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POST-VACCINATION REACTIONS IN DONKEYS VACCINATED WITH AFRICAN HORSE-SICKNESS POLYVALENT VACCINES (*)

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Post-vaccination reactions with fatal results in donkeys, mules and horses have been reported following vaccination with polyvalent mouse neurotropic Horse-Sickness vaccines that have been widely used for many years in Africa and Asia.

During the recent epizootic of African Horse-Sickness in the Middle East, neurologic involvement of vaccinated equines has been reported from several countries (REID [1960], KAVEH [1961], HUQ [1961], ORHAN [1961], SHAH *et al.* [1964]). Also, type 2 Horse-Sickness virus was isolated from the brains of vaccinated equines in Israel (NOBEL and NEUMANN [1961] and India (PAVRI and ANDERSON [1963]. Several cases of post-vaccination blindness in mules have been observed in Iran by Drs. SIADAT, MAURER and OLVEY in 1961 and 1962 (Personal communication).

It has been reported in a previous paper (HAZRATI and OZAWA [1965]) that approximately 10 percent of the donkeys vaccinated with polyvalent mouse-brain vaccine which contained types 1 to 6 virus showed very severe reactions and half of them died. Approximately 5 percent of the donkeys vaccinated with monovalent (type 9) mouse-brain vaccine showed a mild reaction but none of them died. Since it was not always possible to isolate virus from the brains of donkeys showing post-vaccination symptoms, a possible correlation between the neurological post-vaccination reactions and mouse-brain tissue incorporated in the vaccine remained to be investigated.

In the present experiment, polyvalent mouse-brain vaccine, polyvalent tissue-culture vaccine, and normal mouse-brain tissue suspension were inoculated into donkeys to compare their reactions.

MATERIALS AND METHODS

Virus. — Seven serologically different mouse-adapted neurotropic vaccine

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strains (A 501, OD, L, Vryheid, VH, 114 and Karen) were obtained from the Onderstepoort Veterinary Institute. A mouse neurotropic, attenuated, Iranian strain (S 2) was also used as the type 9 vaccine virus. The 100th passage level of each virus in adult mouse brains was stored at — 25° C, and a few additional passages were made in adult mice to prepare fresh seed virus to make mouse-brain vaccine or to adapt to cell cultures.

Cell Cultures. — The monkey kidney cell line, commonly known as MS cells, was obtained from the National Institute of Health, Japan. The methods of cultivating MS cells, constituents of media used, and adapting Horse-Sickness virus to the cells have been described (10, 11).

Preparations of vaccine. — The method of producing polyvalent mouse-brain vaccine was basically the same as that established by the Onderstepoort Veterinary Institute (1). The 103rd passage in 5-week-old Swiss albino mice of the 8 strains was used. Five brains of each strain were mixed and macerated in 200 ml of sterile distilled water kept in a chilled tissue grinder. The brain suspension, without centrifugation, was mixed with an equal volume of freeze-drying diluent, lactose-peptone-Tris buffer (LPTB) solution. As a control, normal mouse-brains from the same colony of mice were treated in the same manner and freeze-dried at the same time.

The freeze-drying diluent, LPTB, was prepared by mixing the following two solutions: solution (*a*) 200 g lactose and 40 g peptone dissolved in 1,000 ml of distilled water; and solution (*b*) 250 ml of 0.2 M Trishydroxymethyl-amino methane mixed with 207 ml of 0.2 M HCl and 543 ml of distilled water. The final *p*H was approximately 7.4. Four hundred units/ml penicillin and 400 μ g/ml streptomycin were added to the diluent, which was sterilized by filtration.

Polyvalent tissue-culture vaccine was prepared in a similar manner using the 7th tissue-culture passage in MS cell cultures of the same 8 strains attenuated by 103 mouse brain passages. The maintenance media of infected cell cultures were centrifuged at 2,500 r.p.m. for 15 minutes, and the supernatants were used. Ten ml of each of 8 strains were mixed.

To the mixture, 20 ml of sterile distilled water and 100 ml of freeze-drying diluent, LPTB, were added.

Both mouse-brain and tissue-culture vaccines were dispensed in 30 ml bottles in volumes of 5 ml and freeze-dried overnight. Dried vaccines were vacuum sealed and stored at 4° C.

All freeze-dried vaccines and normal mouse-brain suspensions were reconstituted by adding 5 ml of sterile distilled water, and 1 ml of the suspension was inoculated intramuscularly into each donkey.

Virus isolation. — Blood samples from 6 donkeys kept in the Razi Institute stable were collected in equal volumes of O.C.G. solution (50 percent aqueous glycerin containing 0.5 percent potassium oxalate and 0.5 percent carbolic acid). The O.C.G.-blood specimens were diluted with sterile distilled water in equal parts and inoculated into 3-day-old suckling mice, each receiving 0.03 ml intracerebrally.

Brain tissue from donkeys was macerated in a tissue grinder with five

volumes of physiological saline containing 10 percent calf serum. The brain suspension was centrituged at 3,000 r.p.m. for 30 minutes at 4° C, and the supernatant was inoculated intracerebrally into suckling mice. Brains of suckling mice which died more than 2 days after inoculation were harvested. A 10 percent mouse brain suspension in serum-saline was prepared, centrifuged, and the supernatant inoculated intracerebrally into adult mice. The brains harvested from these mice were used either for neutralization tests or for adapting the virus to MS cell cultures.

Neutralization Tests. — To identify the viruses isolated from donkeys, neutralization tests were carried out both in adult mice and MS cell cultures. Preliminary neutralization tests were made in adult mice by inoculating them with 10-fold dilutions of infected mouse brain suspension mixed with each of a different antiserums in the manner described in a previous paper (3). After this screening test, mouse brain suspension containing approximately 100 MLD_{50} of virus was mixed with 5-fold dilutions of each of the antiserums under test. The mixtures were incubated 60 minutes at 37° C and inoculated into mice. To make sure that the suspension contained no other virus types, brains of sick mice that had been inoculated with the virus suspension mixed with higher concentrations of the antiserum were harvested and the neutralization tests were repeated with the isolates in the same manner.

The strains adapted to MS cell cultures were also tested serologically as previously described (3).

Donkeys. — Healthy Iranian donkeys of mixed breed in villages near the Razi Institute were used for the experiment. They were approximately 1 year or older. None of them had been vaccinated against horse sickness. Animals were inoculated in October 1966 and engaged in ordinary labour by the owners, who were instructed to pay particular attention to the health of these animals during the following two months.

Any signs of abnormalities of these animals were reported, and most of the donkeys showing reactions were brought into the Institute for close observation.

RESULTS

Among 137 donkeys vaccinated with polyvalent mouse-brain vaccine, post-vaccination reactions were observed in 10 donkeys starting approximately 4 weeks after vaccination (Table I). Within a few days 3 of them, cases 1, 2 and 3, died showing signs of neurologic involvement. Six of seven other sick donkeys were brought into the Razi Institute stable for closer observation. Three of them (cases 4, 5 and 6) showed general reactions and 3 others (cases 7, 8 and 9) were blind. Case 10 was reported to be sick with reactions similar to cases 4 and 5 but recovered within a few weeks.

When cases 4 and 5 were brought into the Institute stable 5 weeks after vaccination, both of them showed anorexia and discharge from their eyes. Respiration and body temperatures were normal. They recovered within 3 weeks.

TABLE I

Post-vaccination reaction in 137 donkeys following vaccination with polyvalent mouse-brain vaccine.

CASE No	TYPE OF REACTION	REACTION COMMENCED	COURSE OF DISEASE	VIRUS ISOLATION	
				FROM BLOOD	FROM BRAIN
1	Neurologic	27th day(*)	Died 2 days later (**)	ND (***)	ND
2	Neurologic	27th day	Died 3 days later	ND	ND
3	Neurologic	28th day	Died 3 days later	ND	ND
4	General	34th day	Recovered 20 days later	Negative	ND
5	General	34th day	Recovered 16 days later	Negative	ND
6	General	37th day	Recovered 28 days later	Negative	ND
7	Neurologic	28th day	Killed 1 day later	Negative	Type 1
8	Neurologic	33rd day	Killed 4 days later	Negative	Type 2
9	Neurologic	46th day	Killed 12 days later	Negative	Negative
10	General	40th day	Recovered 14 days later	ND	ND

(*) Days after vaccination.

(**) Number of days from the day on which vaccination reactions commenced.

(***) Not done.

Case 6 was brought into the same stable 38 days after vaccination. Slight incoordination of gait was noticed and the animal took very little feed and water. There was a sign of constipation. Occasional twitch of muscles between thigh and abdomen was observed. There were a few scars on the head and a slight discharge from the left eye, but the vision appeared to be normal. Within 3 weeks, these abnormalities disappeared gradually, and recovery was complete at the end of the following week.

When case 7 was brought into the Institute stable 30 days after vaccination, the animal was completely blind with a few scars on its head and ears. The animal kept the head down, sticking the tongue out of the mouth due to the paralysis of the muscles. There were no signs of incoordination of gait. Other clinical findings were slight nasal discharge, very shallow breathing and irregular weak pulse. The body temperature was 37.5° C. A necropsy was performed for patohlogical examination and virus isolation. Gross pathological findings were slight subendocardial haemorrhages, edematous swelling in a part of the lung, and slight congestion on the surface of the brain. All other organs appeared normal.

Case 8 was first examined 5 weeks after vaccination. Despite blindness of one eye, the animal still appeared normal. Next day, anorexia was noticed and on the following day the animal was found down with both eyes completely blind. The animal lost appetite completely, and the pulse and breathing were very weak. Body temperatures during these 3 days were between 36.5. and 37°C. The animal was killed on the same day. The only pathological change found in the organs was slight subendocardial haemorrhage as seen in the previous case. The brain was harvested for virus isolation.

Case 9 was first examined 48 days after vaccination. There was discharge from the left eye which was blind. The other eye appeared to be normal. Slight anorexia was observed, but other clinical records were normal. The same condition lasted for 10 days, when the animal was necropsied. There were no apparent pathological changes in the organs of the animal.

Affected eyes of cases 7, 8 and 9 showed no response to artificial light and no reflex reaction to external movements. Their pupils were completely dilated showing no response to bright light.

Two viral agents were isolated from the brains of cases 7 and 8. No virus was isolated from the blood of the donkeys studied. Neutralization tests confirmed that the virus isolated from case 7 was type 1 Horse-Sickness virus, and the virus from case 8 was type 2. Attempts to identify other AHS virus types from these isolates failed. It was evident that these two strains were mouse adapted neurotropic vaccine strains of virus as the first passage killed most of adult mice within a week after intracerebral inoculation.

No post-vaccination reactions were observed either in the 95 donkeys inoculated with normal mouse brain tissue suspension, or in the 36 donkeys vaccinated with polyvalent tissue-culture vaccine.

DISCUSSION

Post-vaccination reactions were seen only among the group of donkeys vaccinated with polyvalent mouse-brain vaccine. The morbidity (7.3 percent) and

mortality (4.4 percent) rates were within the ranges of those reported previously. The addition of type 7 (Karen) and type 9 (S 2) vaccine strains to the 6-type polyvalent vaccine did not cause more severe reactions in vaccinated donkeys. There was no reaction in the 95 donkeys inoculated with normal mouse-brain tissue suspension which suggest that the mouse-brain tissue incorporated in the polyvalent vaccine is not the primary cause of neurologic reactions.

Isolation of type 1 and type 2 neurotropic viruses from 2 different donkey brains indicates that these strains may multiply in the brains of donkeys producing blindness and other neurologic disorders in vaccinated donkeys. Isolation of type 1 neurotropic virus from the brain of a vaccinated donkey has not been reported previously, but type 2 vaccine virus has been isolated from the brains of vaccinated horses in India (14) and Israel (8).

It is an interesting coincidence that type 2 (OD) virus attenuated by mouse-brain passages produced encephalitic reactions both in the horse and donkey. Therefore, the use of this strain in polyvalent mouse-brain vaccine can only be recommended with reservation. However, the same strain of virus, after 7 additional passages in MS cell cultures, has been employed in the polyvalent tissue-culture with satisfactory results not only in donkeys but also in horses and mules in Iran and in North African countries. This might be due to the additional passage in tissue cultures modifying its neurotropism for donkeys or to the absence of mouse-brain tissue which may act as the primary inducer of encephalitis as demonstrated with pertussis vaccine by LEVINE and WENK (1963). Unfortunately this could not be determined due to the limited number of donkeys available.

According to the findings of ERASMUS (1963), type 2 (OD) virus was one of the least neurotropic strains when tested in guineapigs by intraperitoneal and/or intranasal infection. However, it appears that the degree of tropism in guineapigs does not always correlate with that in equines.

No virus was isolated from the blood of these donkeys even though virus types 1 and 2 were recovered from the brains of two showing encephalitic symptoms. The reason may be that these blood samples were collected from donkeys more than one month after vaccination. It should, however, be noted that none of these 6 donkeys with either encephalitic or general reactions had body temperatures higher than 38° C during our observation period.

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SUMMARY

Of 137 donkeys vaccinated with polyvalent (8 types) AHS mouse-brain vaccine, 3 died approximately one month after vaccination. Three other donkeys that had become blind were sacrified for virus isolation. Type 1 virus was isolated from one of them, and type 2 virus from another. Both types had very short incubation periods in adult mice indicating that they were neurotropic vaccine strains. Four other donkeys showed reactions between the 4th and 6th week after vaccination but they recovered within a few weeks.

There were no signs of abnormality among 95 donkeys inoculated with normal mouse-brain tissue prepared in the same manner as polyvalent mouse-

brain vaccine, nor among 36 donkeys vaccinated with polyvalent (8 types) tissue-culture (MS) vaccine.

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RÉSUMÉ

Sur 137 ânes vaccinés contre la Peste équine africaine avec le vaccin polyvalent (contre 8 types de virus) préparé sur cerveau de souris, 3 moururent approximativement un mois après la vaccination. Trois autres ânes qui étaient devenus aveugles furent abattus aux fins d'isolement du virus. Le type 1 fut isolé chez l'un d'eux et le type 2 chez les autres. Les deux types eurent des périodes d'incubation très courtes chez la souris adulte, indiquant qu'il s'agissait de souches vaccinales neurotropes. Quatre autres ânes firent des réactions 4 à 6 semaines après la vaccination mais guérirent en quelques semaines.

On n'observa pas de signes anormaux parmi 95 ânes auxquels on inocula du tissu cérébral normal de souris, préparé de la même manière que le vaccin polyvalent à base de cerveau de souris, ni parmi 36 ânes vaccinés avec du vaccin polyvalent (contre 8 types de virus) préparé sur culture de tissu (MS).

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RESUMEN

De 137 burros vacunados contra la Peste equina africana con vacuna polivalente (contra 8 tipos de virus) preparada en cerebro de raón, 3 murieron aproximadamente un mes después de la vacunación. Otros tres burros que quedaron ciegos fueron sacrificados para fines de aislamiento del virus. Se aisló en uno de los mismos, el tipo 1 y en los demás el tipo 2. Ambos tipos tuvieron períodos de incubación muy breves en el ratón adulto, indicando que se trataba de cepas vacunales neurátropas. Otros cuatro burros tuvieron reacciones de 4 a 6 semanas después de la vacunación, aunque se curaron en algunas semanas.

No se observaron signos anormales entre 95 burros en los que se inoculó tejido cerebral normal de ratón, preparado de igual modo que la vacuna polivalente a base de cerebro de ratón, ni entre 36 burros vacunados con vacuna polivalente (contra 8 tipos de virus) preparada en cultivo de tejido (MS).

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