Arch. Inst. Razi, 1963 15, 73-81

THE CONTRIBUTION OF IRAN IN COMBATING RECENT EPIZOOTIC OF AFRICAN HORSESICKNESS IN THE MIDDLE EAST

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Introduction

In the summer of 1959 a fatal disease broke out in the South Eastern regions of Iran and caused some mortality among horses. This disease which was unknown in the country was taken under consideration and systematic study. In the meantime the disease was reported by veterinary services of the neighbouring countries and from samples submitted by Pakistan government to the Onderstepoort Laboratory, an African Horsesickness virus was isolated.

The spread of this outbreak was not so extensive and it subsided in all the affected areas in the winter of 1959.

In the spring of the following year, the weather being hot and humid in the Southern part of Iran, outbreaks of the disease were reported to be much more intense and spreading in all over Persian Gulf areas. Immediately a group of Razi Institute workers and the field veterinarians were sent to that part and the result of their clinical and post-mortem examination showed undoubtedly the disease to be African Horsesickness. This was reported to the Ministry of Agriculture and O.I.E. in April 1960.

To confirm the diagnosis, adequate blood samples from the affected animals were sent to the Onderstepoort Laboratory, South Africa, and to the Razi Institute, Hessarak, Iran.

(1) *U.N. Conf. on the App. of Sci. & Techn. for the Benefit of the less Deve. Areas, Geneve. E/conf.*, 39/c/423, 1963.

The first attempt to isolate the virus at the Razi Institute proved successful and the virus was isolated in May 1960.

Meanwhile information was received from the Onderstepoort Laboratory confirming the presence of the A.H.S. virus in the submitted blood samples.

Confirmation of the presence of Horsesickness in Iran as well as in Pakistan, Afghanistan, India, Turkey, in this time, made this disease a subject of great importance as regard to its international and economical aspect.

The necessary information was collected, in Razi Institute, about the different aspect of the disease in order to work out an efficient vaccine for its control.

Fortunately at this stage theFood and Agricutlure Organization of the United Nations decided to send Dr. Howel, an expert on A.H.S. from the Onderstepoort Laboratory, to Iran, to conduct a training course concerning Horsesickness at Razi Institute in which representatives from India, Iraq, Pakistan, Afghanistan, Turkey, Jordan, Syria and Iran took part.

In addition to the technical demonstrations and lectures given during this course, an ideal opportunity presented itself to discuss the general problem of the disease in this area. These discussions gave rise to an interesting exchange of views and emphasized the need for international coordination to put into effect the basic sanitary policies to prevent further spread of the disease.

During this period the disease progressed rapidly over the infected areas and gradually appeared in the countries besides the above mentioned ones, so that by October 1960 all countries stretching from Turkey to India, having a horse population of about 13,000,000 were overrun with the disease.

Such being the case, in 1960, all countries in this area were turned into a vast combating zone to control and eradicate the disease. Sanitary control measures were applied and a mass vaccination carried out.

Although the cost of this campaign was heavy and thousands of soliped animals were lost, nevertheless the result was satisfactory, the disease, more or less, came under control and the danger of its spreading into Europe passed.

Horsesickness was probably introduced to the Middle East via the Persian Gulf and Arabian peninsula. The mode of transmission of the disease from the enzootic region in Africa to these new foci has not been definitely determined, but introduction of the infected animals or infected vectors by means of sea or air transport must not be overlooked.

The appearance of A.H.S. in this part of the world, as well as its occurence in Palestine, Lebanon and Syria, in 1944, shows that the disease, which is usually regarded as being confined to the continent of Africa can

appear in any part of the world where the climatic and possibly other conditions are favourable for the insect vectors of this disease. This outbreak also stresses, once more, the fact that the danger to the unaffected countries has not been erased and in the future, as McIntosh anticipated in 1958, "...outbreaks may well occur in continents hitherto free from the disease..." and that in this case the importance of increasing our knowledge of this disease should not be overlooked.

The nature of the disease and its present distribution

Horse sickness is an epizootic, insect-born, infectious disease of equine animals characterized by hyperthermia, cardiovascular changes, pulmonary and subcutaneous oedema. Clinically A.H.S. may be divided into four distinct forms: cardiac, pulmonary, mixed and horsesickness fever. The cardiac form predominated in the recent outbreak in the Middle East.

The disease has been known in South Africa for many centuries and now it is widely distributed in the continent of Africa and in Cyprus, Afghanistan, India, Iraq, Iran, Jordan, Lebanon, Pakistan, Turkey and Syria.

In Africa the disease is found only in certain localities, occurring under definite telluric and climatic conditions (Henning 1949).

Informations concerning A.H.S. in Asia is sufficiently comprehensive to show that the climatic and environmental conditions are closely related to onslaught and disappearance of the disease as it has been found in Africa.

The similarity is seen at first in the appearance of the disease in the hot humid climate of the Persian Gulf during the spring, its spreading nature throughout the hot summer reason, and its negligible incidence during the cold winter months. It was also noted that the horses kept housed in mosquito-proof stables and those moved to high mountainous pastures were not affected by the disease.

Horsesickness is caused by a filtrable virus which is present, in a high concentration, in the blood during the febrile stage, and it also exists in the internal organs and tissue fluids of the affected animals.

There exists an antigenic plurality of strain of H.S. virus (Alexander 1935). McIntosh 1958, using 42 strains, showed that these strains could be grouped in 7 main immunological types. The virus can be isolated from infected materials by intracerebral injection of baby or adult mice. The virus as isolated from the equine cases, has viscerotropic properties which can be demonstrated by the inoculation of susceptible solipeds. In addition to horses, mules and donkeys; dogs and goats are readily infected, either by injection

(goats and dogs), or by feeding on infected meat (dogs). The virus in these animals keeps its viscerotropic nature.

In 1932 Nieschlutz reported the susceptibility of the white mice to the virus by intracerebral route and this was confirmed later on by Alexander in 1933. Alexander also recorded the full susceptibility of the guinea-pig and rat to the virus and offered a valuable technique for the neurotropic fixation and attenuation of the virus. McIntosh, in 1958, showed that ferret, being susceptible to the viscerotropic virus, is the most suitable laboratory animal for isolation of A.H.S. virus from previously immunized sick horses.

Adult mice are the most suitable animals for neurotropic fixation of Horsesickness virus. These animals when injected intracerebrally, become easily infected. In the first two or three passages the virus is not virulent enough to cause a high mortality in mice, i.e. only some of the inoculated mice show the symptoms of the disease and the incubation period may be prolonged up to three weeks or more. As the serial passages progress the virus becomes adapted and accustomed to neurotropic propagation and the incubation period decreases gradually so that after a few passages the mortality is usually 100 per cent and the interval between injection and death reaches a constant minimum, (4-5 days).

Apparently the virulence increase of the virus for mice corresponds to the decrease of its virulence in horses, so that through successive passages the virus loses its pathogenicity for solipeds without losing its antigenic and immunizing potentialities.

The neurotropic virus is pathogen to mice, guinea-pigs, and rats and the only satisfactory method of infecting is by direct intracerebral injection. In these animals the disease assumes neurotropic characteristics exclusively and the virus multiplies, remaining concentrated in the brain. The neurotropic virus does not produce a specific encephalitis or mortality in horses even by intracerebrally inoculation. The virus keeps its neurotropic nature and after at least one passage in equines, retains its attenuation, (Alexander, 1935).

A locally isolated virus has recently been adapted to the primary hamster kidney cells in Razi Institute, (Mirchamsy and Taslimi, 1962). The pathogenicity of the adapted virus, for mice, seems to be slightly decreased after 12 serial passages, (Mirshamsy, unpublished).

Sanitary measures and prevention

Although stabling susceptible animals during night, moving them to areas free from the disease or to high mountainous pastures, application of

insectisides, destruction of affected animals and isolation of the infected zone by quarantine regulations, and so on, help to prevent the introduction of carriers from infected to clean areas, and to protect individual animals; it is absolutely impractical to depend on these measures on a large scale basis.

Therefore, in order to prevent, control and eradicate the disease and to build up an immune equine population in an affected area, besides the above sanitary measures, an annual mass vaccination scheme of solipeds by a suitable vaccine is of absolute necessity. Among the different methods of vaccination, use of the neurotropic polyvalent vaccine has so far yielded the best result.

Neurotropic polyvalent vaccine

The polyvalent neurotropic H.S. vaccine, prepared at the Razi Institute according to the technique developed in Onderstepoort Laboratory, consists of pooled culture materials of 6 attenuated antigenically different H.S. virus strains propagated in mice brain. Each of these 6 virus strains which we call vaccine strains, is a virulent H.S. virus of the field having undergone a complete attenuation through one hundred or more intracerebral passages into adult mice.

These vaccine strains were kindly supplied by the Onderstepoort Laboratory and adequate numbers of freeze-dried ampoules were prepared from each after 2 passages in adult mice.

To prepare the vaccine, each vaccine strain has to be separately treated as follows : 10-20 mice are injected intracerebrally by reconstituted freeze-dried ampoule of the vaccine strain in 10 per cent horse or rabbit serum saline. After an incubation period of 3-4 days, the mice, while in extermis, are killed by etherization and the brain tissue harvested. A ten per cent suspension is then prepared from the infected mice brain in serum saline. The supernatant of this suspension after centrifugation at 3000 r.p.m. for 30 minutes and testing for bacteriological sterility, is used as the working seed virus which can be kept at 4°c. for a few months.

To propagate the virus in mice brain, the required number of Swiss Albino adult mice are injected intracerebrally by 0.05 ml. of diluted working seed virus in serum saline. The mice are kept in suitable cages, labelled with the type of virus, the number of inoculated mice contained in the cage and the date of inoculation, under close observation. The incubation period in mice varies between 60 and 96 hours according to the strain of virus.

The disease which is exclusively neurotropic appears in different forms

depending mainly on the amount of inoculated virus and somewhat on the type of the virus itself. The sick mice, while in extremis, are etherized and then pinned on a dissection board with their backs uppermost. Each mouse is saturated with surgical alcohol. With a sharp pair of scissors and forceps the skin covering the wither and cranium is removed exposing the skull which is again sprayed with alcohol. Using a sterile straight-pointed pair of scissors and forceps, the calvaria is cut and pushed upwards and the brain removed on the point of the scissors. The harvested brains are put in a suitable sterile tube, which is labelled, stoppered and stored at -20° C until required.

As mentioned before the vaccine is a polyvalent one, incorporating 6 different virus strains. Therefore, the infected mice brain of all different types of virus are added in equal amount in the vaccine. The desired amount of infected brain (6 x n) is collected from deep-freeze, allowed to thaw, mixed and then macerated in an Atomix apparatus with sufficient chilled distilled water for 2-3 minutes. The resultant fine suspension is diluted with chilled distilled water and buffered-Lactose-Peptone solution so that the volume of each one of the distilled water and buffer added to the brain tissue reaches (5 x 6 x n) ml.

Penicillin, 1.000 units and streptomicin, 1 Milgm. per ml. of the final suspension are added. This virus suspension which should be bacteriologically sterile is the A.H.S. vaccine.

The vaccine is distributed in bottles and then freeze dried in a Stockes machine. The bottles containing the dessicated vaccine are evacuated, stoppered, sealed and random samples from each batch are then taken for final titration in mice, bacteriological sterility and the safey tests. When the results of these tests are satisfactory, the bottled vaccine is labelled and stored at 4° C. till the time of distribution (Rafyi, 1961, - Hazrati, 1961).

The vaccine at Razi Institute is actually issued in 25 c.c. bottles containing each 5 doses of freeze dried material. Each vaccine dose contains not less than 20,000 mouse m.l.d. of virus from each one of the neurotropic strains. The dose is the same for mule, horse and donkey no matter what age, size or breed. The vaccine must be transported in iced thermos flasks, wherever possible and must be stored in a refrigerator. It should be used as soon as possible, but not after a lapse of four weeks from the time of its removal from the refrigerator, and in this interim it must be stored in a dark cool place.

More than 1,600,000 doses of polyvalent neurotropic vaccine have been prepared at Razi Institute, since May 1960. Part of this amount of vaccine has been used inside the country and the remaining 650,000 doses, issued to the following neighbouring countries; free and reduced to Afghanistan, at reduced cost to Pakistan and at cost price to others :

Name of the country	Amount of the vaccine
Turkey	375,000 doses
Pakistan	21,500 "
Afghanistan	77,000 "
Syria	30,000 "
India	30,000 "
Cyprus	51,000 "
Jordan	29,000 "
Lebanon	5,000 "
Iraq	30,000 "
Other	1,500 "
Total	650,000 doses

As by the time of the disease onset, there was no, or not enough, facility for preparing the vaccine in the above mentioned countries; the importance of the Razi Institute's contribution in providing the necessary vaccine for the disease campaign in this part of the world can be realized.

Vaccination

The vaccine contained in each bottle must be dissolved in 25 ml. sterile saline, and as the vaccine loses its activity very rapidly after being dissolved, this must be done immediately before use. The vaccinal dose is 5 ml. of dissolved vaccine, given subcutaneously.

The solipeds can be inoculated at any age. Mares may be inoculated two weeks before and after foaling, and foals from immune dams should not be vaccinated before seven months old. Livestock owners are advised to rest their vaccinated animals for 3 weeks following inoculation.

Among the military horses and mules, and those of the Razi Institute, where this advice has been followed, no severe post vaccination-reaction except the rise in temperature on about the seventh day after vaccination has been seen.

However, a severe reaction due to H.S. vaccination has been reported from the field showing in many cases a mortality varying from 0.1. per cent of vaccinated animals in horses to 5 per cent in donkeys. This high accident rate, especially in donkeys, encountered with the use of polyvalent neurotropic vaccine, caused much difficulty in combating and eradicating A.H.S. in the Middle East. Some work has been done to clarify the causes of this severe

post-vaccination reaction, which occurs particularly in donkeys, but the result has not been satisfactory. (Hazrati, 1961).

Immunity

The reports received from the field, although limited, are sufficiently comprehensive to show that vaccination results, even under field conditions, have so far been satisfactory.

The immunity is easily demonstrable four weeks after vaccination and is durable for at least one year. The neutralization index of several sera, 3-4-5 months after being vaccinated, was found to be about 100 against type six, one of the six vaccine strains taken as an example. This titre indicates that a solid and complete immunity develops 3-5 months after vaccination (Hazrati 1962).

The occurence of the disease among the vaccinated horses, especially inside and around the infected area where the disease is active, must be attributed to this slow immunity development. It is worth noting that no single case of H.S. has been seen, among the military horses and those of the provinces far from the affected area, after immunization in Iran. Nearly the same result has been obtained from the other affected countries confirming that this vaccine is the most satisfactory one for practical and economical purposes and affords adequate immunity against the virus strains present in this part of the world. The virus strain isoloted at Onderstepoort Laboratory from a blood sample received from Pakistan was found to have an antigenic similarity with type six of H.S. virus. This has also been shown by cross immunization in Iran, using a local Iranian strain (Rafyi, 1961). Later on by "intracerebral neutralization test in mice", using 5 mouse adapted local strains, selected from a total of 53 strains isolated in Iran, the same result was obtained. As this antigenic relation was not complete, it was decided to use a local strain in polyvalent vaccine. Strain S2 was selected for this purpose and it was found to be fully attenuated, possessing relatively a good immunizing potentiality at its 65th generation in adult mice, (Hazrati & Taslimi). This strain will be incorporated into the present polyvalent vaccine in the future in order to increase the value of the vaccine.

SUMMARY

1. A brief account is given of the epizoology of African Horsesickness and

the recent outbreak of the disease in the Middle East.

2. The nautre of the disease and its present distribution is discussed. Attention is paid to the types of the strains of virus isolated in Iran which was found to be closely related to type 6.
3. The value of vaccinating solipeds, using polyvalent neurotropic vaccine, to control the disease in the recent outbreak is shown.
4. Details of the technique employed for the preparation of polyvatent neurotropic vaccine at Razi Institute, Iran, are given.
5. It is mentioned that a locally isolated strain has been fully attenuated, possessing relatively a good immunizing potentiality, and that this attenuated strain will be, in the future, incorporated into the present polyvalent vaccine in order to increase its potency for the equine population of this part of the world.
6. The contribution of Iran in the disease campaign, in the Middle East, either by organization of the special training course concerning horsesickness, at Razi Institute, or by providing the necessary vaccine to help the neighbouring countries are described.

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