in the thesis entitled "Studies on antigenic improvement of the existing cell culture gel vaccine" was carried out by Shri Ravindra Nath Sharma under my guidance and supervision during the academic session 1968-69.

(N.S. Datt)

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I TRODUCTION

INTRODUCTION

Since the dawn of evolution, man has been making use of animal kingdom for personal benefits. For the subsistance of human life animals are providers of food, clothing and transport etc. In view of the benefits, it is natural that their welfare should receive utmost attention. The health hazards owing to foot and mouth disease are very serious in view of its wide distribution, rapid spread and its damaging effects on productivity.

In India, where cattle development programmes are being carried out on an intensive basis, the loss due to foot and mouth disease is indeed expected to be very high and so the immediate need for control measures. Again in control of this disease, selective vaccination appears to be the only solution.

A dig into the scientific literature reveals that from time to time workers have tried to evolve suitable vaccines. In thirties cattle were immunized with glycerine, colloidal silver and formalin treated infected blood (Bevan, 1933; Brunswick, 1934; Lamikhov, 1952). In 1957, a concentrated chloroform inactivated vaccine was advocated by Roher to immunize the livestock. Frankel (1950) cultivated the virus on explants of bovine tongue epithelium and prepared aluminium hydroxide adsorbed vaccine. Later on, Epsinet (1956), Rivenson (1956), Mackowiak et al. (1959), Bengelsdorff et al. (1964) and lately Andreev et al. (1968) used saponin as an adjuvant for the preparation of this vaccine and observed high grade of immunity using comparatively lesser dose.

In India, goat kidney cell-cultured aluminium hydroxide adsorbed, inactivated vaccine has been developed, which has given

encouraging results under field trials. However, efforts are necessary to improve upon this vaccine, in order to better its immunogenic power.

Further the dose of this polyvalent vaccine appears unwieldy. It is evident from the work done abroad that if saponin is incorporated in the preparation of vaccine, immunogenicity can be increased, while the dose can be reduced at the same time. Thus, if the antigenicity of the cell-cultured (goat kidney) gel vaccine could be improved through the incorporation of adjuvant "Saponin" there was some possibility that even if its dose could not be reduced the improved antigenicity of the vaccine would be able to give a better quantitative advantage to the vaccine to deal with the heterologous field strains. This in itself would mean a step forward in the ultimate control of this disease.

With this objective in view, comparative studies were began with the saponified gel vaccine. Different types of monovalent saponin incorporated vaccines containing varying concentrations of saponin were prepared and for comparative study conventional monovalent gel vaccines of each type provided by the Foot and Mouth Disease Laboratory were included.

The present study was thus involved in the assessment of the quality of the saponified vaccines vis-a-vis the conventional vaccines.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

From time to time workers have been developing vaccines against Foot and Mouth Disease and, therefore, sufficient literature is available on the use of different methods for the preparation of vaccines and their standardization.

Inactivated Vaccines

Galloway (1931) reported satisfactory immunity in guinea-pigs with phenol and bile inactivated vaccines.

Galea (1932) inactivated the virus with chloroform and observed the protection in immunized guinea-pigs upto 172 days.

The infected blood after treating with glycerine (Bevan, 1933), colloidal silver (Brunswick, 1934), formalin (Lamikhov, 1952) had been used for the immunization of animals. The immunity with glycerinated blood was reported upto 14 months and the formalized blood protected 75 per cent of animals.

Graub *et al.* (1939) prepared heat inactivated vaccine from infected blood collected at the height of febrile reaction.

Roher (1957) prepared a concentrated foot and mouth disease vaccine with vesicular epithelium from infected cattle, inactivated with chloroform and used it for the vaccination of one million cattle, in East Germany in the year 1956. He suggested the possibility of further concentration of vaccine by acetone treatment.

Crystal violet vaccines

De-Blieck *et al.* (1942) recorded immunity upto 82 days in guinea-pigs with crystal violet inactivated vaccine. Viera (1944)

vaccinated cattle with infected blood treated with crystal violet. Datta (1951) has reported on the superiority of crystal violet inactivated vaccine over Waldmann's aluminium hydroxide gel adsorbed formalized vaccine 1938.

In India, Dhanda et al. (1954) prepared vaccine using 2½ per cent tongue epithelium, vesicular fluid and virulent blood using the crystal violet in a concentration of 0.03 per cent. The same authors in 1957, modified the procedure for preparing concentrated vaccine containing 15 per cent suspension of vesicular epithelium of cattle tongue, equal amount of blood and buffer phosphate to reduce the dose of the vaccine.

Aluminium hydroxide gel vaccines

Schmidt-Jensen et al. (1936) observed only primary lesions in the challenged guinea-pigs earlier vaccinated with aluminium hydroxide adsorbed vaccine, prepared from foot pads epithelium of infected guinea-pigs.

Schmidt and Hansen (1936) experienced better results, when the gel was incorporated in high concentration in the vaccine.

Schmidt et al. (1936) reported that formalized aluminium hydroxide gel vaccine was better than the formalized vaccine alone. Schmidt (1936) used trivalent formalized adsorbed vaccine and reported a highest degree of immunity against type A and C and lowest against type O.

Waldmann et al. (1937) prepared a formalized (0.15%) vaccine from infected guinea-pig pads and tested this vaccine in guinea-pigs and cattle employing intraperitoneal (i/p) and subcutaneous (s/c)

routes of inoculation respectively. They observed maximum antibody titres at 13 - 17 days post-vaccination.

Waldmann (1933) used for the first time the infected cattle tongue epithelium for the preparation of formalized aluminium hydroxide adsorbed vaccine.

Peterson (1940) reviewed the step wise development of adsorbed and formalized vaccines as follows:

"Willstatters researches on adsorbing substances showed adsorbent value of aluminium hydroxide for enzymes. Schmidt of Copenhagen (1934) advocated the use of aluminium hydroxide as adsorbing agent for the virus. In next few years, Danish workers reported protection in guinea-pigs with an inactivated adsorbed vaccine."

Rushmore (1945) modified the method of Waldmann and prepared vaccine from the virus collected from artificially infected cattle tongue.

The principal limitation in the preparation of foot and mouth disease vaccine was the source of virus. The Schmidt-Waldmann type of vaccine was prepared from the virus taken from the vesicular lesions of the tongue of cattle which were inoculated with the virus intradermolingually (i/dl) for obtaining a large harvest of the virus.

Skinner et al. (1952) used infected mouse tissue for the preparation of formalized adsorbed vaccine.

Waldmann et al. (1955) and Waldmann and Zimmermann (1955) reported that the adsorbed vaccine from the organ suspensions of artificially infected newly born calves were cheaper than the vaccine prepared from cattle tongue grown virus. They obtained satisfactory results with the former vaccines.

Tissue cultured vaccines

Toussieng (1936) prepared aluminium hydroxide gel vaccine from the virus grown in guinea-pigs by Frankel's method.

The foot and mouth disease virus grown in bovine embryo skin and mixed with hyperimmune serum was used for the immunization of animals by intradermal (i/d) inoculation of tongue by Frankel and Vanwavern (1937). After 24 hours of challenge they observed primary lesions in vaccinated animals, while controls reacted severely.

Fogedby (1940a, 1940b) favoured the use of cultured virus for the preparation of vaccine. He cultivated the virus on calf foetal skin epithelium suspended in Tyrode's solution buffered with bicarbonate. When the titre of virus reached 10^{-3} to 10^{-4} after an incubation period of 40 hours, he used the cultured virus for the production of aluminium hydroxide vaccine using heat or formalin as inactivating agents.

Fogedby and Keofoed (1940) carried out studies with Danish aluminium hydroxide gel vaccine in guinea-pigs with different dose levels and concentrations and experienced that lower dose level to be much better than higher dose for higher degree of immunity.

Fogedby and Keofoed (1942) prepared the formalized gel

vaccine with the virus cultivated on calf embryo skin and lung tissue cultures.

Menzani (1944) prepared inactivated vaccine from the filterate of vesicular epithelium by shaking with bentonite gel by incubating the mixture for 48 hours at 28°C. He obtained a high degree of immunity in cattle on vaccination in a dose of 20 ml. by subcutaneous (s/c) route. In the year 1947, he prepared heat and formalin inactivated aluminium hydrosilicate gel adsorbed vaccine and reported better results in comparison to Waldmann and Kobe vaccines.

Hansen et al. (1949) used ultra-violet rays for adsorbed virus as inactivating agent and reported the vaccine to be safe and effective for guinea-pigs.

Frankel (1950) cultivated the foot and mouth disease virus on explants of bovine tongue epithelium for the production of aluminium hydroxide gel adsorbed vaccine.

Fogedby (1952) did not find any difference in the immunogenicity between Schmidt-Waldmann type and formalized aluminium hydroxide adsorbed vaccines.

Frankel (1953) advocated the use of imported bovine tongues from the countries free from foot and mouth disease for virus propagation.

Girard et al. (1952-53) reported the results of two experiments on cattle vaccinated with trivalent vaccines. In their first experiment, out of 17 cattle immunized with trivalent vaccine

(O_2, A_5 and C_1), there was one breakdown to type O and one to type C. In next experiment there was 3 per cent failure, where a foreign vaccine was used.

Michelsen (1953) studied the tetravalent vaccine and could not get satisfactory results.

Pyl (1952, 1953 and 1954) described the method of purification and concentration of virus for the production of an efficient foot and mouth disease vaccine. He reported that the dose can be reduced by 1/5th for cattle and swine and noted the production of immunity within 3 days post-vaccination in cattle. The author used the vaccine in dose as low as 0.6 ml. with sufficient immunity and stated that the dose of 3 ml. contained about 130 mgm. of antigen.

Rosenbusch (1953) also explored the possibility of use of cattle tongue epithelium for the cultivation of virus and the vaccine prepared from which found as effective as from natural virus.

Pyl and Heinig (1955) observed the aluminium salicylate vaccine with similar protective effect as aluminium hydroxide gel vaccine.

Geiger and Otto (1958) prepared five trivalent vaccines containing virus strains, viz., O, Ag and C (Waldmann) from bovine or porcine origin. None of the vaccines gave satisfactory immunity on challenge at 10 and 30 days. In 1959, Geiger claimed satisfactory immunity with Pyl's concentrated trivalent vaccine, if used in a dose of 5 ml.

Armbruster et al. (1960) attempted vaccination of adult pigs with concentrated aluminium hydroxide or salicylate adsorbed

vaccines, inactivated with formalin or hydroxylamine hydrochloride and in oil emulsion adjuvant, but none of these vaccines could give satisfactory results.

Frederick (1961) prepared foot and mouth disease aluminium hydroxide gel adsorbed formalized vaccine from the virus grown on kidney monolayers and reported good results in the field trials in cattle, sheep, goats and pigs.

Michelsen (1961) tried both swine adapted foot and mouth disease virus tissue culture vaccine and conventional bovine origin vaccine, but did not find any satisfactory results in pigs. He tried "tween" like agent used as emulsifier and claimed some protection in pigs. Intracutaneous (i/cut.) route gave somewhat satisfactory results than the subcutaneous route of vaccination.

Caproale et al. (1963) prepared trivalent vaccines from virus propogated in calf kidney cells. These vaccines were formalized, adsorbed; purified concentrated formalized adsorbed and B.Y.A inactivated adsorbed. The authors found the first vaccine to be efficacious but not completely innocuous, while the second one was neither harmless nor immunogenic, but the third one efficacious and innocuous.

Ubertini et al. (1964) prepared type O and C vaccines with virus cultivated on monolayers of calf kidney cells in rotating flasks and another batch of vaccine against type O by propagating the virus on tongue epithelium by Frankel's method. They immunized pigs with 3 or 16 times the dose of cattle and reported immunity to contact infection. Later on they recorded 96 per cent protection

against type O and 83 per cent protection against type C with saponin incorporated vaccine prepared by Pyl's method with virus grown on calf kidney monolayers.

In the year 1964-65, for the production of foot and mouth disease vaccine at the Indian Veterinary Research Institute, Mukteswar, a Cell Culture Unit was established. In 1966, 1967 and 1969, cell-cultured polyvalent vaccine (against O, A, C and Asia 1) was prepared at this Institute on an experimental basis. The virus for the vaccine was cultivated in goat kidney monolayers and adsorbed on aluminium hydroxide gel, inactivated with formalin at 25°C for 43 hours. It was tested in different organized as well as Government farms of the country so as to assess its effectiveness as a protective tool for the imported, cross-bred and other valuable stock of cattle. A prolonged and systematic vaccination programme justified that no untoward post-vaccination reaction was observed (Datt et al., 1966, 1967 and 1968).

Morrow et al. (1966) modified the Frankel's method of virus cultivation by cultivating the virus on mechanically stirred tongue epithelium suspended in Balanced Salt Solution (B.S.S.) containing Lactalbumin hydrolysate and antibiotics. They prepared formalin inactivated aluminium hydroxide vaccine with the virus. The vaccine was reported to have played an important role in combating foot and mouth disease epizootics in the South East Africa and the Middle East.

Saponin vaccines

Epsinet (1956) immunized cattle with saponin vaccine by intradermal and subcutaneous routes with 0.5 ml. to 1.0 ml. doses

respectively. He found that the saponin vaccine to be superior to the alum adsorbed vaccine. The duration of immunity was the same irrespective of the routes of vaccination employed.

Enrique-Palma (1956) stated that vaccine prepared from epithelial tissue to be much superior antigenically. He did the comparative study with various co-adjuvants and claimed better results with saponin and carboxymethyl cellulose. Saponin added vaccine was found to be better in stimulating the production of complement fixing antibodies in guinea-pigs.

Rivensen (1956) also reported the superiority of saponin over the aluminium hydroxide for the production of immunity.

Jivion et al. (1962) prepared concentrated monovalent and bivalent saponin vaccines without any change in safety and efficacy, when the concentration and period of inactivation with formalin was reduced.

Mackowiak et al. (1959) observed a rapid rise of antibody titre in cattle vaccinated with a concentrated trivalent vaccine containing 0.5 per cent saponin. The immunity engendered with this vaccine was more lasting than the aluminium hydroxide vaccine, while the duration of immunity was observed by Olechnowits et al. (1962) as slightly less than adsorbed vaccine.

Schneider et al. (1963) prepared saponin vaccine with type C strain (cell-cultured virus) and vaccinated two groups of 21 and 14 pigs with 5.0 ml. and 3.0 ml. dose respectively. They observed 19 pigs completely immune and two partially in the former group and in the latter group 10 pigs completely, 3 pigs partially immune and one not immune.

Van Bekkum (1963) reported satisfactory protection in pigs with saponin aluminium hydroxide adsorbed formalized vaccine in comparison to conventional aluminium hydroxide gel vaccine.

Bengelsdorff et al. (1964) made observations with adsorbed vaccine prepared with type C virus propagated in calf-kidney or in permanent BHK_{21} cell-lines containing 0.3 per cent purified saponin. Two groups of 51 pigs and 14 pigs were vaccinated with 5.0 ml. and 3.0 ml. of vaccine respectively by subcutaneous route. Only one pig was reported to be susceptible to contact infection in each group. In another experiment with trivalent vaccine prepared with type O virus from cattle lesions and type A and C from tissue culture with saponin in 0.3 per cent concentration of the 6 immunized pigs, one pig was found susceptible after 35 days of vaccination.

Girard et al. (1964) vaccinated pigs with Asia 1 formol inactivated saponin vaccine containing 300 mgm. of virulent epithelium, 25 mgm. saponin diluted in 0.25 ml. phosphate buffer and 0.05 ml. glycocoll-buffer. They reported that 30 per cent of animals resisted the challenge with $_{50}ID_{50}$ of bovine virus.

Schneider et al. (1964) immunized more than 100 cattle with mono, bi, and trivalent (O, A and C) gel adsorbed vaccine containing 0.3 per cent saponin in a dose of 5.0 ml. In trivalent vaccine the virus type O was incorporated from cattle tongue. The immunity was satisfactory even after 6 months, except against type O in cattle, vaccinated with trivalent vaccine. The authors recorded highest serum neutralizing titres after 14 days of vaccination. There was a fall in titres after 8 weeks in the case of monovalent vaccine then gradually in the case of trivalent vaccine.

Throughout the 26 weeks of their observation, the titres against type O was the lowest than against type A and C.

Strobbe et al. (1964) reduced the concentration of aluminium hydroxide gel and added 0.05 per cent saponin in a trivalent vaccine to reduce the dose from 20 ml. to 10 ml. The authors found that saponin did not have any effect on the infectivity of virus titre, milk production, and keeping quality of the vaccine.

Comparative studies with adsorbed and saponin vaccines were carried out in pigs and cattle by Heinig et al. (1965) with satisfactory results in cattle on challenge.

Nathans (1965) immunised pigs with a concentrated type C gel adsorbed saponin vaccine and reported 35.6 per cent animals to be immune to contact infection after 4 weeks but the immunity was temporary. He further observed passive immunity in piglets from vaccinated sows. He emphasised the use of chloroform in extracting out most of the protein the the Frankel's culture for improving the quality of vaccine. The author also concluded that the vaccination of young pigs was of no use due to the presence of maternal antibodies in them upto the age of 19 weeks and advocated the usefulness of vaccine in preventing outbreaks.

Santucci et al. (1965) carried out studies on inactivated saponin vaccine in cattle and considered its immunogenicity to correspond to those of O, A and C vaccines prepared in Europe.

Wisniewski (1965) prepared two batches of trivalent saponin vaccines and immunized 13 cattle in varying doses of 1.2 ml., 4 ml. and 13 ml. Serum samples were tested for neutralizing antibodies

on 3rd, 7th, 14th and 21st day post-vaccination. He noted significant titres on 3rd and highest titres on 21st day in 5 out of 9 cattle. There was no significance difference in antibody titres with the size of dose.

Gagliardi et al. (1966) studied the serum neutralizing antibodies in pigs vaccinated with saponin bentonite gel adsorbed vaccines and recorded the neutralizing titres of $10^{1.04}$/ ml. with O_{63} antigen, while little or no response in similar quantity with type C antigen.

Muntiu et al. (1966) observed that formolised saponin vaccine prepared from type O_2 virus passaged in cattle was unsatisfactory in pigs in doses as large as 32 times of cattle while the vaccine from the virus strain of the same type isolated and passaged in pigs containing less concentration of formol was suitable to immunize 50 - 70 per cent pigs. The second dose of this vaccine after 21 days, immunized 100 per cent population with the immunity of short duration.

Jivoin et al. (1966, 1967) prepared an alum hydroxide adsorbed saponin formol inactivated vaccine to control the epidemic of foot and mouth disease in Roumania during 1963 - 1960. They immunized 3 million cattle and over 2 million sheep, goats and pigs. The authors recorded the breakdown in immunity in only 21 cattle after 3 - 4 months of vaccination. The immunity in pigs was reported to be weaker and of shorter duration but adequate enough to protect against natural infection.

Florent (1967) propogated the virus on tongue epithelium,

bovine foetal lung tissue and bovine kidney cells for the production of vaccines. The addition of saponin was found to improve the vaccine. The author reported that saponin was tolerated better by pigs than cattle. He suggested the replacement of old formalin vaccine by saponin vaccine as it engendered better and lasting immunity for at least 8 months in cattle.

Bayramoglu et al. (1967) carried out studies with vaccines containing 25 mgm. and 10 mgm. of saponin per dose of vaccine and reported no change in potency of vaccine in both the concentration of saponin.

Andreev et al. (1968) made a comparative study with formolized alum adsorbed saponin vaccine(15 mgm. saponin per dose) prepared from lapinized virus and standard alum hydroxide formol vaccine in two groups of cattle. They reported 100 per cent and 71 per cent protection with saponin and standard vaccines respectively on challenge with virulent virus after 20 - 30 days of immunization. They also observed higher virus neutralizing antibody titres with saponin vaccine than the standard vaccine.

Cattle were immunized with type C and O monovalent saponified vaccines prepared from virus grown in calf kidney cell monolayers in 5 ml. and 10 ml. doses. The rise in protecting and neutralizing antibodies was observed upto 3 months but a gradual fall after 5 months.

Potency testing of vaccines

(a) Method of potency testing in naturally susceptible animals:

For the potency test of vaccine in which cattle are to be

used, the method of challenge and interval between vaccination and challenge are important. Three methods have been used, i.e., the contact method of Rossi (1952), of rubbing the virulent virus on tongue by Waldmann et al. (1941) and intradermolingual inoculation with titrated virus by Willems (1953).

Hecke (1961) reported the rubbing of the virus on tongue was advantageous because both cellular and humoral immunity could be determined. In different laboratories the interval between the vaccination and challenge has ranged from 3 - 4 weeks. Mackowiak et al. (1959) and Van Bekkum (1966).

In many countries the method as suggested by the expert committee in Berne in 1947 is adopted (Willems, 1953 and 1966). In this method animals are vaccinated with fixed dose of vaccine and are observed for certain periods before challenge intradermolingually with 10,000 ID_{50} virulent virus along with fully susceptible controls. If none of the vaccinated animals showed generalization, while controls showed both primary and secondary lesions, the vaccine is considered to be of a requisite quality (Gilbert et al., 1964; Rafyi et al., 1962; Willems, 1953).

In France and in some South American countries, the method of potency testing suggested by Lucam and Fedida (1953 and 1960) and Henderson et al. (1962) is preferred. Protection 'K' index in cattle is determined. In this method, where protection 'K' index is determined eight cattle are used. Four animals for virus titration in unvaccinated group and other four for virus titration after vaccination and protection 'K' index is calculated.

Henderson and Galloway (1953) evolved a suitable and more accurate method in which a number of cattle are vaccinated with varying number of doses of vaccine. The vaccinated animals are challenged intradermolingually with 10^{-4} ID_{50} of virulent virus and the protective dose of the vaccine against generalization is calculated. The method is very expensive and requires 24 animals for testing each batch of vaccine (Willems, 1966; Leunen et al., 1960). In France a modified method has been tried where 50 per cent protective dose in guinea-pigs is determined (Terre et al., 1965; Mackowiak et al., 1966).

For testing the potency of the vaccines, attempts have been made to use sheep and swine (Poul et al., 1964; Lucam et al., 1964; Ubertini et al., 1964; Gilbert, 1966; Cardassis et al., 1966; Fontaine et al., 1966). These authors reported the difficulties in using above animals as they did not show uniform reaction post-infection. To overcome this difficulty of uncertain disease picture in sheep, Fontaine et al. (1966) and Oral et al. (1968) challenged the animals and detected the viraemia and claimed satisfactory results

(b) Methods of potency testing in laboratory animals:

Various laboratory animals are used for the potency testing. They are less expensive and easily handled. Mice and guinea-pigs have been used for the purpose. Now French workers pass their 2/3 of the vaccines by testing only in guinea-pigs (Lucam et al., 1959; Lucam et al., 1958; Terre et al., 1965; Mackowiak et al., 1966)

Potency testing methods in guinea-pigs can be grouped in two:-

(1) Qualitative method - not being used anywhere (Palmer,

1937; Kindiakov et al., 1939a and 1939b).

(2) Quantitative method - with following two procedures:

(i) Protection 'C' index procedure - in this method titration of virus is done in vaccinated as well as control animals and protection 'C' index is calculated (Rivenson, 1960 and 1962; Lucas et al., 1963, 1964 and 1966).

(ii) DV_{50} method - in this, guinea-pigs are vaccinated with various dilutions of the vaccine intramuscularly. After a certain period, they are challenged intradermoplanterally by 10^{-4} DV_{50} and the vaccinating dose (DV_{50}) is calculated (Lucas et al., 1962; Fontaine et al., 1963-64 and 1964; Mackowiak, 1966; Olechnowitz et al., 1962; Terre et al., 1965; Ubertini et al., 1956).

The use of adult mice in potency testing of the vaccine had been extensively made (Uhlmann and Traub, 1953; De-Boer and Bachrach, 1961; Cunha, 1960; Shevetsov et al., 1969).

von Bulow (1962 and 1963) inoculated female mice with foot and mouth disease virus before conception. When the litters were born from these mothers, he utilized the litters for the susceptibility of foot and mouth disease virus and reported that with some modifications in the method of potency testing, these litters can be used.

(c) Methods of potency testing by studying antibody response in the vaccinated animals:

Potency testing of the vaccines is conducted either by observing the serum neutralizing antibodies in tissue culture

and/or in baby mice (Davies et al., 1963; Fellows, 1962; Petermann et al., 1961; Mackowiak et al., 1962; Ubertini et al., 1960; Lucam et al., 1962, 1964; Zavagli, 1960; Mackowiak et al., 1965; Martin, 1960; Martin et al., 1961; Marucci, 1958; Rivensen, 1960; Ubertini, 1966 and Ubertini et al., 1964).

Brocksby (1949) reported the use of guinea-pigs for serum neutralization test.

Marucci (1957 and 1958) and Fedida (1961) attempted the use of complement fixation test and protection test to detect post-vaccination antibodies but due to lower sensitivity of these tests did not achieve much success.

Several workers have seen good correlation between serum neutralizing titres and challenge results (Mackowiak et al., 1959; 1962; Lucam et al., 1962; Poul et al., 1964; Patty, 1965; Martin et al., 1961). They suggested on economical grounds certain batches of vaccines can be issued on the basis of high antibody titres in the sera of vaccinated animals.

(d) Combined method of potency test:

In France, the vaccine is firstly tested by serum neutralization test in tissue culture. If the vaccine batch does not pass this test then it is tested in guinea-pigs. Only in doubtful cases, the vaccine is tested in cattle (Mackowiak et al., 1966; Lucam et al., 1964, 1966). With these methods it was possible to issue 65 per cent of vaccines by testing in guinea-pigs, 15 per cent by serum neutralization test in tissue culture and only 20 per cent of the vaccines were tested in cattle.

MATERIALS AND METHODS

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MATERIALS

Virus Strains

All the four immunological types of foot and mouth disease virus strains, viz., O, A, C and Asia 1 since maintained in the foot and mouth disease laboratory in different species of animals were used.

For vaccine production cell-cultured adapted strains were made use of, while the strains maintained in cattle, guinea-pigs and mice were used for challenge purposes in the respective species of animals.

The Experimental Animals

The healthy animals like, sheep, goats, hill-bulls, rabbits, guinea-pigs and mice came from the Veterinary Section of the Institute

The animals used in the study were screened for the presence of neutralizing antibodies. The serum neutralization test was performed in cell-culture tubes with two dilutions of virus, viz., 1/50 and 1/500. Two culture tubes were infected with each serum-virus mixture. If there was observable cytopathogenic effect in all the four tubes the animals were taken as "clean", i.e., not having got observable immunity to any of the specific types of the virus due to some previous experience.

METHODS

Preparation of Monolayer

Goat kidney cortical monolayer (primary cell-cultures) were set up as per the technique employed in the foot and mouth disease laboratory at this Institute (I.V.R.I., Annual Reports, 1966-67, 1967-68).

Culture of Virus strains

The large culture flasks (Provitsky's) showing complete monolayers were removed from the incubator. These were washed with phosphate buffer saline (P.B.S.) and seeded separately with 25 - 30 ml. of cell-cultured virus suspension respectively. These were left at the bench for the virus to adsorb for a period of 15 - 30 minutes, during which period an occasional shake was given. After that maintenance medium (without serum) was added in 500 ml. quantity into each of the flask and transferred to the incubator (37°C.). These were examined for cytopathogenic effect (C.P.E.) and after about 18 hours, when almost all the cells were involved, the flasks were given a vigorous shake. The supernatant from the flasks was then harvested and stored at 4°C.

Clarification of virus

The virus harvested was filtered through clarifying sheet of seitz to remove the cellular debris.

Virus titration

The viruses so grown were titrated in clean hill-bulls

as described by Henderson (1949), in unweaned mice (Skinner, 1951) and goat kidney cell-culture tubes.

For titration in mice, unweaned mice (6-7 days of age) were inoculated intramuscularly with each dilution of virus in 0.05 ml. dose. These were watched for characteristic paralytic symptoms and resultant mortality. The titre of virus (ID_{50}) was calculated by the method of Reed and Muench (1938).

For titration in cell-cultures, three tubes of goat kidney monolayers were infected with each dilution of virus in 0.2 ml. dose, keeping control tubes seeded with phosphate buffer saline. After infection, 2.0 ml. of maintenance medium (with 2% serum) was replaced in each tube. The tubes were examined after 24 and 48 hours of infection for cytopathogenic effect. $TCID_{50}$ of virus was calculated by the above method.

Purity test

The virus material collected from the above inoculated animals and infected culture tubes, was tested for its purity and type specificity by complement fixation test as described by Brooksby (1952).

Preparation of Vaccines

During the present study monovalent vaccines against types O, A, C and Asia 1 were prepared.

For the preparation of vaccine the following ingredients were used:

Aluminium hydroxide gel (Alhydrogel):

This was taken from the foot and mouth disease laboratory, where it was imported from Denmark. It was sterilized by autoclaving at 15 lb. pressure for 30 minutes.

Phosphate buffer and glycocollbuffers were prepared as follows:

Phosphate buffer:

Disodium hydrogen phosphate Na_2HPO_4 (Anhydrous)	..	0.937 gm.
Sodium dihydrogen phosphate $NaH_2PO_4 2H_2O$	..	106.50 gms.
Distilled water	..	1000.0 ml.
pH	..	.. 7.6

Glycocoll buffer:

Sodium hydroxide	..	25.5 gms.
Sodium chloride	..	52.0 gms.
Glycine	..	73.0 gms.
Distilled water	..	414.0 ml.
pH	..	.. 9.5 to 10.5

Glycerine:

Analar grade glycerine was used.

Buffers and glycerine were sterilized by autoclaving at 15 lb. pressure for 30 minutes.

Formalin water:

It was prepared by mixing 6.0 ml. of 40 per cent formaldehyde solution into 94.0 ml. of sterilized distilled water.

Saponin solution:

It was prepared as a 10 per cent solution in phosphate buffer saline, centrifuged, adjusted pH to 7.4, filtered through E.K.S. Seitz pad and stored at 4°C.

Procedure for the preparation of vaccines

Vaccines were prepared with the virus having an infectivity of 10^{-5} to 10^{-6}/ml. in hill-bulls, 10^{-5} to 10^{-6}/0.05 ml. in unweaned mice and 10^{-6} to 10^{-7}/0.2 ml. in culture tubes.

Monovalent vaccines with each strain were prepared with the following composition:

Aluminium hydroxide gel	-	50 per cent
Virus suspension	-	40 per cent
Glycocoll buffer	-	1 per cent
Phosphate buffer	-	2 per cent
Glycerine	-	1 per cent
Formalin water	-	1 per cent

Saponin in variable amounts in the final concentration of 0.2 per cent and 0.3 per cent.

Distilled water to make - 100.

With sterile precautions, these ingredients were mixed and kept under constant stirring for a period of 48 hours at 25°C.

for ageing of the vaccine. After this period, the vaccines were bottled and stored at 4°C. pending tests.

In addition, certain modifications were made for the preparation of some batches of vaccines against type O and C strains, with the following composition:

(1) Vaccine with 40 per cent virus, without gel:

Virus suspension	- 40 per cent
Glycocoll buffer	- 1 per cent
Phosphate buffer	- 2 per cent
Glycerine	- 1 per cent
Formalin water	- 1 per cent
Saponin	- 0.3 per cent
Distilled water to make 100.	

(2) Vaccine with 55 per cent virus and 40 per cent gel:

Aluminium hydroxide gel	- 40 per cent
Virus suspension	- 55 per cent
Glycocoll buffer	- 1 per cent
Phosphate buffer	- 2 per cent
Glycerine	- 1 per cent
Formalin water	- 1 per cent
Saponin	- 0.3 per cent
Distilled water to make 100.	

(3) Vaccine with 55 per cent virus, 40 per cent gel and without saponin:

Aluminium hydroxide gel	- 40 per cent
Virus suspension	- 55 per cent

Glycocoll buffer - 1 per cent

Phosphate buffer - 2 per cent

Glycerine - 1 per cent

Formalin water - 1 per cent

Distilled water to make 100.

Standardization of vaccines

Sterility test : Bacterial sterility of vaccines was tested by seeding on blood agar, serum agar, plain agar, plain broth, glucose agar and Robertson's anaerobic media followed by incubation for 7 days at 37°C.

Safety test : Safety and innocuity of the vaccines were carried out in homologous species as well as in laboratory animals on the following lines and the animals were observed for a week following vaccination:

Species of animals	Number of animal used	Dose	Route
Rabbits	2	2.0 ml	s/c
Guinea-pigs	2	2.0 ml	s/c
Adult mice	6	0.5 ml	s/c
Cattle	2	18.0 ml 2.0 ml	s/c i/dl.
Sheep	2	3.0 ml 2.0 ml	s/c i/dl
Goats	2	3.0 ml 2.0 ml	s/c i/dl

Potency test in cattle, sheep and goats

Clean hill-bulls, sheep and goats were vaccinated with monovalent vaccines given as below:

Species of animals	Number of animals	Dose	Route	Site of inoculation
Hill-bulls	3	2.0 ml	s/c	Dewlap
Do.	3	5.0 ml	s/c	Do.
Do.	3	10.0 ml	s/c	Do.
Sheep	3	1.0 ml	s/c	Shoulder region
Do.	3	2.0 ml	s/c	Do.
Do.	3	5.0 ml	s/c	Do.
Goats	3	1.0 ml	s/c	Do.
Do.	3	2.0 ml	s/c	Do.
Do.	3	5.0 ml	s/c	Do.

Serum samples from vaccinated animals were collected at 7th, 14th and 21st day post-vaccination and stored at -20°C. without adding any preservative.

To determine the development of neutralizing antibodies in the vaccinated animals, sera from the animals vaccinated with each group of monovalent vaccine were pooled. Each sample of serum was diluted to 1:5 or 1:10 in phosphate buffer saline and inactivated at 56°C. for 30 minutes. Ten-fold serial dilutions of the respective virus types were made in P.B.S. ranging from 10^{-1} to 10^{-6}. Each virus dilution and a required dilution of homologous serum were mixed in equal quantity and incubated at room temperature for one hour.

Three tubes showing complete monolayers were inoculated using 0.4 ml. dose of each serum virus mixture. Simultaneously, three culture tubes were infected in 0.2 ml. dose with each virus dilution. Similarly three tubes each were included as P.B.S. and

media control. After the adsorption period of 20 - 30 minutes, each tube received 2.0 ml. of maintenance medium containing 2 per cent serum and replaced in incubator at 37°C. for 48 hours before reading results.

In the event of control tubes showing no cytopathogenic effect, other tubes were examined at 24 and 48 hours respectively for noting the cytopathogenic effect. The extent of C.P.E. was graded as follows:

100 per cent cytopathogenic effect - +++

60 per cent cytopathogenic effect - ++

30 per cent cytopathogenic effect - +

No cytopathogenic effect - ---

$TCID_{50}$ was calculated by the Reed and Muench formula (1938).

To calculate the log serum neutralization index, the virus titre in presence of serum was deducted from the virus titre without serum. The log S.N.I. was corrected to 1.0 ml. by multiplying the appropriate dilution factor of serum and virus used.

Challenge test

(i) Cattle : The vaccinated cattle against type A, C and Asia 1 vaccines were challenged after 3 weeks of vaccination intradermolingually with 10,000 ID_{50} virus/ml. along with three controls. These animals were observed for primary reaction and generalization or any rise of temperature for a period of 10 days.

(11) Sheep and goats : The vaccinated sheep and goats with 'Asia 1' and 'O' vaccines could not be challenged. The protective value of the vaccines was estimated by the development of neutralising antibodies at different intervals.

Potency test in adult mice

Two-fold serial dilution of the vaccine were made in P.B.S. from 1:2 to 1:16 and ten mice with each dilution were vaccinated subcutaneously each with 0.2 ml. dose.

These vaccinated mice were challenged 21 days post-vaccination with mice adapted virus strains. The challenge dose used was 10,000 ID_{50} by intramuscular route along with ten unvaccinated mice as control and were observed for a period of 10 days.

RESULTS

RESULTS

Titration of Tissue Culture Adapted Virus Strains and Virulent Virus Strains and Their Purity

The virus strains used for vaccine production and for the challenge test of the vaccinated animals were titrated and their purity tested by complement fixation test. The results are presented in Table I.

TABLE I

Showing Results of Titration of Virus Strains and Their Antigenic Purity

Type of virus strain	Purpose used for	No. of animal used	Titre/ ml.	Results of C.F. Test
"A" T.C. Virus P. 18	Vaccine production	2 hill-bulls	10^5	Pure "A"
"A" Cattle tongue epithelium	For challenge test	2 hill-bulls	10^5	Pure "A"
"A" Adult mice strain	For challenge of vaccinated mice	24 adult mice	10^6	Pure "A"
"Asia 1" T.C. virus P. 10	Vaccine production	2 hill-bulls	$10^{5.5}$	Pure"Asia 1"
"Asia 1" Cattle tongue epithelium	For challenge test	2 hill-bulls	10^5	Pure"Asia 1"
"Asia 1" Adult mice strain	For challenge of vaccinated mice	24 adult mice	10^7	Pure"Asia 1"
"C" T.C. Virus P. 33	Vaccine production	2 hill-bulls	10^5	Pure "C"
"C" Cattle tongue epithelium	For challenge test	2 hill-bulls	10^5	Pure "C"
"O" T.C. Virus P. 12	For vaccine production	2 hill-bulls	10^6	Pure "O"
"O" Adult mice strain	For challenge of vaccinated mice	24 adult mice	10^7	Pure "O"

Vaccination of Animals with Different Monovalent Vaccines

Vaccination with Type 'A' vaccines

Hill-bulls were vaccinated each with 2 ml., 5 ml. and 10 ml. doses of 0.2 per cent, 0.3 per cent saponin gel and the conventional gel vaccines respectively. These animals were observed for a period of 21 days before being challenged. They were normal during the period of observation, except for a swelling at the site of inoculation.

In order to assess the status of neutralizing antibodies, sera withdrawn from the vaccinated animals on different days following vaccination were tested. Thus, the development of neutralizing antibodies could be detected on the 7th day following vaccination in pooled sera of the animals vaccinated with 5 ml. dose of vaccine containing 0.2 per cent saponin and with 2 and 10 ml. dose of vaccine containing 0.3 per cent saponin respectively in addition to 2 ml. dose of conventional gel vaccine without the incorporation of saponin.

The log serum neutralizing (S.N.) titre/ml. in animals vaccinated with 0.2 per cent saponin gel ranged from 0 on 7th day to 3.9 on 21st day in 2 ml. group, 2 to 4.10 in 5 ml. group and 0 on 7th day to 4.99 on 21st day in 10 ml. group, while with 0.3 per cent saponin gel vaccine log S.N. titre/ml. at 2 ml., 5 ml. and 10 ml. were 0.33 to 4.11, 0 to 3.69 and 0.44 to 4.76 respectively. The highest serum neutralizing antibody titre was observed in animals vaccinated with 0.3 per cent saponin gel in 10 ml. group, on 21st day following vaccination. The log S.N. titre/ml. in

animals vaccinated with conventional gel vaccine were not encouraging. The maximum S.N. titre was recorded in 5 ml. group, i.e., 4.19 on 21st day, while in 2 ml. and 10 ml. group the log S.N. titre/ml. were 0.78 to 2.69 and 0 to 3.69 respectively.

The peak titres in all the vaccinated animals were observed between 14th to 21st day of vaccination. The serum neutralization index (S.N.I.) was higher with saponin gel vaccines than with the conventional gel vaccine irrespective of the dose used.

All the immunized animals were challenged after 21 days of vaccination along with three hill-bulls as control with 10,000 ID_{50} doses. Control animals developed extensive primary lesions on tongue and secondary lesions on gums, lips and all the feet. The animals vaccinated with saponin gel vaccines in 2 ml. group showed mainly primary lesions on tongue except hill-bull Nos. 55, 362, 540, 39 and 477 which also showed lesions on gums and lips. The animals vaccinated with conventional gel vaccine in 2 ml. group, i.e., hill-bull Nos. 44, 348, 33 developed severe primary as well as secondary lesions. In 5 ml. group hill-bull Nos. 391 and 335 showed mild secondary lesions. The animals vaccinated with 10 ml. dose were found to be strongly immune, except hill-bull No. 311, which showed very mild lesion on the lips. It is, therefore, evident that gel vaccines reinforced with saponin gave better protection when compared to the conventional gel vaccine.

The results of these experiments are shown in Tables II_a, II_b and II_c and Fig. 1.

TABLE II_a

Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with 0.2% saponin gel Type 'A' vaccine						Challenge results of animals vaccinated with 0.2% saponin gel Type 'A' vaccine						
			Log serum neutralization index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
134	2 ml.	0	0	3.93	3.90	+++	-	-	-	-	-	-
55	2 ml.	0				++	+	-	-	-	-	-
362	2 ml.	0				++	+	-	-	-	-	-
132	5 ml.	0	2.0	4.33	4.1	++	-	-	-	-	-	-
104	5 ml.	0				+	-	-	-	-	-	-
427	5 ml.	0				+	-	-	-	-	-	-
204	10 ml.	0	0	3.93	4.99	+	-	-	-	-	-	-
340	10 ml.	0				-	-	-	-	-	-	-
179	10 ml.	0				+	-	-	-	-	-	-
Controls	Unvaccinated contact controls											
327						+++	+	+	+	+	+	+
238						+++	+	+	+	+	+	+
421						+++	+	+	+	+	+	+

0 = No antibodies.

TABLE II_b

Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with 0.3% saponin gel Tyne 'A' vaccine						Challenge results of animals vaccinated with 0.3% saponin gel Tyne 'A' vaccine						
			Log serum neutralisation index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
540	2 ml.	0				+++	+	+	-	-	-	-
39	2 ml.	0	0.33	3.3	4.11	+++	-	-	+	-	-	-
447	2 ml.	0				++	+	-	-	-	-	-
170	5 ml.	0				++	-	-	-	-	-	-
537	5 ml.	0	0	4.24	3.69	-	-	-	-	-	-	-
37	5 ml.	0				+	-	-	-	-	-	-
530	10 ml.	0				+	-	-	-	-	-	-
165	10 ml.	0	0.44	3.33	4.76	-	-	-	-	-	-	-
26	10 ml.	0				+	-	-	-	-	-	-
Controls		As in Table II_a.										

0 = No antibodies.

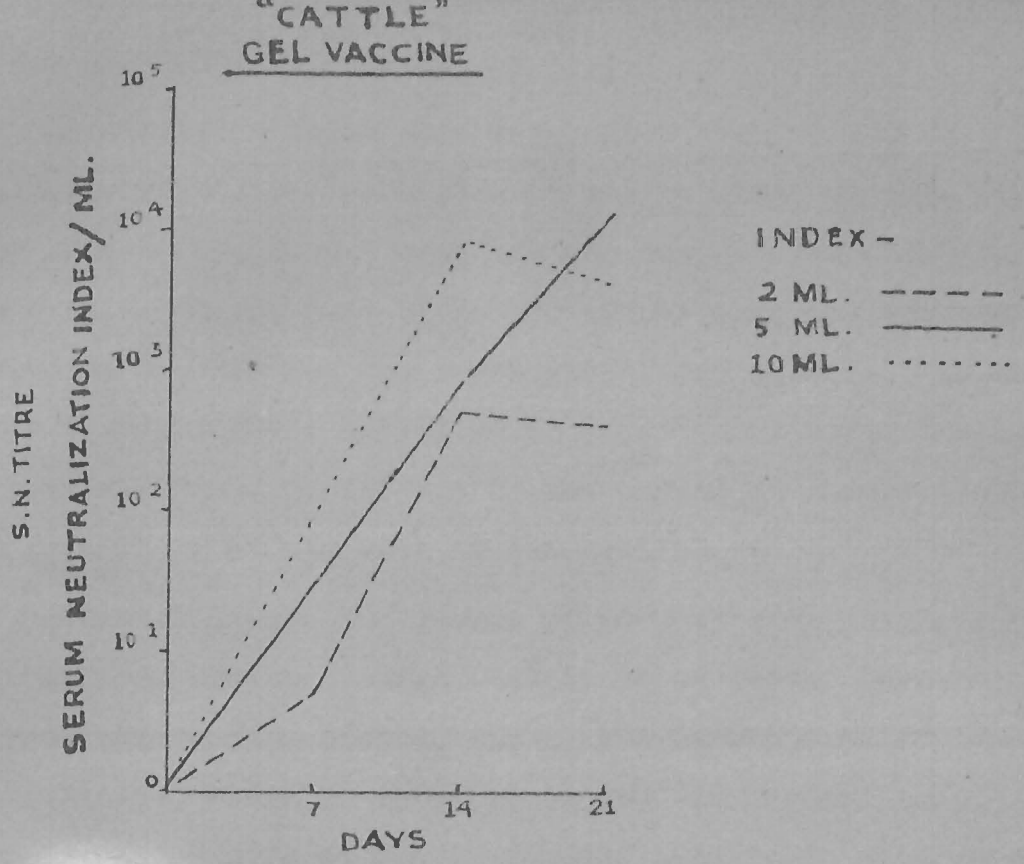
TABLE II_c

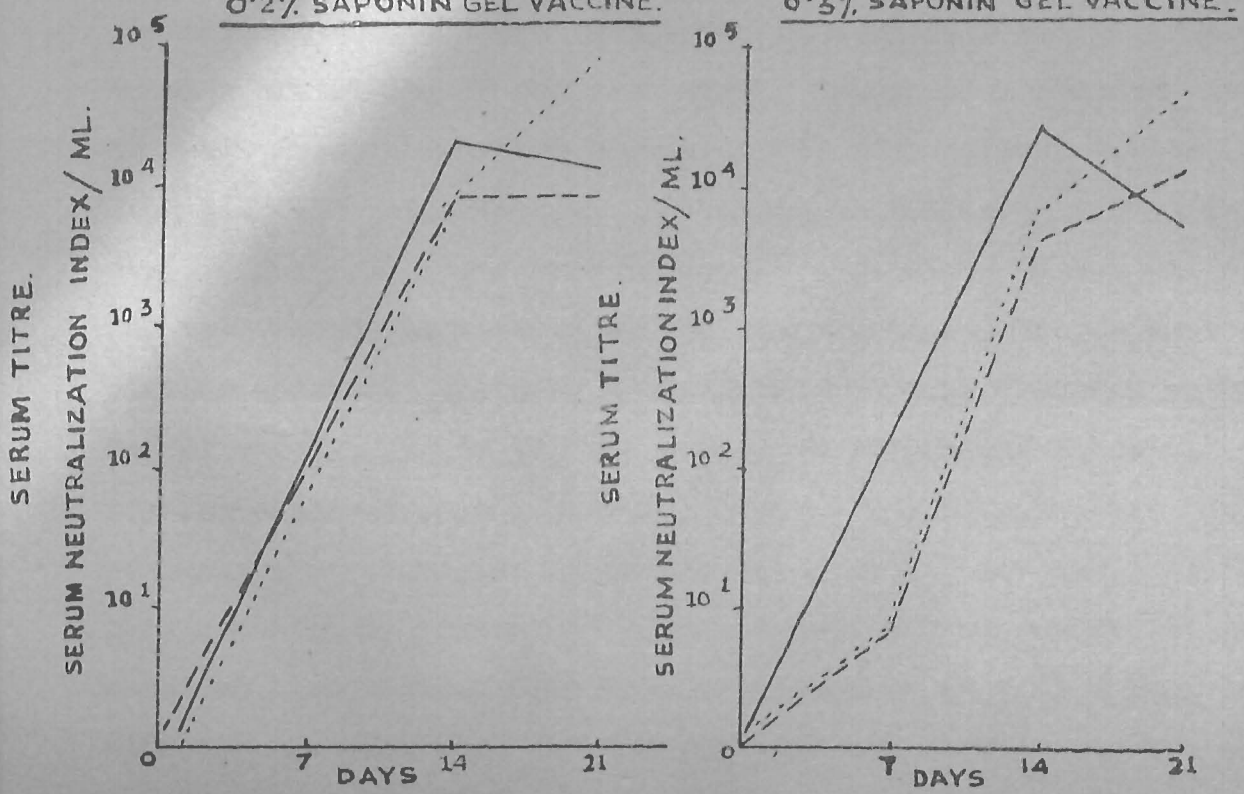
Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with conventional gel Type 'A' vaccine						Challenge results of animals vaccinated with conventional gel Type 'A' vaccine						
			Log serum neutralization index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before Vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
44	2 ml.	0				+++	+	+	+	+	-	-
343	2 ml.	0	0.78	2.73	2.69	+++	+	+	-	+	-	-
98	2 ml.	0				+++	+	+	+	-	+	-
391	5 ml.	0				+++	+	-	+	-	-	-
501	5 ml.	0	0	2.93	4.19	++	-	-	-	-	-	-
385	5 ml.	0				++	+	-	+	-	-	-
311	10 ml.	0				++	-	+	-	-	-	-
65	10 ml.	0	0	3.98	3.69	++	-	-	-	-	-	-
331	10 ml.	0				++	-	-	-	-	-	-
Controls		As in Table II_a										

0 = No antibodies.

FIG.1





NEUTRALIZING ANTIBODY TITRE AGAINST TYPE "A" VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "A" VACCINES.

Vaccination with Type 'C' vaccines

Batches of hill-bulls were vaccinated each with 2 ml., 5 ml. and 10 ml. doses of 0.2 per cent saponin gel vaccine, vaccine containing 55 per cent virus, 40 per cent gel and saponin (Vaccine No. I), vaccine containing 55 per cent virus and 40 per cent gel without saponin (Vaccine No. II) and the conventional gel vaccine. These animals were observed for a period of 21 days before being challenged. They were all found normal during the period of observations, except for a swelling at the site of inoculation.

In order to assess the status of neutralizing antibodies, sera withdrawn from the vaccinated animals on different days following vaccination were tested. Thus, the development of neutralizing antibodies could be detected on the 7th day following vaccination in pooled sera of the vaccinated animals in each group. The animals vaccinated with 0.2 per cent saponin gel vaccine and vaccine containing 55 per cent virus, 40 per cent gel and saponin in 5 ml. doses showed the highest serum neutralizing titres, i.e., 3.91 and 4.25 respectively. The serum neutralizing titres in 2 ml. and 10 ml. doses were higher in comparison to the gel vaccine and the vaccine containing 55 per cent virus and 40 per cent gel without saponin. The peak antibody titres were recorded on 21st day of vaccination in all the groups of vaccinated animals. The vaccine prepared with a higher concentration of virus and less percentage of aluminium hydroxide gel also did not prove to be much superior in respect of high antibody titres, while the gel vaccines incorporated with saponin seemed to provoke a much better antibody response than the conventional gel vaccine and the vaccine

with 55 per cent virus and 40 per cent gel, but without the incorporation of saponin.

These animals were challenged after 21 days following vaccination along with three control hill-bulls. Control animals reacted severely. Hill-bull No. 551 vaccinated with a dose of 2 ml. of 0.2 per cent saponin gel vaccine developed mild secondary lesions on gums and lips, while hill-bull Nos. 553 and 576 in 5 ml. doses showed very mild secondary lesions only on one of the feet. Hill-bull No. 566 vaccinated with vaccine containing 55 per cent virus, 40 per cent gel and saponin in 5 ml. dose also developed mild lesions on gums and lips. Of the hill-bulls vaccinated in 5 ml. dose with the vaccine containing 55 per cent virus and 40 per cent gel, one hill-bull No. 535 developed secondary lesions on lips and one foot, while the animals vaccinated with conventional gel vaccine showed severe primary lesions on gums and lips in 2 ml. dose group. Hill-bull No. 563 developed secondary lesions on two feet. The hill-bull No. 579 vaccinated with 10 ml. dose of conventional gel vaccine also showed secondary lesions on two feet.

The results are shown in Tables III_a, III_b, III_c and III_d and Fig. 2.

TABLE III$_a$

Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with 0.2% saponin gel Type 'C' vaccine						Challenge results of animals vaccinated with 0.2% saponin gel Type 'C' vaccine						
			Log serum neutralization index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
551	2 ml.	0				+++	+	+	-	-	-	-
532	2 ml.	0	1.75	Could not be tested	2.36	+++	-	-	-	-	-	-
574	2 ml.	0				+++	-	-	-	-	-	-
550	5 ml.	0				+++	-	-	-	+	-	-
576	5 ml.	0	2.51	Do.	3.91	++	-	-	-	-	-	+
543	5 ml.	0				++	-	-	-	-	-	-
546	10 ml.	0				+++	-	-	-	-	-	-
583	10 ml.	0	2.90	Do.	3.92	-	-	-	-	-	-	-
560	10 ml.	0				++	-	-	-	-	-	-
Controls	Unvaccinated contact control.											
540						++++	+	+	+	+	+	+
575						+++	+	+	+	+	+	+
56						+++	+	+	+	+	-	+

0 = No antibodies.

TABLE III_b

Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with Type 'C' vaccine (55% virus, 40% gel and saponin Vaccine No. I)						Challenge results of animals vaccinated with Type 'C' vaccine (55% virus, 40% gel and saponin)						
			Log serum neutralization index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
564	2 ml.	0				+++	-	-	-	-	-	-
548	2 ml.	0	3.16		3.25	++	-	-	-	-	-	-
554	2 ml.	0				+	-	-	-	-	-	-
544	5 ml.	0				++	-	-	-	-	-	-
566	5 ml.	0	3.52		4.25	++	+	+	-	-	-	-
552	5 ml.	0				+	-	-	-	-	-	-
542	10 ml.	0				++	+	-	-	-	-	-
534	10 ml.	0	2.50		2.61	++	-	-	-	-	-	-
530	10 ml.	0				++	-	-	-	-	-	-
Controls		As in Table III_a										

0 = No antibodies.

TABLE III$_c$

Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with Type 'C' vaccine (55% virus and 40% gel, Vaccine No. II)						Challenge results of animals vaccinated with Type 'C' (55% virus and 40% gel) vaccine						
			Log serum neutralization index / ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
573	2 ml.	0				+++	-	-	-	-	-	-
556	2 ml.	0	1.75		2.99	+++	+	-	-	-	-	-
541	2 ml.	0				+++	+	-	-	+	-	-
535	5 ml.	0				+++	-	+	-	-	+	-
568	5 ml.	0	2.33		3.16	+++	-	-	-	-	-	-
547	5 ml.	0				++	-	-	-	-	-	-
570	10 ml.	0				++	-	-	-	-	-	-
571	10 ml.	0	0		2.45	-	-	-	-	-	-	-
569	10 ml.	0				+++	-	-	-	-	-	-
Controls		As in Table III$_a$										

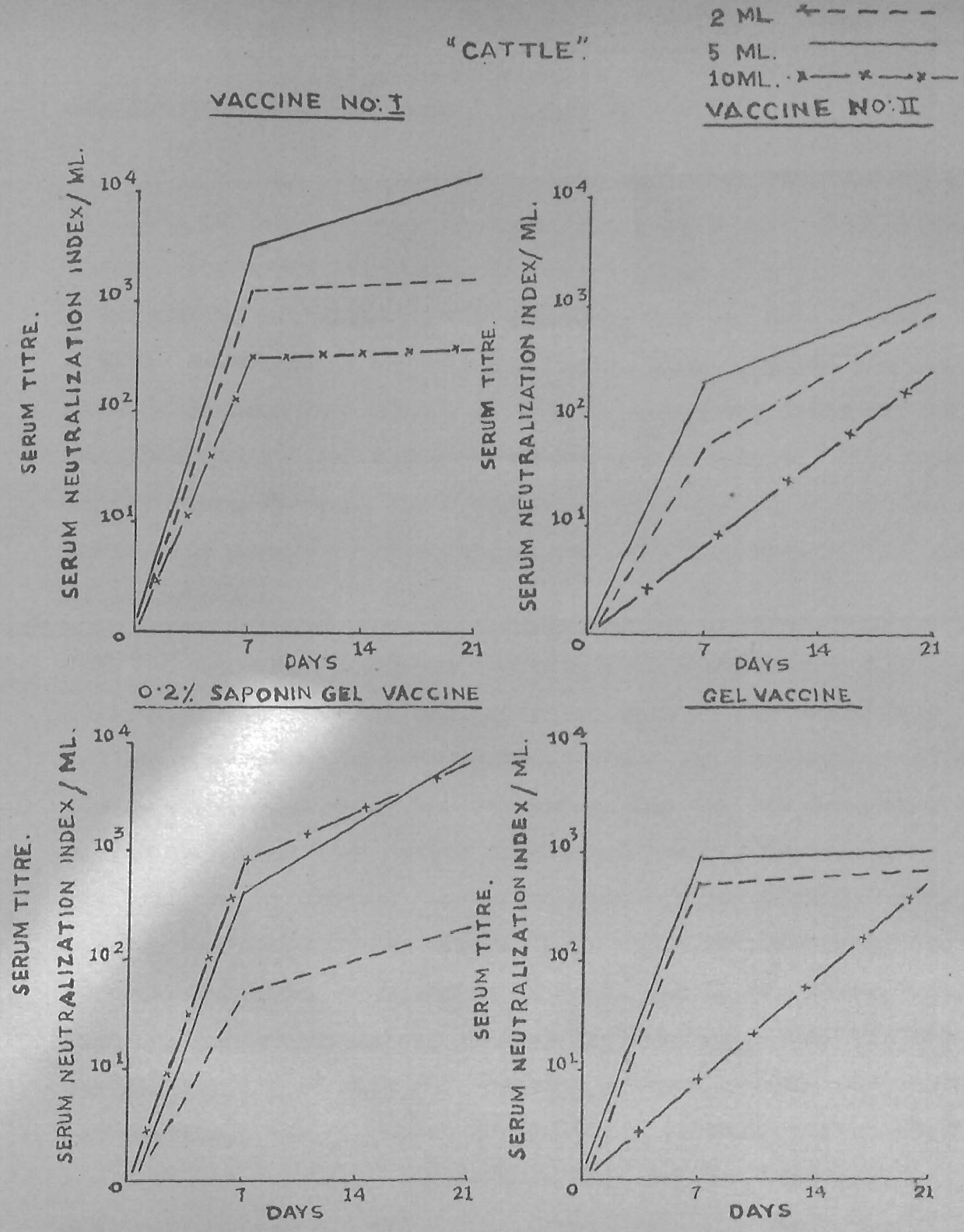
0 = No antibodies.

TABLE III$_d$

Showing Immuno Response and Challenge Results

Antibody response in hill-bulls vaccinated with Type 'C' conventional gel vaccine						Challenge results of animals vaccinated with Type 'C' conventional gel vaccine						
			Log serum neutralization index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
549	2 ml.	0				+++	+	+	-	-	-	-
545	2 ml.	0	2.73		2.36	+++	+	+	-	-	-	-
563	2 ml.	0				+++	+	+	+	-	+	-
565	5 ml.	0				+++	-	-	-	-	-	-
572	5 ml.	0	2.36		3.01	+++	-	-	-	-	-	-
539	5 ml.	0				+++	-	-	-	-	-	-
579	10 ml.	0				+++	+	-	-	-	+	+
567	10 ml.	0	0		2.75	-	-	-	-	-	-	-
562	10 ml.	0				+++	+	-	-	-	-	-
Controls		As in Table III$_a$										

0 = No antibodies.



NEUTRALIZING ANTIBODY TITRE AGAINST TYPE 'C' VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE 'C' VACCINES.

Vaccination with Type 'Asia 1' vaccines

Batches of hill-bulls, sheep and goats were vaccinated with the various vaccines against Asia 1 type employing different doses. Sheep and hill-bulls were vaccinated each with 2 ml., 5 ml. and 10 ml. doses of 0.2 per cent, 0.3 per cent saponin gel and the conventional gel vaccines respectively, while the goats were vaccinated each with 1 ml., 2 ml. and 5 ml. doses of 0.3 per cent saponin gel and the conventional gel vaccines. All these animals were observed for a period of 21 days. They were normal during the period of observation, except a swelling at the site of inoculation.

In order to assess the status of neutralizing antibodies, sera withdrawn from the vaccinated animals on different days following vaccination were tested. Thus, the development of neutralizing antibodies could be detected on the 7th day following vaccination in pooled sera of the vaccinated animals in each group. In the group of hill-bulls vaccinated with 0.2 per cent saponin gel vaccine maximum antibody titres (4.09 and 4.69) were recorded on 14th day following vaccination in 2 ml. and 10 ml. doses, while on 21st day post-vaccination, gradual fall was recorded. In the animals vaccinated with 0.3 per cent saponin gel and conventional gel vaccines, maximum serum neutralizing antibody titres -3.97, 4.90 and 2.69 and 3.99 were observed on 21st day and 14th day following vaccination in 2 ml. and 10 ml. doses. The log serum neutralization index in animals vaccinated with gel vaccine did not show significant difference with different doses.

The hill-bulls vaccinated with gel vaccines incorporated with saponin has shown better development of antibodies as compared to the conventional gel vaccine.

All the vaccinated hill-bulls were challenged after 21st days of vaccination along with three hill-bulls as control with 10,000 ID_{50} virulent virus. Control animals showed extensive primary and secondary lesions. The hill-bull No. 327 vaccinated with 10 ml. dose of 0.2 per cent saponin gel vaccine and hill-bull Nos. 253, 263, 244 and 304 vaccinated with 2 ml., 5 ml. and 10 ml. doses of 0.3 per cent saponin gel vaccine respectively developed mild lesions on gums and lips, while the animals vaccinated with the conventional gel vaccine reacted severely. Hill-bull Nos. 364, 321, 345 in 2 ml. group developed severe secondary lesions on gums, lips and feet. Hill-bull Nos. 324 and 344 each in 5 ml. and 10 ml. group also developed mild secondary lesions on one of the foot.

The vaccinated sheep and goats also showed the presence of neutralizing antibodies on the 7th day following vaccination. The peak antibody titres were observed on 14th day following vaccination in sheep vaccinated with 0.2 per cent saponin gel vaccine in 2 ml. and 10 ml. groups, while in 5 ml. dose on 21st day. In sheep vaccinated with 0.3 per cent saponin gel vaccine, maximum serum neutralizing titres were detected 4.10 and 4.99 in 2 ml. and 5 ml. groups on 21st day respectively, while in 10 ml. group on 14th day. In the group of sheep vaccinated with 10 ml. dose of conventional gel vaccine, maximum serum neutralizing titre 4.99 was observed on 14th day while with other doses the S.N. titres were not as high as with saponin gel vaccines.

Goats vaccinated with saponin gel vaccine developed high serum neutralizing antibodies with all the doses. In 2 ml. group log serum neutralization titre of 5.1 was observed on 14th day. The peak antibody titres were recorded on 14th or 21st day following vaccination. In the group of goats vaccinated with 1 ml. of conventional gel vaccine, antibodies could not be detected on 7th day following vaccination. As the group of goats vaccinated with 5 ml. dose of saponin gel vaccine, had already suffered from the disease, the booster effect of the vaccine was observed.

The results of these experiments are presented in Tables IV_a, IV_b, IV_c, IV_d, IV_e, IV_f, IV_g, IV_h and Fig. 3, 4, 5.

TABLE IV$_A$

Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with 0.2% saponin gel vaccine Type 'Asia 1' vaccine						Challenge results of vaccinated animals with 0.2% saponin gel 'Asia 1' vaccine						
			Log serum neutralization index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
356	2 ml.	0				+++	-	-	-	-	-	-
331	2 ml.	0	2.21	4.09	3.82	++	-	-	-	-	-	-
322	2 ml.	0				+	-	-	-	-	-	-
351	5 ml.	0				++	-	-	-	-	-	-
374	5 ml.	0	2.38	3.50	3.69	-	-	-	-	-	-	-
372	5 ml.	0				+	-	-	-	-	-	-
327	10 ml.	0				++	+	-	-	-	-	-
196	10 ml.	0	2.46	4.69	4.66	-	-	-	-	-	-	-
240	10 ml.	0				-	-	-	-	-	-	-
Controls		Unvaccinated contact control										
252						+++	+	+	+	+	+	+
313						+++	+	+	+	+	+	+
200						+++	+	+	+	-	+	+

0 = No antibodies.

TABLE IV_b

Showing Immune Response and Challenge Results

Antibody response in bill-bulls vaccinated with 0.3% saponin gel vaccine Type 'Asia 1' vaccine						Challenge results of vaccinated animals with 0.3% saponin gel Type 'Asia 1' vaccine						
			Log serum neutralisation index / ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
258	2 ml.	0				++	+	+	-	-	-	-
259	2 ml.	0	2.15	3.89	3.97	++	-	-	-	-	-	-
262	2 ml.	0				++	-	-	-	-	-	-
263	5 ml.	0				++	+	+	-	-	-	-
244	5 ml.	0	2.68	3.99	3.42	++	+	+	-	-	-	-
267	5 ml.	0				++	+	-	-	-	-	-
304	10 ml.	0				++	+	+	-	-	-	-
307	10 ml.	0	2.36	3.49	4.90	++	-	-	-	-	-	-
312	10 ml.	0				++	-	-	-	-	-	-
Controls		As in Table IV_a										

0 = No antibodies.

TABLE IV_c

Showing Immune Response and Challenge Results

Antibody response in hill-bulls vaccinated with conventional gel Type 'Asia 1' vaccine						Challenge results of vaccinated animals with conventional gel Type 'Asia 1' vac.						
			Log serum neutralization index/ml.			Lesions present after challenge						
Hill-bull number	Vaccination	Antibodies before vaccination	7th day	14th day	21st day	Tongue	Gum	Lip	Left fore foot	Left hind foot	Right fore foot	Right hind foot
364	2 ml.	0				+++	+	+	-	-	-	+
321	2 ml.	0	2.44	2.69	3.32	+++	-	+	+	+	-	-
345	2 ml.	0				+++	-	-	+	+	-	-
329	5 ml.	0				++	-	-	-	-	-	-
324	5 ml.	0	2.39	3.39	3.32	++	-	-	-	-	-	+
353	5 ml.	0				+	-	-	-	-	-	-
344	10 ml.	0				++	-	-	-	-	-	+
316	10 ml.	0	2.67	3.99	3.49	+	-	-	-	-	-	-
319	10 ml.	0				+	-	-	-	-	-	-
Controls		As in Table IV_a										

0 = No antibodies.

TABLE IV_d

Antibody Response in Sheep Vaccinated with 0.2% Saponin gel Type 'Asia 1' Vaccine

Sheep number	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml.		
			7th day	14th day	21st day
59	2 ml.	0			
163	2 ml.	0	2.62	3.69	3.69
179	2 ml.	0			
97	5 ml.	0			
130	5 ml.	0	2.39	3.43	3.99
176	5 ml.	0			
196	10 ml.	0			
190	10 ml.	0	3.39	4.15	3.69
164	10 ml.	0			

0 = No antibodies.

TABLE IV.

Antibody Response in Sheep Vaccinated with 0.3% Saponin gel Type 'Asia 1' Vaccine

Sheep number	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml.		
			7th day	14th day	21st day
193	2 ml.	0			
188	2 ml.	0	2.67	2.93	4.10
192	2 ml.	0			
200	5 ml.	0			
191	5 ml.	0	2.77	4.39	4.99
182	5 ml.	0			
170	10 ml.	0			
186	10 ml.	0	3.85	4.99	3.69
173	10 ml.	0			

0 = No antibodies.

TABLE IV$_f$

Antibody Response in Sheep Vaccinated with Conventional gel Type 'Asia 1' Vaccine

Sheep number	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml.		
			7th day	14th day	21st day
134 (old)	2 ml.	0			
410	2 ml.	0	2.57	2.36	2.49
477	2 ml.	0			
187	5 ml.	0			
167	5 ml.	0	1.68	2.87	2.59
197	5 ml.	0			
198	10 ml.	0			
134 (new)	10 ml.	0	2.46	4.98	3.70
177	10 ml.	0			

0 = No antibodies.

TABLE IV$_g$

Antibody Response in Goats Vaccinated with 0.3% Saponin gel Type 'Asia 1' Vaccine

Goats number	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml.		
			7th day	14th day	21st day
221	1 ml.	0			
204	1 ml.	0	2.69	3.72	4.06
206	1 ml.	0			
208	2 ml.	0			
217	2 ml.	0	3.90	5.10	3.69
222	2 ml.	0			
215	5 ml.				
211	5 ml.	3.2	4.19	4.90	5.34
205	5 ml.				

0 = No antibodies.

RET 1851

TABLE IV_h

Antibody Response in Goats Vaccinated with Conventional gel Type 'Asia 1' Vaccine

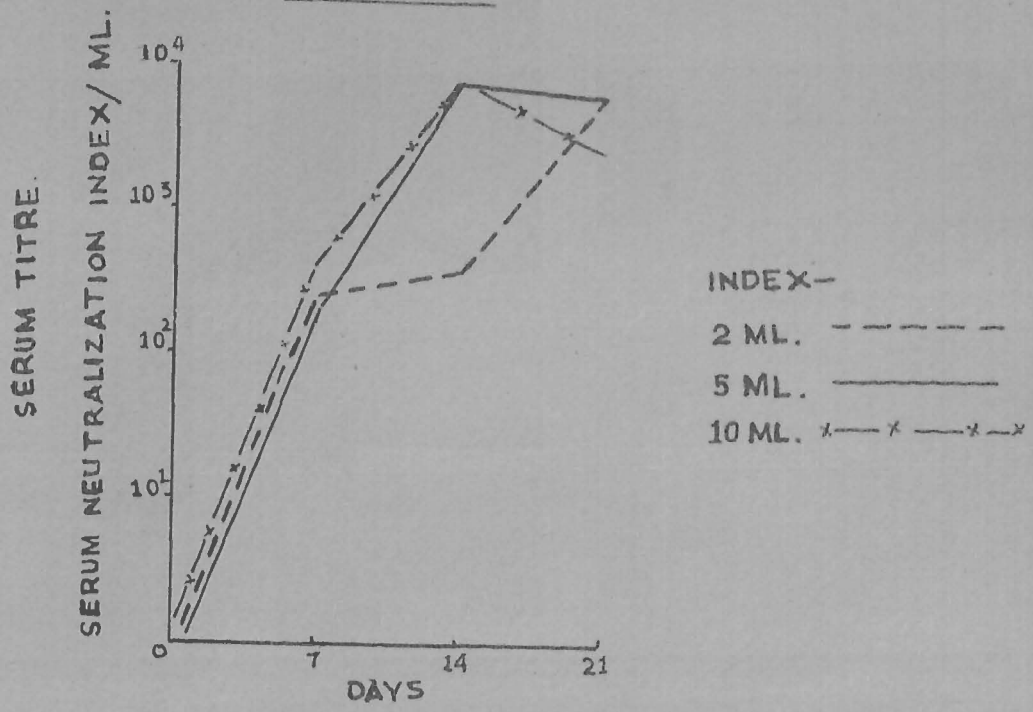
Goats number	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml.		
			7th day	14th day	21st day
213	1 ml.	0			
196	1 ml.	0	0	4.20	3.79
219	1 ml.	0			
198	2 ml.	0			
197	2 ml.	0	2.69	3.59	4.43
195	2 ml.	0			
220	5 ml.	0			
194	5 ml.	0	2.59	4.57	3.30
203	5 ml.	0			

0 = No antibodies.

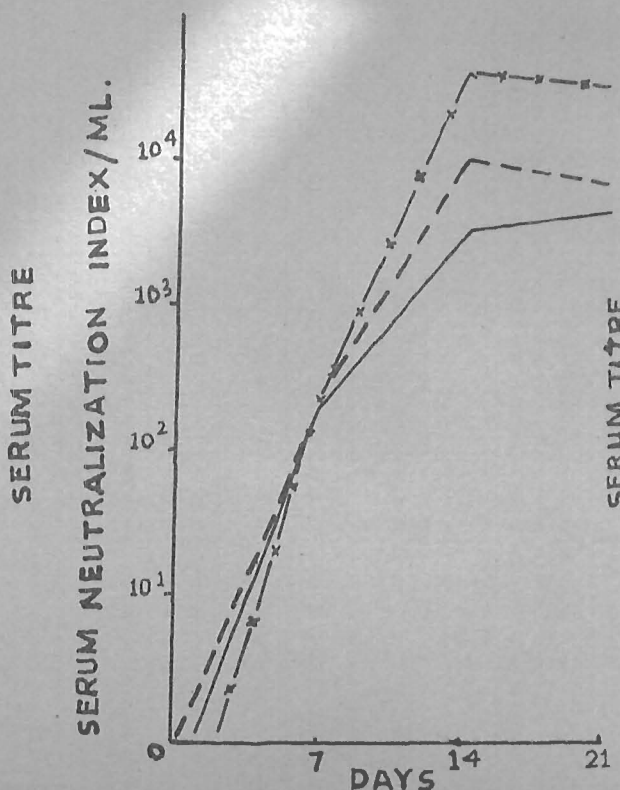
FIG. 3

'CATTLE'

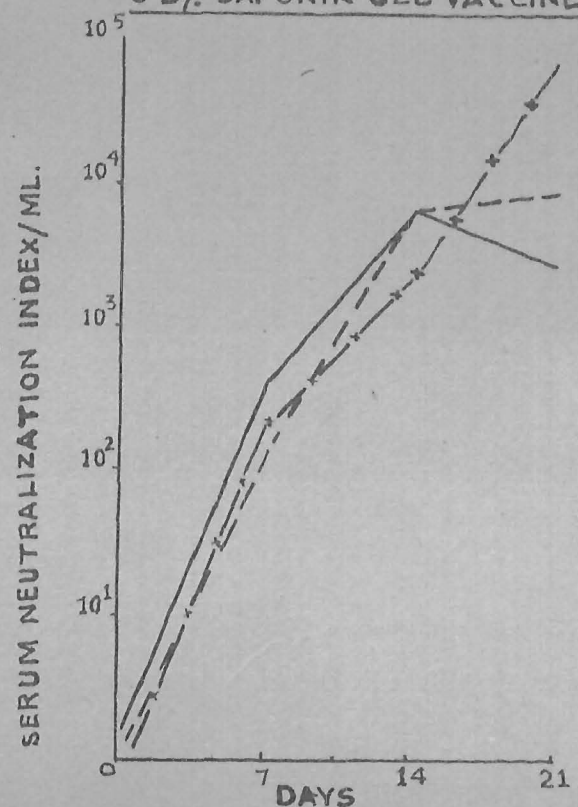
GEL VACCINE



0·2% SAPONIN GEL VACCINE.



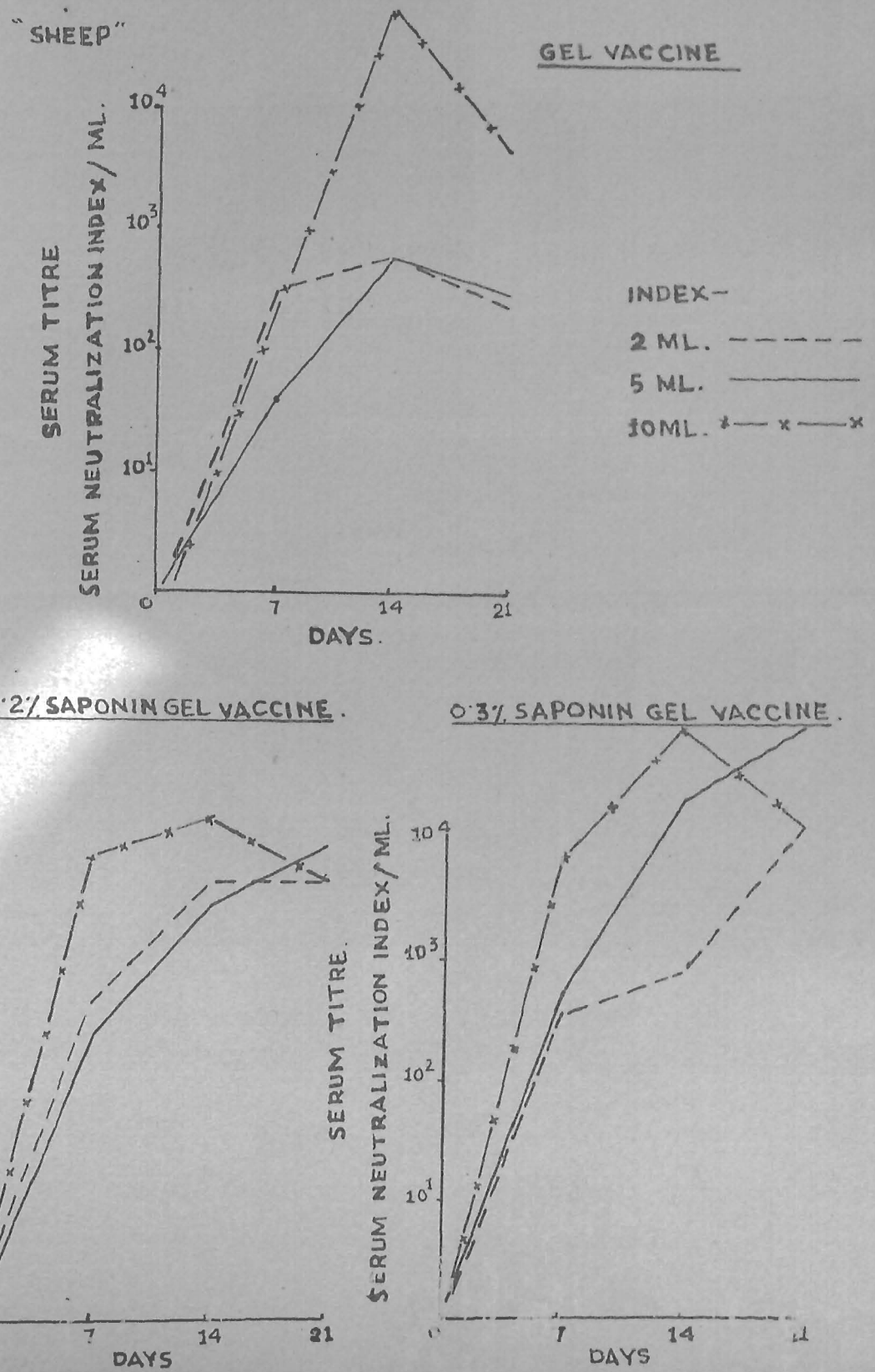
0·3% SAPONIN GEL VACCINE.



NEUTRALIZING ANTIBODY TITRE AGAINST TYPE "ASIA 1" VIRUS

FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "ASIA 1" VACCINES

FIG.4.



NEUTRALIZING ANTIBODY TITRE AGAINST TYPE "ASIA 1" VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "ASIA 1" VACCINES.

FIG. 5

'GOAT'

INDEX –

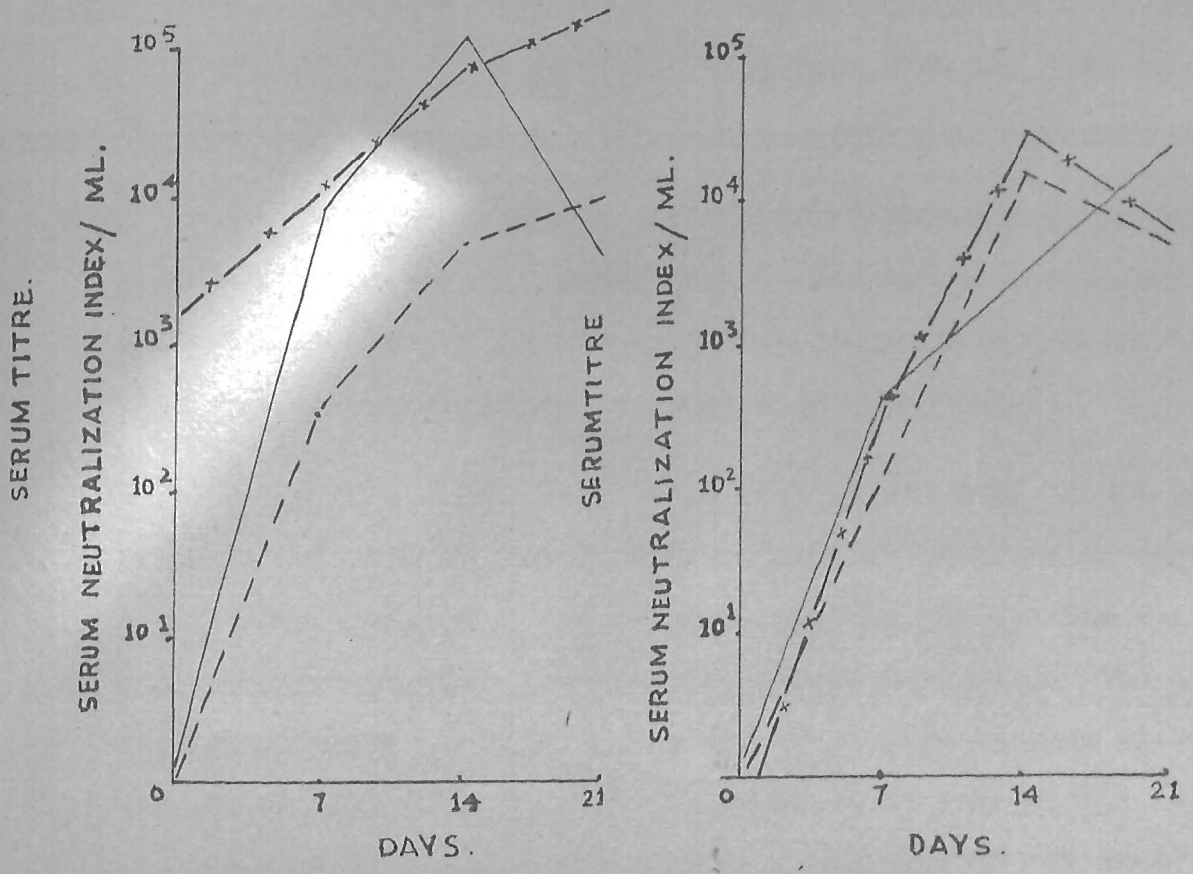
2 ML. – – – – –

5 ML. ————

10ML. –×–×–×–

0·3% SAPONIN GEL VACCINE.

GEL VACCINE.



NEUTRALIZING ANTIBODY TITRE AGAINST TYPE 'ASIA 1' VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE 'ASIA 1' VACCINES.

Vaccination with Type 'O' vaccines

Batches of heifer calves and sheep were vaccinated with various vaccines against type 'O' using different doses. Heifer calves above one year of age and with clean history were vaccinated with only 2 ml. and 10 ml. doses as shown in Tables V_a, V_b and V_c.

In addition, sheep were vaccinated each with 1 ml., 2 ml. and 5 ml. of different vaccines (Tables V_d, V_e and V_f). The vaccinated animals were normal during the observation period of 21 days, except for a moderate sized nodule at the site of vaccination.

In order to assess the status of neutralizing antibodies, sera collected from the vaccinated heifer calves before vaccination and on 21st day and from sheep before vaccination and on 7th and 21st day post-vaccination, respectively were tested.

Heifer calves vaccinated with 10 ml. dose of 0.2 per cent saponin gel vaccine showed maximum serum neutralization index, i.e., 2.93, while with 0.3 per cent saponin gel vaccine peak antibody titres were 2.93 irrespective of the dose used. The group of heifer calves vaccinated with 2 ml. of saponin vaccine without gel showed the S.N.I. 4.19, while in 10 ml. dose only 2.79. Likewise the animals vaccinated with vaccine containing 55 per cent virus, 40 per cent gel and 0.3 per cent saponin (Vaccine No. I) also developed higher serum neutralizing antibodies (S.N.I. 2.99) in 2 ml. than in 10 ml. dose. Heifer calves vaccinated with vaccine containing 55 per cent virus and 40 per cent gel did not appear to

give satisfactory serum neutralization titres. However, the animals vaccinated with 10 ml. of conventional gel vaccine showed S.N.I. of 2.93.

Due to extreme shortage of time sheep sera taken before accepting them into experiment could not be screened. These serum samples were, however, tested at a later date for serum neutralizing antibodies and were, unfortunately, indicative of a previous infection in these animals, except in groups of sheep vaccinated with 1 ml. of vaccine No. II and 5 ml. of the conventional gel vaccine where no antibodies could be detected in the pre-vaccination sera. In the former group booster effect of the vaccine on the status of neutralizing antibodies was in evidence (Tables V_d, V_e and V_f) but no such effect was witnessed in so called "clean" groups. In the animals vaccinated with saponin gel vaccines, the booster effect of the vaccines was very high irrespective of the dose used, while the amnestic response with the conventional gel vaccine and the vaccine containing 55 per cent virus and 40 per cent gel (Vaccine No. II) was not so. In the groups of sheep vaccinated with vaccine No. II and conventional gel vaccine in 1 ml. and 5 ml. doses respectively, the serum neutralization index was quite high on 21st day, following vaccination, i.e., 4.8 and 4.65 respectively.

The results of experiments are also shown in Fig. 6, 7, 8, 9 and 10.

TABLE V_a

Antibody response in Heifer Calves vaccinated with 0.2% saponin gel Type 'O' Vaccine				Antibody response in Heifer Calves vaccinated with 0.3% saponin gel Type 'O' Vaccine			
Heifer calves No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 21st day	Heifer calves No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 21st day
154	2 ml.	0	1.93	161	2 ml.	0	2.93
111	2 ml.	0		165	2 ml.	0	
146	10 ml.	0	2.93	156	10 ml.	0	2.93
170	10 ml.	0		160	10 ml.	0	

0 = No antibodies.

TABLE V_b

Antibody response in Heifer Calves Vaccinated with Type 'O' Vaccine 0.3% Saponin Without gel				Antibody response in Heifer Calves Vaccinated with Type 'O' Vaccine Containing 40% gel, 55% Virus and 0.3% Saponin (Vaccine No. I)			
Heifer calves No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 21st day	Heifer calves No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 21st day
144	2 ml.	0		153	2 ml.	0	
			4.19				2.99
162	2 ml.	0		153	2 ml.	0	
140	10 ml.	0		159	10 ml.	0	
			2.79				1.93
175	10 ml.	0		167	10 ml.	0	

0 = No antibodies.

TABLE V.

Antibody Response in Heifer Calves Vaccinated with Type 'O' Vaccine Containing 55% Virus and 40% gel (Vaccine No. II)				Antibody Response in Heifer Calves Vaccinated with Type 'O' Conventional gel Vaccine			
Heifer calves No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 21st day	Heifer calves No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 21st day
160	2 ml.	0	1.93	164	2 ml.	0	1.93
141	2 ml.	0		166	2 ml.	0	
147	10 ml.	0	1.93	138	10 ml.	0	2.93
173	10 ml.	0		168	10 ml.	0	

0 = No antibodies.

TABLE V_d

Antibody Response in Sheep Vaccinated with 0.2% Saponin gel Type 'O' Vaccine					Antibody Response in Sheep Vaccinated with 0.3% Saponin gel Type 'O' Vaccine				
Sheep No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 7th day	21st day	Sheep No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 7th day	21st day
352	1 ml.				301	1 ml.			
337	1 ml.				349	1 ml.			
343	1 ml.	1.95	2.75	5.66	348	1 ml.	2.55	4.09	6.19
236	1 ml.				233	1 ml.			
303	2 ml.				344	2 ml.			
234	2 ml.				293	2 ml.			
287	2 ml.	1.85	3.15	6.0	296	2 ml.	2.70	3.79	5.34
364	2 ml.				290	2 ml.			
354	5 ml.				346	5 ml.			
335	5 ml				359	5 ml.			
355	5 ml.	2.0	3.25	5.32	361	5 ml.	3.12	4.99	6.19
351	5 ml.				332	5 ml.			

TABLE V.

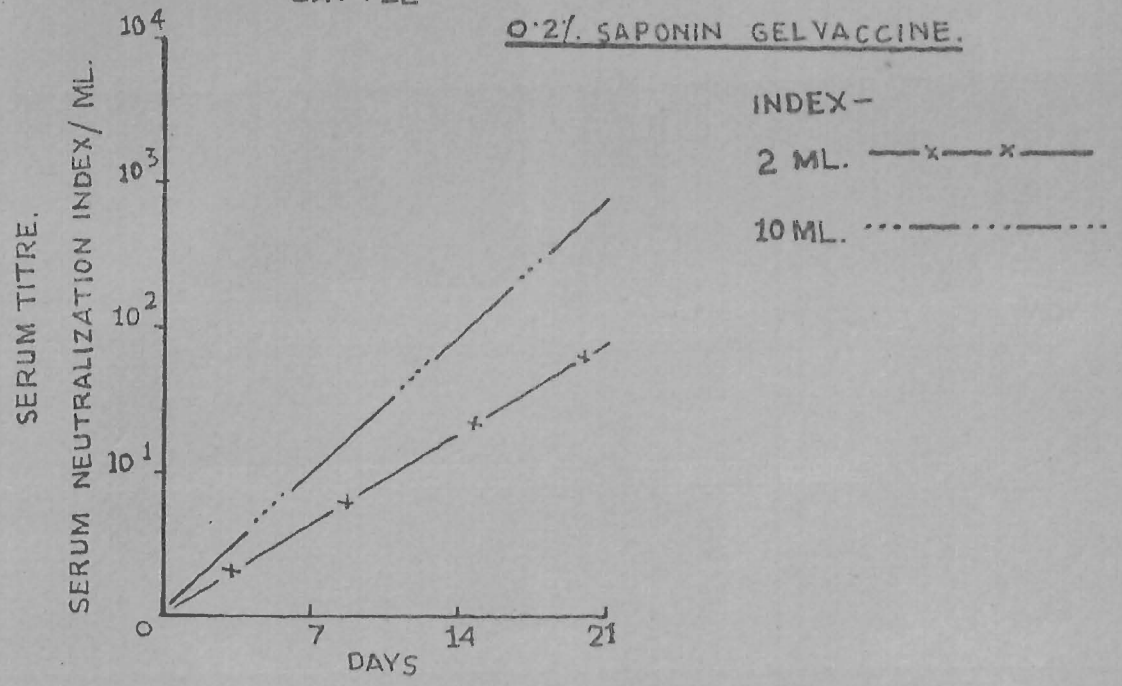
Antibody Response in Sheep Vaccinated with Type 'O' Vaccine Containing 40% gel, 55% Virus and 0.3% Saponin (Vaccine No. I)					Antibody Response in Sheep Vaccinated with Type 'O' Vaccine 0.3% Saponin Without gel				
Sheep No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 7th day	21st day	Sheep No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 7th day	21st day
239	1 ml.				353	1 ml.			
333	1 ml.				336	1 ml.			
231	1 ml.	3.11	4.09	5.92	273	1 ml.	3.21	4.39	5.59
363	1 ml.				309	1 ml.			
362	2 ml.				334	2 ml.			
272	2 ml.				342	2 ml.			
279	2 ml.	1.35	3.69	4.61	340	2 ml.	1.85	3.76	5.92
308	2 ml.				356	2 ml.			
277	5 ml.				341	5 ml.			
338	5 ml.				275	5 ml.			
345	5 ml.	Nil	2.79	4.65	305	5 ml.	3.11	4.17	4.33
350	5 ml.				271	5 ml.			

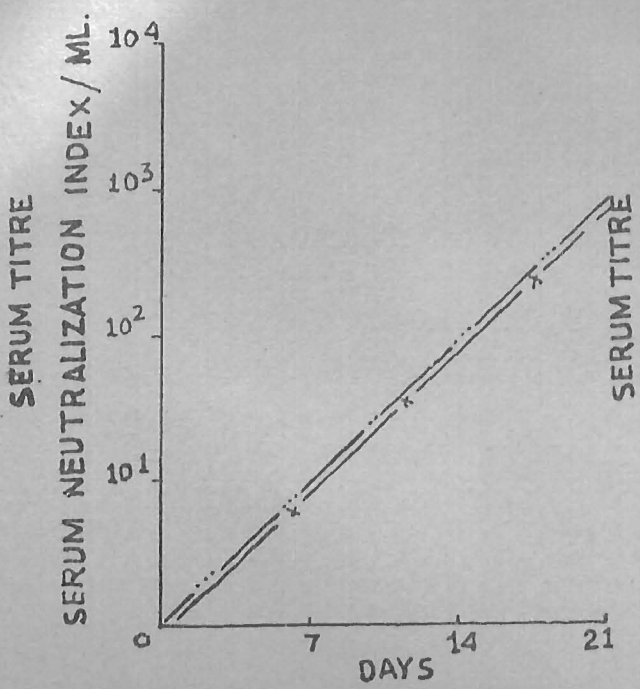
TABLE V$_f$

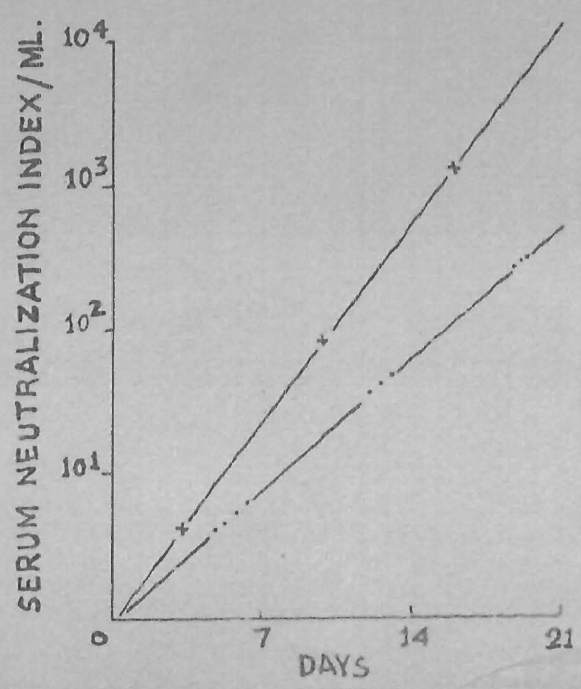
Antibody Response in Sheep Vaccinated with Type 'O' Vaccine Containing 55% Virus and 40% gel (Vaccine No. II)					Antibody Response in Sheep Vaccinated with Type 'O' Conventional gel Vaccine				
Sheep No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 7th day	21st day	Sheep No.	Vaccination	Antibodies before vaccination	Log serum neutralization index/ml. 7th day	21st day
360	1 ml.				276	1 ml.			
357	1 ml.	Nil	2.99	4.3	282	1 ml.	2.0	3.27	5.11
306	1 ml.				235	1 ml.			
339	1 ml.								
304	2 ml.				230	2 ml.			
272	2 ml.	1.35	3.96	4.32	295	2 ml.	3.55	4.0	4.69
353	2 ml.				30	2 ml.			
278	2 ml.								
298	5 ml.				293	5 ml.			
288	5 ml.	3.22	4.10	4.80	302	5 ml.	2.15	3.49	4.96
300	5 ml.				20	5 ml.			

FIG. 6

"CATTLE"





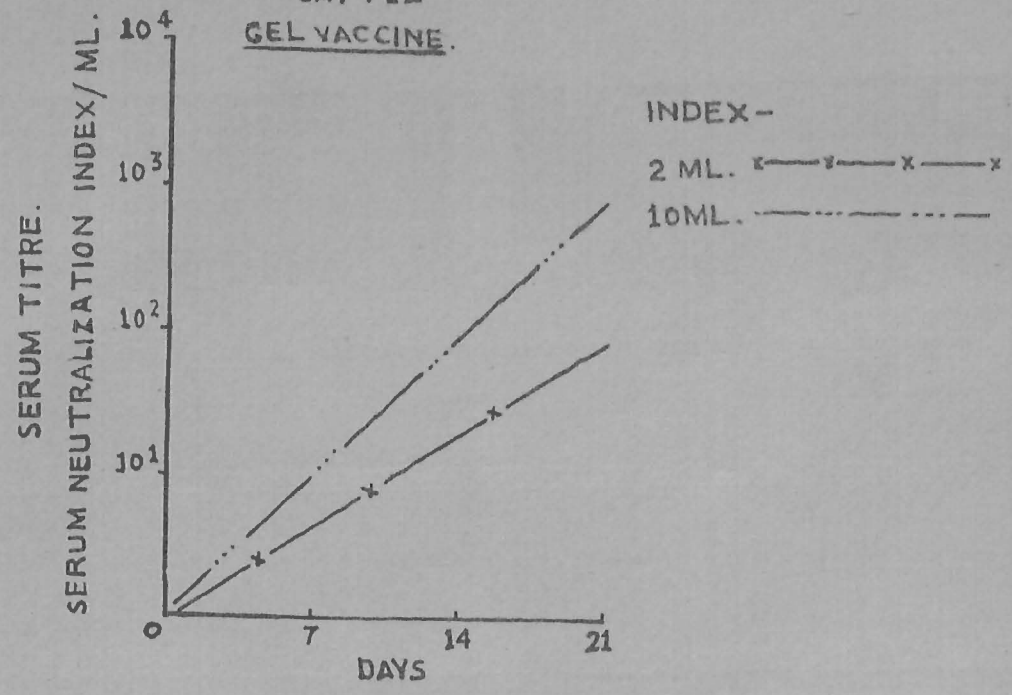


NEUTRALIZING ANTIBODY TITRE AGAINST TYPE "O" VIRUS FOLLOWING FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "O" VACCINES.

FIG. 7

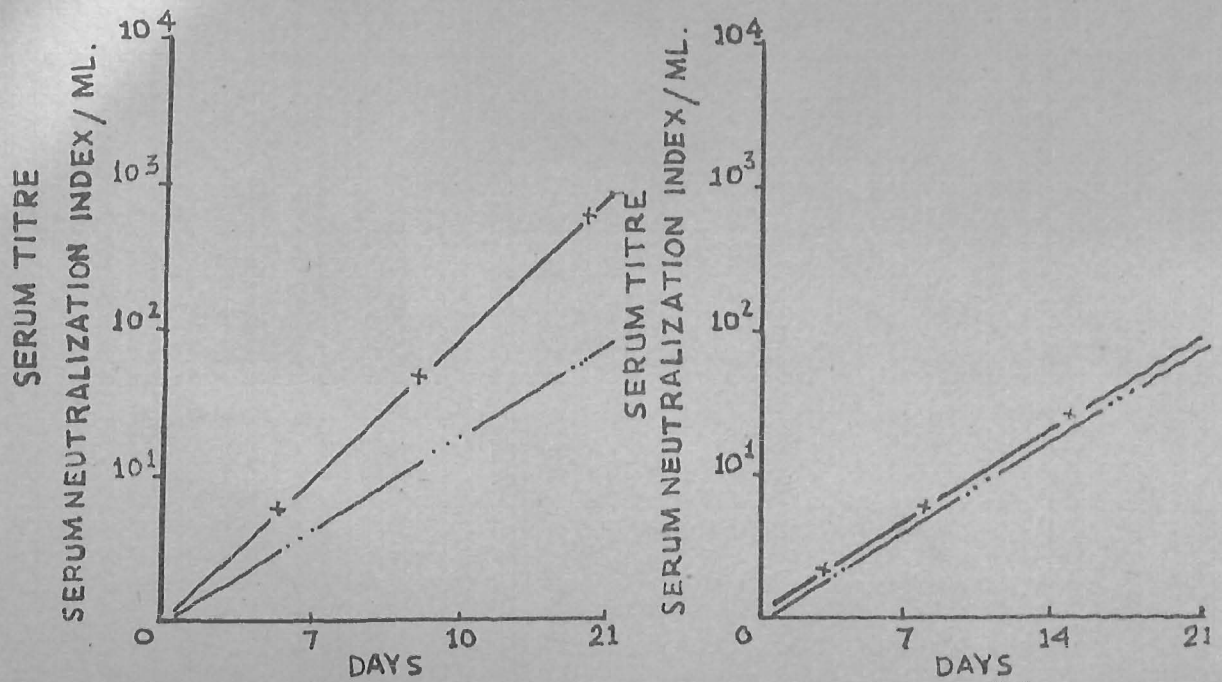
"CATTLE"

GEL VACCINE.



VACCINE NO: I

VACCINE NO: II



NEUTRALIZING ANTIBODY TITRE AGAINST TYPE "O" VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "O" VACCINES.

FIG. 8

"SHEEP"

INDEX—

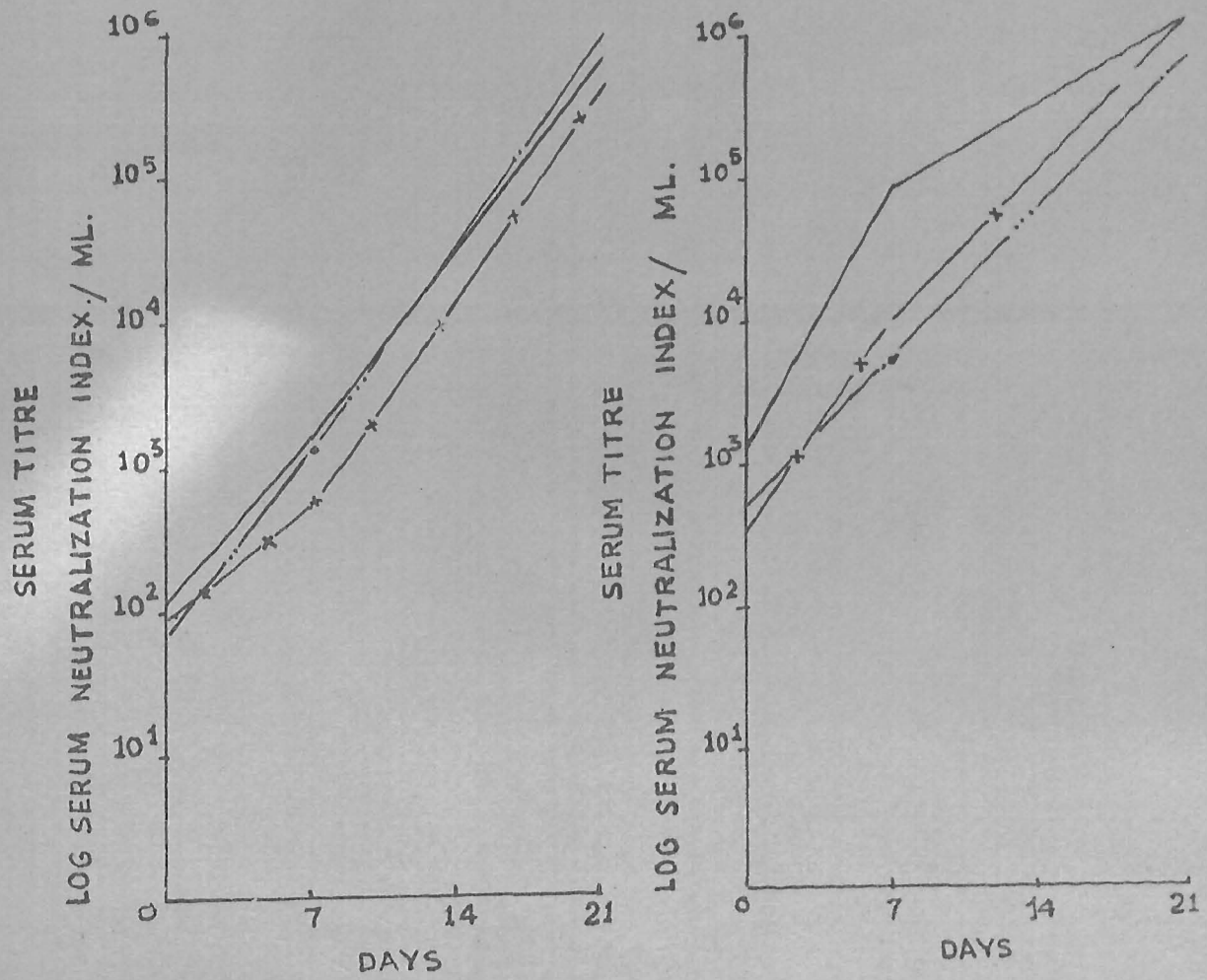
1 ML. — x — x —

2 ML. — ··· — ···

5 ML. ————

0·2% SAPONIN GEL VACCINE.

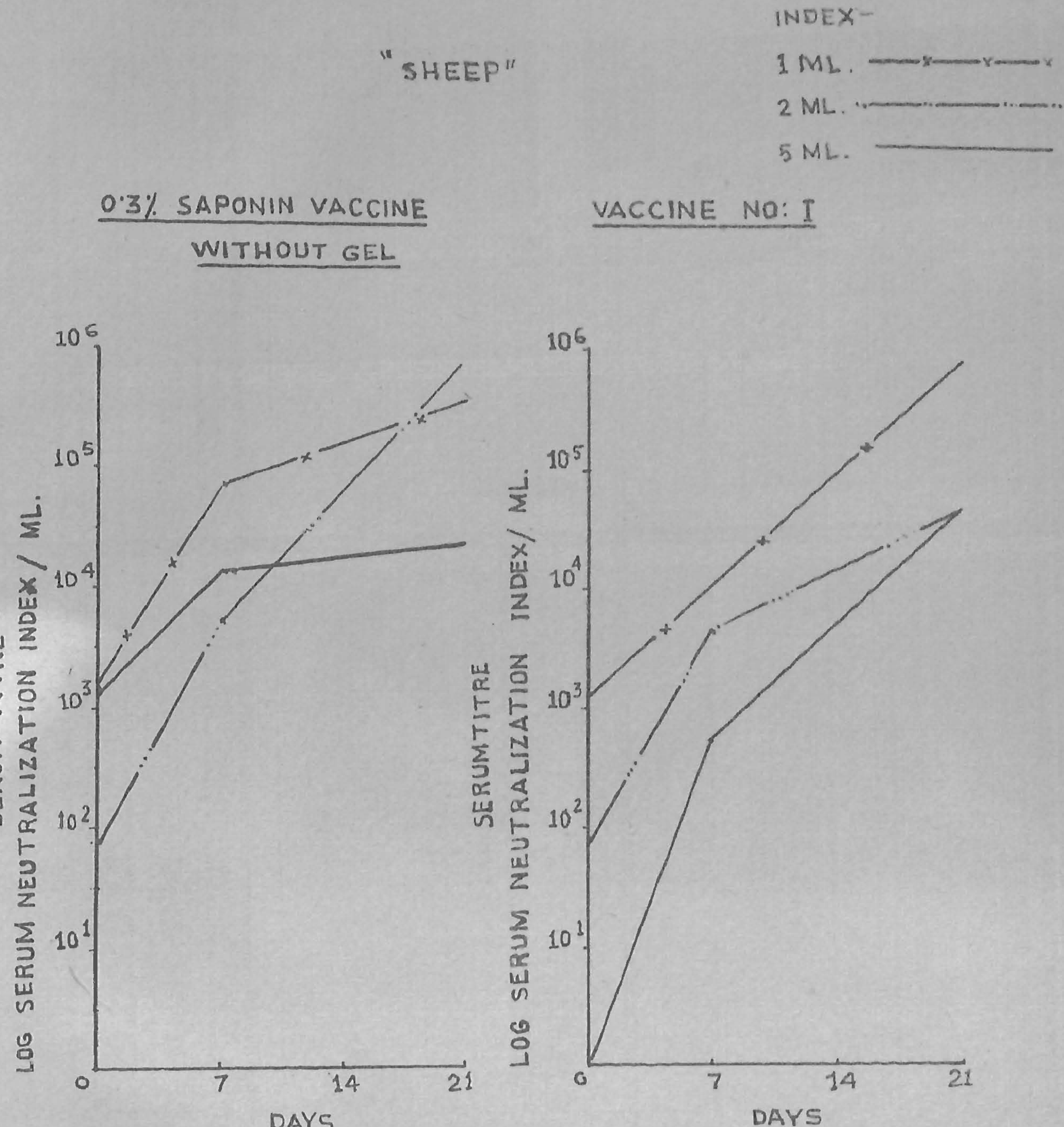
0·3 SAPONIN GEL VACCINE.



NEUTRALIZING ANTIBODY TITRE AGAINST "O" VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "O" VACCINES.

FIG. 9

"SHEEP"



NEUTRALIZING ANTIBODY TITRE AGAINST "O" VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "O" VACCINES.

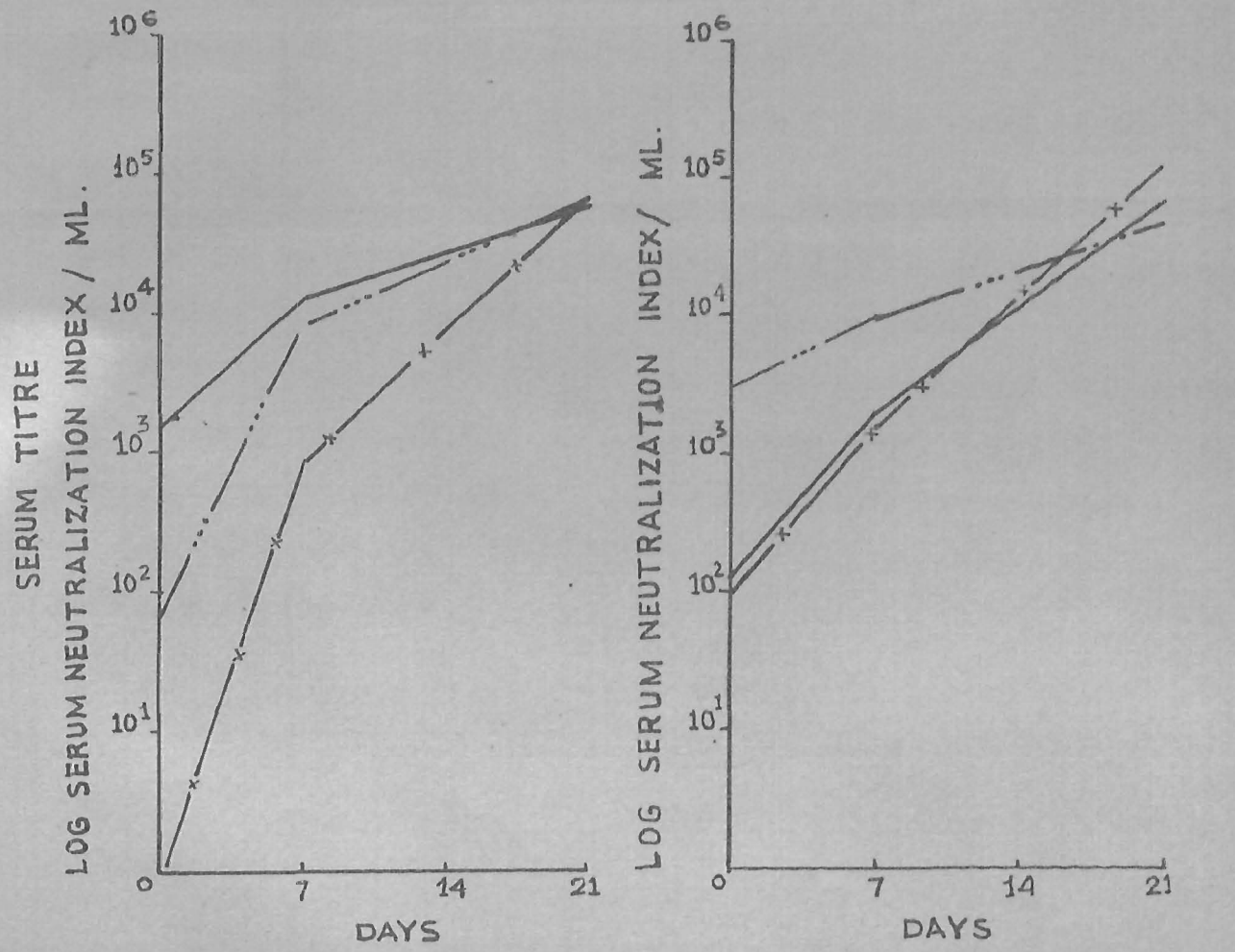
FIG. 10.

"SHEEP"

INDEX –
1 ML. x——x——x——x
2 ML. ····——····——····
5 ML. ————————

VACCINE NO: II

CONVENTIONAL GEL VACCINE



NEUTRALIZING ANTIBODY TITRE AGAINST "O" VIRUS FOLLOWING VACCINATION WITH VARIOUS DOSES OF TYPE "O" VACCINES.

Potency testing of Type 'A', 'O' and 'Asia 1' vaccines in adult mice

For testing the immunogenicity of various vaccines, a group of adult mice were vaccinated with each vaccine using different dilutions in 0.2 ml. dose subcutaneously. These mice were observed for a period of 21 days before being challenged. After 21 days following vaccination all the mice were challenged with 10,000 ID_{50} of homologous virus strains along with controls. The protection dose 50 (PD_{50}) was calculated in vaccinated mice by Read and Muench method (1938). The results are shown in Table VI.

From the above mentioned table it is observed that more than 50 per cent protection was obtained in all the groups of mice vaccinated with 1:4 or 1:8 dilution of the vaccines as also the degree of protection conferred seemed to increase with the increased amount of virus in the vaccines (Vaccine Nos. I and II). The immunity conferred by saponin gel vaccines and the vaccines containing higher percentage of virus in all the cases was better than the conventional gel vaccines.

TABLE VI

Vaccination and Challenge Results

in Adult Mice

Vaccine	Dilution of vaccine used	No. of mice used	Dose and route	Percentage of mice after surviving challenge	Protection dose (PD_{50})
1	2	3	4	5	6
Type 'A' 0.2% saponin gel vaccine	1:2	10	0.2 ml s/c	80	
	1:4	10	Do.	70	1/60 or 0.0166 ml.
	1:8	10	Do.	30	
	1:16	10	Do.	30	
Type 'A' 0.3% saponin gel vaccine	1:2	10	0.2 ml	90	
	1:4	10	Do.	60	1/40 or 0.025 ml.
	1:8	10	Do.	50	
	1:16	10	Do.	20	
Type 'A' conventional gel vaccine	1:2	10	0.2 ml	70	
	1:4	10	Do.	50	1/25 or 0.08 ml.
	1:8	10	Do.	20	
	1:16	10	Do.	0	
Controls		10	10,000 ID_{50} virus, i/n	All died	
Type 'Asia 1' 0.2% saponin gel vaccine	1:2	10	0.2 ml	80	
	1:4	10	Do.	60	1/25 or 0.04 ml.
	1:8	10	Do.	40	
	1:16	10	Do.	20	
Type 'Asia 1' 0.3% saponin gel vaccine	1:2	10	0.2 ml	60	
	1:4	10	Do.	80	More than 1:80 or 0.0125 ml.
	1:8	10	Do.	70	
	1:16	10	Do.	60	
Type 'Asia 1' gel vaccine	1:2	10	0.2 ml	60	
	1:4	10	Do.	50	1/20 or 0.05 ml.
	1:8	10	Do.	40	
	1:16	10	Do.	0	
Controls		10	10,000 ID_{50} virus, i/n	All died	

TABLE VI (Continued)

Vaccine	Dilution of vaccine used	No. of mice used	Dose and route	Percentage of mice after surviving challenge	Protection dose (PD_{50})
1	2	3	4	5	6
Type '0' 0.2% saponin gel vaccine	1:2	10	0.2ml.	100	
	1:4	10	Do.	80	1/30 or 0.033 ml.
	1:8	10	Do.	40	
	1:16	10	Do.	40	
Type '0' 0.3% saponin without gel vaccine	1:2	10	0.2ml.	90	
	1:4	10	Do.	80	1/25 or 0.04 ml.
	1:8	10	Do.	0	
	1:16	10	Do.	30	
Type '0' vaccine containing 55% virus, 40% gel & saponin	1:2	10	0.2ml	60	
	1:4	10	Do.	90	1/25 or 0.04 ml.
	1:8	10	Do.	50	
	1:16	10	Do.	20	
Type '0' vaccine containing 55% virus & 40% gel	1:2	10	0.2ml	90	
	1:4	10	Do.	80	1/65 or 0.0153 ml.
	1:8	10	Do.	80	
	1:16	10	Do.	40	
Type '0' gel vaccine	1:2	10	0.2ml	90	
	1:4	10	Do.	50	1/20 or 0.05 ml.
	1:8	10	Do.	0	
	1:16	10	Do.	0	
Controls		10	10,000 ID_{50} virus, i/m	All died	

DISCUSSION

DISCUSSION

Vaccines against foot and mouth disease prepared from time to time in different countries have been reported to have a varying degree of antigenicity and, therefore, a variable capacity to immunize. This has been mainly ascribed to the antigenic plasticity of the virus and, especially the quantitative antigenic differences within the main types. Thus, attempts have always been directed towards effective improvement in the vaccines, so that these could be effective against the field virus in the minimum dose possible.

In this country, the present cell-cultured gel vaccine, as is being manufactured at the Indian Veterinary Research Institute, although, has been found fairly satisfactory against foot and mouth disease, further investigations were undertaken in order to improve its antigenicity to cope with the wild (field) strains of the virus, presently prevalent in the country.

For this purpose type specific vaccines (as already mentioned in the text) using different compositions of the virus, stabilizer and adjuvant, were prepared and tried out. The method of testing the vaccines has been by assessing the status of neutralizing antibodies and followed by challenge tests where possible with the virulent virus to ascertain the degree/nature of immunity.

'A' Type Vaccine

From the results presented in Tables II_a, II_b and II_c, no significant difference could be observed in three doses of vaccines used in assessing the antibody titres. With each of the vaccine, it was observed that animals vaccinated with smaller doses of vaccine appeared to give a higher level of serum neutralizing antibody titres than those vaccinated with larger doses. The exact significance of this phenomenon is not clear and, therefore, more critical work is needed to confirm this.

Further, while assessing the serum neutralizing antibodies in serum samples withdrawn on different days following vaccination, it was observed that these antibodies were consistently higher in animals vaccinated with saponified gel vaccines than the conventional gel vaccine. These findings appeared in agreement with those of Mackowiak et al. (1959) and Florent (1967).

The maximum antibody titres in each group of vaccinated animals was recorded either on 14th or 21st day. The minimum log serum neutralization titre/ml. of the serum of hill-bulls which withstood challenge was found 3.3 in 2 ml. group of 0.3 per cent saponin gel vaccine, which indicated that serum neutralization titre/ml. 3.3 or above was protective.

Schneider et al. (1964) mentioned that peak titres were seen on 14th day following vaccination.

'C' Type Vaccine

Hill-bulls vaccinated with type 'C' vaccines and challenged after 21 days following vaccination gave satisfactory results. Each group of vaccinated animals showed the presence of serum neutralizing antibodies in pooled sera collected on 7th day following vaccination, except hill-bulls vaccinated with 10 ml. dose of the conventional gel vaccine and the vaccine containing 55 per cent virus and 40 per cent gel without saponin. No significant difference was observed in the three doses of vaccines used as far as the production of serum neutralizing antibodies and the protection against generalized infection were concerned. These findings are in accord with Wisniewski (1965).

While assessing the serum neutralizing antibodies in sera from vaccinated animals, it was found that the production of antibodies was higher with saponified gel vaccines than with the conventional gel vaccine, and the vaccine containing 55 per cent virus and 40 per cent gel without saponin. Schneider et al. (1963) also obtained similar findings with vaccines prepared from cell-cultured virus type 'C' in pigs.

From the findings, it was also observed that the quantity of aluminium hydroxide gel does play some specific role in producing a high level of antibodies. However, if the quantity of gel was reduced and some other adjuvant like 'Saponin' incorporated, the level of serum neutralizing antibodies produced was higher.

Schmidt and Hansen (1936) also reported that higher percentage of aluminium hydroxide gel in the vaccine provided better results.

'Asia 1' Type Vaccine

The hill-bulls, sheep and goats vaccinated with 'Asia 1' gel vaccines incorporated with saponin showed encouraging results. Serum neutralizing antibodies were detected in pooled sera on 7th day following vaccination. It is evident from the Tables IV_a, IV_b and IV_c that there was no significant difference in antibody titres in animals vaccinated with different doses with each vaccine, although a smaller dose appeared to produce a better antibody response than a bigger dose.

A minimum serum neutralization titre/ml. of 2.15 can be stated as protective index upon challenge with 10,000 ID_{50} of virulent virus. Therefore, it may be concluded that the saponified gel vaccines provided more encouraging results than the conventional gel vaccine.

The vaccinated sheep and goats could not be challenged because of the non-availability of the species adapted virulent types of virus and also because of the fact that these animals do not develop constant secondary lesions. Oral (1968) challenged vaccinated sheep and calculated the protection index by observing viraemic phase in blood withdrawn at different hours

post-infection. Unfortunately present study was not made by this method.

'O' Type Vaccine

Neutralizing antibodies against type 'O' virus in vaccinated heifer calves were found satisfactory (Tables V_a, V_b and V_c). No difference in serum neutralization titres was observed with different dose level.

Sheep vaccinated with various vaccines against type 'O' could not be screened before accepting them into experiment. Later, when the sera collected before vaccination and post-vaccination periods were tested for determining the immune status, an evidence of amnestic response was forthcoming which again was better elicited in animals receiving saponin vaccine. Here again, small doses appeared to give better antigenic response.

Thus, taking an over all assessment of the efficacy of the vaccine against the four prevailing types of the virus, encountered in this country, it was observed that the saponified vaccines gave better results than the conventional gel vaccines as well as the vaccines containing higher percentage of virus and correspondingly less percentage of aluminium hydroxide gel. This indicated a definite superiority of the saponin in improving the antigenicity of the vaccines.

In a group of heifer calves and sheep, vaccine with an incorporation of 0.3 per cent saponin but no gel gave rather

encouraging may better results than any of the vaccines used. This is a crucial finding and needs to be investigated further to confirm its authenticity since if found correct, it will be a big step forward in the economics of foot and mouth disease vaccine manufacture.

Again the observation (which was almost uniformly seen in the case of all the four types of the saponified gel vaccine used) that a small dose appeared to elicit a better antigenic response holds great significance. Although, in the present state of knowledge it may be difficult to furnish an explanation for it, the findings appear in accord with those of Fogedby and Keofoed (1940).

More work is thus needed on the use of saponin as an adjuvant in the manufacture of foot and mouth disease vaccines particularly if in some way the dose of the vaccine could be reduced, while keeping a higher level of antigenicity at the same time, when used as polyvalent immunizing agent. This is essential both from the practical administrative as well financial considerations so vitally connected with the control of foot and mouth in India.

SUMMARY

SUMMARY

Comparative studies were carried out with saponified gel monovalent and the existing monovalent aluminium hydroxide gel foot and mouth disease vaccines to find out the immunogenic response of the vaccines in hill-bulls, sheep and goats.

For studying the immunogenic response of monovalent vaccine containing 0.2 per cent and 0.3 per cent saponin with aluminium hydroxide gel, clean hill-bulls and heifer calves were vaccinated with 2 ml., 5 ml. and 10 ml. doses and 2 ml. and 10 ml. doses respectively.

Sheep were vaccinated with 2 ml., 5 ml. and 10 ml. doses of 'Asia 1' saponified gel vaccine and with 1 ml., 2 ml. and 5 ml. of type 'O' vaccine. Goats were vaccinated with 1 ml. 2 ml. and 5 ml. doses of these vaccines.

In addition, three modifications of type 'C' and 'O' vaccines were also studied. The immune status of the vaccinated animals in each group was assessed in pooled sera collected on 7th, 14th and 21st day before challenge with 10,000 ID_{50}/ml. of the corresponding virulent viruses. Virulent virus challenge was conducted in all the cattle, except those vaccinated against type 'O' of the virus.

In most of the vaccinated animals antibodies were detected on 7th day and the maximum antibody titre in all animals with different doses reached by 14th or 21st day following vaccination.

The saponified gel vaccines appeared to elicit a better antigenic response than the conventional aluminium hydroxide gel vaccine against all the types of the virus as judged by serum neutralization as well as challenge tests.

An attempt was also made to test potency of the vaccines in adult mice with satisfactory results.

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APPENDIX

APPENDIX

Solutions Used for the Preparation of Goat Kidney Cell-Culture

1. Hank's solution I

Sodium chloride	..	80 gm.
Potassium chloride	..	4 gm.
Magnesium sulphate	..	2 gm.
$CaCl_2$ (Calcium chloride)		1.4 gm.
Distilled water	..	1,000 ml.

Sterilized at 15 lb. pressure for 30 minutes in autoclave.

2. Hank's solution II

Disodium hydrogen phosphate (Na_2HPO_4, $7H_2O$)		1.12 gm.
Pot. dihydrogen phosphate (KH_2PO_4)		0.6 gm.
Dextrose	..	20 gm.
Phenol red solution 0.04%		50 ml.
Distill water	..	950 ml.

Sterilized at 15 lb. pressure for 30 minutes in autoclave.

3. Hank's solution III

Sodium bicarbonate ($NaHCO_3$)	..	7.0 gm.
Distilled water	..	800 ml.

Sterilized at 15 lb. pressure for 10 minutes in autoclave.

4. Phosphate buffer saline (P.B.S.)

Solution 'A':

Sodium chloride	..	32 gm.
Potassium chloride	..	0.8 gm.
Disodium hydrogen phosphate (Na_2HPO_4, $7H_2O$)		8.68 gm.
Potassium dihydrogen phosphate (KH_2PO_4)	..	0.8 gm.
Distilled water	..	3,200 ml.

Solution 'B':

Calcium chloride ($CaCl_2$)	..	0.4 gm.
Distilled water	..	400 gm.

Solution 'C':

Magnesium chloride ($MgCl_2$, $6H_2O$)	..	0.85 gm.
Distilled water	..	400 gm.

The above three solutions were autoclaved at 15 lbs. pressure for 30 minutes when cool solutions 'B' and 'C' were serially mixed to solution 'A' and shaken well. Penicillin and dihydro-streptomycin were added at the rate of 1 lac units of penicillin and 0.1 gm. streptomycin per litre.

Phenol red (0.04%)

Four grammes of phenol red (B.D.H.) was dissolved in sufficient N/5 sodium hydroxide solution with the help of

pestle mortar. The volume was made to 1,000 ml. using glass distilled water (the colour of phenol red solution should remain cherry red).

Trypsin solution (0.25%)

Trypsin (Difco 1:250)	..	1.25 gm.
Phosphate buffer saline		500 ml.

The suspension was stirred over night on magnetic stirrer in refrigerator. Next day it was filtered through Seitz E.K.S. pad and stored at 4°C.

Horse serum

Growth medium with 12% horse serum:

Lactalbumin hydrolysate	..	5.0 gm.
Yeast extract	..	0.5 gm.
Hank's I	..	100 ml.
Hank's II	..	100 ml.
Horse serum	..	120 ml.
Crystalline penicillin	..	1 lac I.U.
Dihydrostreptomycin sulphate	..	0.1 gm.
Mycostatin		50,000 Units.
Hank's III	..	25 ml.
Distilled water	..	655 ml.

pH adjusted to 7.2 to 7.4

The growth medium was sterilized by filteration through Seitz E.K.S. pads.

Maintenance medium I (without serum)

The composition of this medium is same as that of growth medium except it did not contain any serum. The volume was made good by adding distilled water.

Maintenance medium II (2% horse serum)

This medium contained 2% horse serum also in addition to maintenance medium I. Other details were as in maintenance medium I.