STUDIES ON THE IMMUNE RESPONSE OF FOOT-AND-MOUTH DISEASE VACCINE TYPE ASIA-1 IN PREGNANT EWES, LAMBS AND EVALUATION OF TYPE O VACCINE BY CHALLENGE

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Summary. – Seven pregnant ewes at the 10th to 12th week of pregnancy were vaccinated with foot-and-mouth disease (FMD) vaccine type Asia-1. All pregnant animals responded well with antibody production without any adverse effects. The maximum antibody titer was noted 3 to 4 weeks after the vaccination. In the colostrum a high level of maternal antibodies persisted from 12 hrs to 6 weeks after birth. Irrespective of the presence of the maternal antibodies, the vaccinated lambs responded with antibody production within the first week of vaccination. The antibodies persisted up to the 12th week of vaccination. In another experiment five sheep were vaccinated with FMD type O vaccine and challenged with 10,000 $TCID_{50}$ of virulent type O cell culture-adapted virus. The antibody titers in the vaccinated animals prior to challenge ranged between 1.26 to 1.65, while the four control sheep remained free from detectable antibody against virus type O. Pyrexia and viraemia developed present in all the control sheep but were absent in the vaccinated ones. Charasteristic primary lesions on the dorsum of the tongue were observed after 48 hrs of virus challenge in the control sheep but were absent in the vaccinated ones.

Key words: foot-and-mouth disease virus; types Asia-1 and O; vaccine; sheep; immune response

Introduction

FMD, which affects cloven-footed animals is endemic in India. Lot of work has been carried out to control this disease in cattle and swine, but very little in sheep. It is known that FMD in adult sheeps tends to be relatively mild or even inapparent (Geering, 1967). In addition, FMD virus (FMDV) infection of adult sheep invariably produces a prolonged carrier state (Burrow, 1968). Therefore, to reduce its incidence and hasten its eventual control, it is important to vaccinate the entire sheep population in endemic areas against FMD.

But the information on vaccination effects in pregnant ewes and their immune response is lacking. Similarly, the protective quality of passive antibodies acquired by lambs from FMD vaccinated ewes, as well as the effect of these antibodies on the immunogenic response in such lambs after FMD vaccination is not clearly known. In due consideration of these points, we contemplated a study on the immune response of pregnant sheep to FMD vaccine type Asia-1 to know the status of maternal antibodies in lambs and the immune response of such lambs to FMD vaccination.

At present the vaccine used for cattle is also recommended for sheep, but adequate information about the potency of FMD vaccine in sheep is not available. The reason may be that the evaluation of FMD vaccine in sheep cannot be done by a needle challenge as in the case of cattle. Thus also a study was conducted to elaborate a suitable method of challenge for vaccinated sheep.

Materials and Methods

Cells. BHK-21 C-13 (Glasgow) and BHK-21 Razi suspension cell lines were used.

Media. Eagle's MEM-G with 10% bovine serum was used for growing cells in monolayer as well as in suspension. For virus infection MEM with with 5% tryptose phosphate broth and 2% bovine serum (PEG-treated) was used.

Cell culture. Both BHK-21 monolayers and suspension cells maintained at IVRI, Bangalore were used in the present study. The BHK-21 monolayer cells were prepared in test tubes for serum

neutralization tests. For vaccine preparation, the virus was grown in suspension cells as described by Kadoi *et al.* (1975).

Virus. The 63/72 strain of FMDV type Asia-1, original isolated from 6 month-old bull calf in Maharashtra State in 1972 was used. The cattle tongue virus adapted to BHK-21 monolayer cells (6 passages) and BHK-21 suspension cells (2 passages) was used for the infection of BHK-21 suspension cells. The Madras strain of FMDV type O isolated in 1975 during an outbreak in Tamil Nadu was also used in this study.

Virus assay. The infectivity titration ($TCID_{50}$) was performed in BHK-21 C-13 monolayer cells and the titre was calculated in a standard way. The complement fixing (CF) antigen titer was determined by a microtest as described by Telling (1975).

Preparation of vaccine. The virus was harvested from BHK-21 suspension cells 20 hrs post infection (p.i.). Suitability of the clarified virus antigen for vaccine was assessed by infectivity and CF titers and the virus was inactivated by BEI. The CF antigen titer was 160 CFU/ml and the infectivity titer was $10^{6.2}$ $TCID_{50}$/ml of the virus suspension used for vaccine preparation.

Binary ethyleneimine (BEI) inactivation of FMDV. 2-bromo-ethylamine hydrochloride (BEA) (Aldrich Chemical Co.) was used for preparation of BEI. 0.1 mol/l BEA was prepared in 0.2 N NaOH and the cyclization was allowed to proceed in at 37 °C for 60 mins as described by Bahnemann *et al.* (1974).

The temperature of a required volume of viral antigen initially adjusted to 37 °C. BEI (1% v/v) was added to virus suspension to obtain a final concentration of 0.001 mol/l after cyclization. Inactivation of BEI was done for 20 hrs at 37 °C after which cold sodium thiosulphate was added to a final concentration of 2%.

Formulation of the vaccine. BEI-inactivated gel vaccine was formulated by using 70 parts of virus, 30 parts of gel and 1 part of glycine buffer to adjust pH 8.7 and stirred for 12 hrs. Then the vaccine was kept at 4 °C. After two weeks, 50 per cent of the supernatant from the top was removed. The vaccine was tested for sterility, safety and potency. Saponin was added to the final product (5 mg/dose).

Experimental animals. Albino-guinea pigs of either sex weighing 450 – 500 g were obtained from Animal Experimentation Station Yelahanka, Bangalore. Local breed of 1 – 2 years-old pregnant sheep unvaccinated against FMD obtained from local villages were screened against FMDV type Asia-1 and O antibodies. Those found negative for antibodies were selected for experiments.

Vaccination of ewes. Seven pregnant ewes in the middle of pregnancy were screened against virus type Asia-1 antibodies and found negative. These animals were grouped into two batches and each batch was vaccinated as follows.

Batch 1. Five pregnant sheep were vaccinated with 1.25 ml of BEI-inactivated aluminium hydroxide gel saponified vaccine type Asia-1. Four lambs born out of these vaccinated mothers were fed with colostrum. They were used for the study of persistence of antibodies up to 4 weeks. Three of these lambs were vaccinated at 4 weeks of age with 0.5 ml dose of the same vaccine.

Batch 2. Two pregnant sheep were vaccinated with 1.25 ml of BEI-inactivated aluminium hydroxide gel saponified vaccine type Asia-1. The lambs born out of these vaccinated mothers were fed with colostrum. These lambs were used to study the persistence of maternal antibodies up to 6 weeks and then vaccinated w 0.5 ml of the same vaccine at the sixth week of age.

Post-vaccination serum samples from the ewes were collect at different intervals for assessing antibody titers. Blood sampl from lambs were collected at 12, 15, 24 and 48 hrs, and 1 to weeks after their birth for assessing the maternal antibody tite Serum samples were collected from these lambs to assess t antibody level 1 – 12 weeks post vaccination.

Challenge of sheep. All the vaccinated and unvaccinated she were challenged with 10,000 $TCID_{50}$ of tissue culture adapt virus type O by intradermolingual route.

Serum neutralization (SN) test. A constant amount of vi (100 $TCID_{50}$) and variable serum dilution method of Cas (1967) was employed. Twofold dilutions of the tested sera w mixed with standard virus and allowed to react for 1 hr at 37 °

The mixtures were then assayed for residual infectivity BHK-21 monolayer cell cultures. SN titers were expressed as l values of reciprocals of the highest positive dilutions.

ELISA. Dynatech Immulon 96 well plates were used in t indirect ELISA performed according to Abu Elzein and Crowth (1978) with slight modification. Antibody levels in sera w quantitated by titration. The highest dilution which gave a disti measurable difference between the control and the test serum w taken as the titer.

Results and Discussion

The antibody response in pregnant ewes vaccinated w vaccine type Asia-1 at the 12th week of pregnancy is d picted in Fig. 1. There is shown also the persistence maternal antibodies against virus type Asia-1 in three colo trum-fed lambs from 15 hrs up to 4 weeks after birth. At t 15th hr, an average SN titer of 1.84 and ELISA titer 50 – 100 were observed. At the 24th hr, both the tit slightly rose, but thereafter they gradually declined. At t 4th week the SN and ELISA titers were 1.62 and 4 respectively.

The above mentioned lambs were vaccinated at the 4 week of their age. After the first week of vaccination avera SN and ELISA antibody titers were 1.83 and 66, respe tively. At the 2nd week the titers of SN and ELISA increas to 2.03 and 100, and then gradually declined to 1.83 and at the 8th week of vaccination (Fig. 1).

The antibody response of two pregnant ewes an anoth experiment is depicted in Fig. 2. The persistance of matern antibodies in the two colostrum-fed lambs of the abo mentioned ewes from 12 hrs up to 6 weeks after birth w studied. An average SN antibody titer of 1.82 was observ at the 12th he. At the 48th hr, the titers increased to 2. and 150 in SN test and ELISA, respectively. The tit gradually decreased and reached 1.22 and 13 at the 6 week.

The above mentioned lambs were vaccinated at the 6 week of their age. An average antibody response af

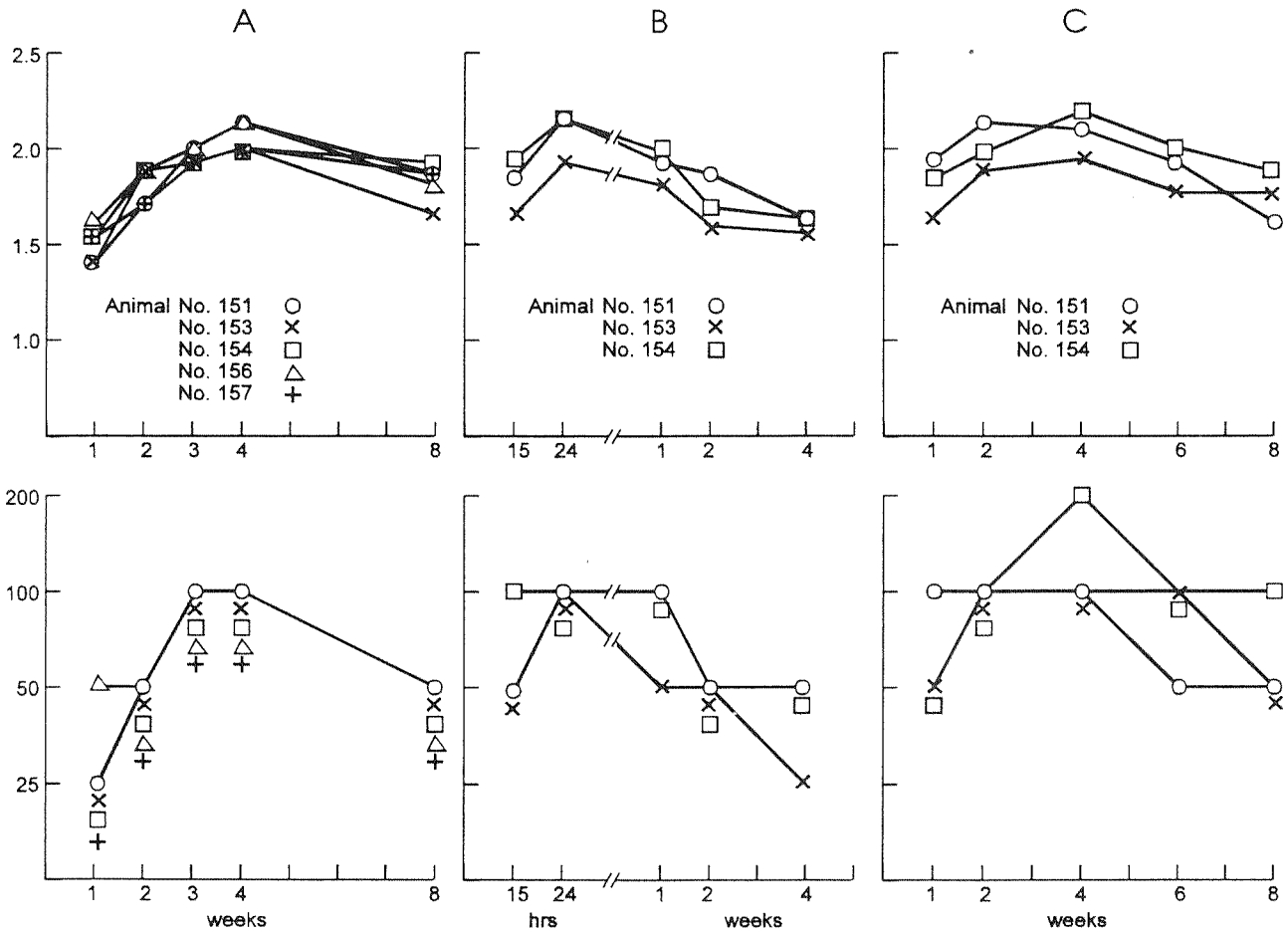

Fig. 1

Antibody response in pregnant ewes (batch 1) vaccinated during pregnancy (A) and in their lambs fed on colostrum vaccinated mothers (B) and in the same lambs after booster vaccination (C)

Abscissa: time after vaccination (A,C) or birth (B); ordinate: antibody titer assayed by SN test (upper part) and ELISA (lower part).

vaccination at the 1st week was 1.48 and 37 in SN test and ELISA, respectively. The titer steadily increased and reached a maximum of 2.05 at the 6th week and then declined to 1.78 at the 12th week.

All the five vaccinated sheep showed an SN antibody titer of 1.80, 1.69 and 1.59 at the 21st, 40th and 60th day, respectively.

Animals were examined after challenge for characteristic FMD lesions in the mouth and feet and found negative except primary lesions on the dorsum of the tongue. None of the vaccinated sheep showed any lesions on the tongue at the site of inoculation. Necropsy and histological examinations were also negative.

In the case of viral diseases threatening young animals, maternal immunity in the form of colostrum acts as a preventive factor. Therefore, to protect young animals, it is convenient to vaccinate their dams to ensure inheritance of adequate maternal antibodies. It has been observed in the field that calves after 4 months of age are susceptible to FMDV but FMD is uncommon in younger calves (Khukhorov *et al.*, 1973). It is generally considered that colostral immunity protects them up to 4 months age. This may also be true in the case of lambs derived from vaccinated ewes or from recovered animals for which adequate data are not available. It is also not clear, whether FMD colostral immunity can adversely affect immunization of young lambs. It has been demonstrated that the presence of colostral antibodies inhibited the serological response to trivalent vaccine (Graves, 1963; Wisniewski and Jankowski, 1971; Prudovsky, 1963).

In the present study the immune response of pregnant sheep to aluminium hydroxide gel vaccine type Asia-1 inoculated at the 12th week of pregnancy indicated that there was no interference in antibody production. All the vaccinated pregnant sheep produced a good immune response without any ill effect. The maximum antibody titers were

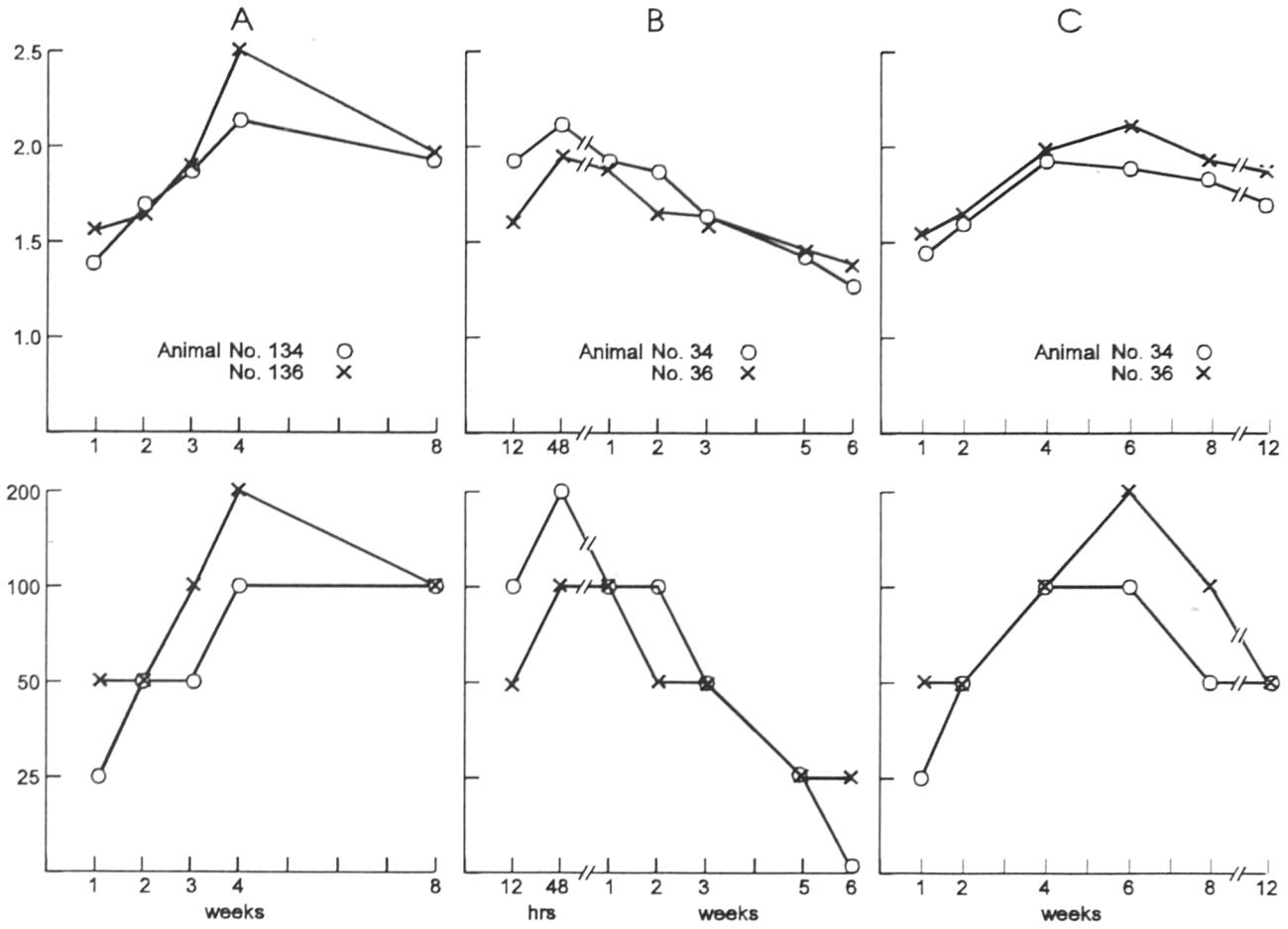

Fig. 2
Antibody response in pregnant ewes (batch 2) vaccinated during pregnancy (A) and in their lambs fed with colostrum from immunized mothers (B), and the booster response (C)
For legend see Fig. 1.

observed 3 – 4 weeks after vaccination. The titers declined after 8 weeks post vaccination.

We have also noticed that the antibody titers in dams declined in later part of pregnancy although the fall of the titer was neither sharp, nor sudden. It was noticed that the lambs fed with colostrum showed a high rise of neutralizing antibodies within first 24 – 48 hrs. In most cases the latter titers were much higher than those of the dams.

During the month before parturition a decrease in serum antibody titers of dams can be found, so that by the time of calving, the colostrum antibody titers are generally higher than the serum antibody titers (Graves, 1963; Šrubar and Jironová, 1966; Shankar and Uppal, 1981).

After ingesting colostrum, serum antibodies in a calf reached peak titres in 4 – 5 hrs (Šrubar and Jironová, 1966). A serum titer of the young may sometimes be as high as that of its mother (Larenaudie *et al.,* 1975). In the present study with lambs a similar trend of neutralizing antibodies was observed. An adequate immune response was observed in pregnancy when the pregnant ewes were vaccinated at the 12th week of pregnancy.

Colostrum-fed lamb born of vaccinated mothers showed an adequate level of antibodies even 12 – 15 hrs after birth. Satisfactory antibody titers could be maintained in them up to 4 – 5 weeks. Cunliffe and Graves (1979) observed that passively immunized lambs resisted infection during the first 30 – 40 days of life.

According to Larenaudie *et al.* (1975) maternal antibodies persisted for more than 5 months in calves. In the present investigation adequate maternal antibody level was noticed in lambs up to 6 weeks. However, the maternal antibodies declined rapidly from the 5th week onwards. No colostral antibodies were detected in the lambs born of unvaccinated mothers.

From our study it can be concluded that pregnant ewes can be vaccinated at 12 weeks of pregnancy without any

untoward reaction. Antibody titers in the dams declined appreciably before lambing. The colostrum fed lambs from vaccinated mothers showed a high level of neutralizing antibodies 12 – 48 hrs after birth. A satisfactory level of maternal antibodies persisted up to 4 weeks of age.

It has been reported by various workers that pyrexia, viraemia and primary lesions are the main clinical features in sheep detected after needle challenge (Fontaine *et al.,* 1966; Rivera *et al.,* 1969; Grosso and Gaggino, 1971).

According to Cardasis *et al.* (1966) there was a correlation between the antibody titer and the degree of immunity in sheep. According to them an antibody titer above 1.80 conferred and absolute protection while titers between 1.80 and 1.40 provided a protection against generalization, and those between 1.40 and 1.20 corresponded to a doubtful protection. But in the present challenge studies it has been noted that sheep with antibody titers of 1.26 and 1.40 were protected against generalization. So on the basis of this limited study it may be concluded that a development of primary lesion, viraemia and pyrexia may be taken as the criteria of the breakdown of immunity in the vaccinated sheep.

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References

Abu Elzein, E.M.E., and Crowther, J.R. (1978): Enzyme labelled immunosorbent assay technique in foot and mouth disease research. *J. Hyg. Camb.* **80**, 391–399.

Bahnemann, H.G., Auge D mello, P., Abaracon, D., and Gomes, I. (1974): Immunogenicity in cattle of foot and mouth disease vaccines inactivated with binary ethyleneimine. *Bull off. int. Epizoot.* **81**, 1335–1343.

Burrow, R. (1968): Excretion of FMD virus prior to the development of lesions. *Vet. Rec.* **82**, 387–388.

Cardassis, J., Pappous, C., Broves, D., Stouraltis, P., and Seimenis, A. (1966): Test of infectivity and dosage of FMD vaccine in sheep. *Bull. off. int. Epizoot.* **65**, 427–439.

Casals, J. (1967): Immunological techniques for animal viruses. *Meth. Virol.* **3**, 146–163.

Cunliffe, H., and Graves, J.N. (1979): Immunological responses of lambs to emulsified foot and mouth disease vaccine. *Arch. Virus Forch.* **32**, 261–268.

Fontaine, J., Dubouclard, C., and Bornarel, P. (1966): Vaccination of sheep against foot-and-mouth disease: study of methods for testing immunity. *Bull. off. int. Epizoot.* **65**, 195–212.

Geering, W.A. (1967): Foot and mouth disease in sheep. *Aust. vet. J.* **43**, 485–494.

Graves, J.H. (1963): Transfer of neutralizing antibodies by colostrum to calves born of foot and mouth disease vaccinated dams. *J. Immunol.* **91**, 251–256.

Grosso, A.M., and Gaggino, O.P. (1971): Experimental foot-and-mouth disease in the sheep. Detection of the virus in the nasal cavity. *Revista de Investigaciones Agro.* **3**, 109–116.

Kadoi, K., Zoletto, R., and Kobori, S. (1975): Studies on BHK cell culture suspension. Use of a new model of mini vessel. *Bull. cell. agric. vet. Med. Nihon Univ.* **32**, 41–46.

Khukhorov, V.M., Zubov, I.V., Muravev, V.K., Onufriev, V.P., Proninia, M.A., Smirnov, V.I., and Filatov, I.P. (1973): Evaluation de la fevre aphteuse dans les regions on vaccination a ete pratiquee sur une large echelle. *Veterinariya* **I.** (Moscow), 50–51.

Larenaudie, B., Remond, M., Guerin, D., and Bordas, C. (1975): A study of the persistance of foot and mouth disease antibodies in calves born to vaccinated mothers. *Bull off. int. Epizoot.* **83**, 337–342.

Prudovsky, S. (1963): Some aspects of the immune response of cattle to foot and mouth disease vaccines. *Refuah. Vetes. Israel* **30**, 77–85.

Rivera, R., Ardo Gaimarini, C.R., and Pereeira Gomes, J. (1969): Fiebro aftosa en ovinos. Estudios experimentales. *Patologia Gac. Vet. Bs. Aires* **32**, 226–236.

Shankar, H., and Uppal, P.K. (1981): Transfer of antibodies through colostrum in calves born to FMD vaccinated cows. *Indian J. anim. Sci.* **6**, 622–626.

Šrubar, B., and Jironová, M. (1966): On the study of specific colostrol immunity of calves of cows that had been vaccinated against foot and mouth disease. *Vet. Med.* (Prague) **11**, 303–310.

Telling, R.E. (1975): Industrial production of foot and mouth disease vaccine using BHK suspension cells. Some comparative results relating *in vitro* assays and cattle potency. *Rep. Mtg. FAO, Byasoia, Italy,* 95–100.

Wisniewski, J., and Jankowski, J. (1971): Passive immunity in calves acquired from the colostrum of ewes vaccinated against foot and mouth disease. *Med. Vet.* **27**, 591–593.