Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Studies on the Neurotropic Virus of Horsesickness V.

The Antigenic Response of Horses to Simultaneous Trivalent Immunization.

By R. A. ALEXANDER, Section of Protozoology and Virus Diseases, Onderstepoort.

It has been shown previously that there exists a plurality of antigenically different strains of horsesickness virus. Consideration of this important fact, in the light of results obtained with the neurotropic virus vaccine both in the laboratory and in the field during the season 1934-35, has shown that the problem of immunization has become one of the development of complete polyvalent immunity. On purely theoretical grounds it is reasonable to believe that this might be accomplished most satisfactorily by a series of injections each of which comprised a certain infecting dose of a different virus strain, but the practical difficulties attached to such a procedure are so vast that it is essential to limit the number of injections to a minimum. If possible immunization should be confined to a single subcutaneous injection of a mixture of the different strains. This is the procedure which has been adopted but it is necessary to record the antigenic response in horses as determined by in vitro serum neutralization tests and by in vivo immunity tests.

From time to time during the course of routine vaccine production by the method described (this journal) horses were injected with material taken at random from cold storage and kept at room temperature (±80° F.) for periods up to 14 days so as to approximate the conditions under which immunization was carried out in the field. Details of 5 such injections are the following:—

2/8/34.	Horse 20987.	10 cc. subcutaneously vaccine batch 8 prepared 24/7/34.
2/8/34.	Horse 20991.	10 cc. subcutaneously vaccine batch 10 prepared 1/8/34.
3/10/34.	Horse 20985.	10 cc. subcutaneously vaccine batch 24 prepared 27/9/34.
3/10/34.	Horse 20968.	10 cc. subcutaneously vaccine batch 27 prepared 3/10/34.
22/11/34.	Horse 20941.	10 cc. subcutaneously vaccine batch 40 prepared 20/10/34.

Each of the animals showed the anticipated mild febrile reaction to the vaccine and until required for bleeding were stabled in company with a number of susceptible horses, exercise being restricted to running in a bare dry paddock from 10 a.m. to 3 p.m. each day. During this period no cases of natural horsesickness occurred in the stables so that it is believed that between the time of injection and the time blood was tapped for the collection of serum the horses were not exposed to natural infection.

Since it has been shown that the antibody content of serum reaches a maximum approximately 6 months after the immunizing injection, neutralization tests were conducted with serum collected at this stage using the intra-cerebral protection test in mice as described previously (Alexander, 1935). The results are given in tabular form in Tables I, II and III on pages 11-13.

It will be noticed that unit volume of the serum dilutions were required to neutralize ± 100 minimal infective doses of virus strain 449 but only ± 50 M.I.D. of strains O and 464 B. In the tables no adjustment has been made as compensation for this slight variation in titre of the antigens since the results are so clear that this was considered both unnecessary and undesirable.

Consideration of the tables shows that each of the 5 horses developed antibodies against each of the three virus strains. There was some slight variation in the respective titres notably in the case of horse 20987 whose serum neutralized 449 virus only to a titre of $^1/_{16}$ but in the case of horsesickness even this low titre indicates that a solid immunity has been induced. The significance of these results will become clear if the tables are read in conjunction with those indicating the antigenic diffrences of the virus strains published previously (Alexander, 1935, pp. 369-371).

After the completion of the experiment the 5 horses used were exposed to natural horsesickness on the farm Kaalplaas which is notorious as a bad horsesickness farm. Throughout the season they remained in perfect health.

To confirm the results of this experiment by direct immunity test 8 other horses were immunized in a similar manner. Four weeks after immunization they were divided into 3 groups; group 1 (3 horses) received 10 cc. of virulent O virus intravenously; group 2 (3 horses) received 10 cc. of virulent 449 virus; group 3 (2 horses) received 10 cc. intravenously of a mixture of strains O and 449. There were no reactions. The virulence of the strains used for the immunity test was demonstrated subsequently by routine passage through susceptible horses all of which died. Unfortunately it was not possible to carry out an immunity test against strain 464 as this strain in its virulent form has been lost.

TABLE I.—*Neutralization of Virus Strain* 449.

Antigen Titration.

Date.	Virus Antigen.	Conc.	Antigen Dilutions.							
			V.	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.
5.2.35.....	Generation 181, strain 449.....	1 : 100	44444	44555	4455x	44560	55600	66000	00000	00000
29.3.35.....	,, ,, ...	,,	44444	44450	44555	45556	44550	60000	00000	00000
22.5.35.....	,, ,, ...	,,	44	50	45	45	44	70	00	00

Neutralization Test.

Date.	Serum.	Interim.	Serum Dilutions.									
			1/2.	1/4.	1/8.	1/16.	1/32.	1/64.	1/128.	1/256.	1/512.	1/1024.
		Days.										
5.2.35	Horse 20987, bled 21.1.35.....	172	00	00	0000	*4500*	*5550*	4550	4550	4445	44	44
5.2.35	Horse 20991, bled 21.1.35.....	172	00	0000	0000	0000	000x	5000	0000	*5566*	46	4x
29.3.35	Horse 20968, bled 27.3.35.....	176	00	00	0000	0000	0000	6000	6000	*4455*	44	44
29.3.35	Horse 20985, bled 27.3.35.....	176	00	00	0000	0000	6000	0000	*4600*	*4445*	45	55
22.5.35	Horse ——, bled 8.5.35.....	180	—	—	00	00	00	00	00	*44*	44	44

TABLE II.—*Neutralization of Virus Strain O.*

Antigen Titration.

Date.	Virus Antigen.	Conc.	Antigen Dilutions.							
			V.	1/10.	1/20.	1/40.	1/80.	1/160.	1/329.	1/640.
10.4.35	Generation strain O.....	1 : 10	5666	5567	6660	6670	0000	x000	0000	0000
25.4.35	,, ,, ...	1 : 10	4556	5660	6660	5600	7000	0000	0000	0000
9.5.35	,, ,, ...	1 : 10	45	45	56	67	00	00	00	00

Neutralization Test.

Date.	Serum.	Interim.	Serum Dilutions.									
			1/2.	1/4.	1/8.	1/16.	1/32.	1/64.	1/128.	1/256.	1/512.	1/1024.
		Days.										
10.4.35	Horse 20987, bled 21.1.35.....	172	0000	0000	0000	0000	6000	0000	7000	*5580*	—	—
10.4.35	Horse 20991, bled 21.1.35.....	172	0000	0000	0000	0000	0000	*5700*	*5670*	6677	—	—
25.4.35	Horse 20968, bled 27.3.35.....	176	0000	8000	7000	*6600*	*5670*	4660	5667	66	—	—
25.4.35	Horse 20985, bled 27.3.35.....	176	0000	0000	0000	*5680*	*6700*	6678	5660	5677	—	—
9.5.35	Horse 20941, bled 8.5.35.....	180	00	00	00	00	*67*	77	57	56	—	—

TABLE III.—*Neutralization of Virus Strain 464 B.*

Antigen Titration.

Date.	Virus Antigen.	Conc.	Antigen Dilutions.							
			V.	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.
26.4.35	Generation 168, strain 464B.....	1 : 90	3444	4450	3450	4450	6000	0000	0000	0000
17.5.35	,, ,, ...	1 : 90	3444	4550	4444	4456	0000	0000	0000	0000
7.6.35	,, ,, ...	1 : 90	34	44	50	46	50	00	00	00

Neutralization Test.

Date.	Serum.	Interim.	Serum Dilutions.									
			1/2.	1/4.	1/8.	1/16.	1/32.	1/64.	1/128.	1/256.	1/512.	1/1024.
		Days.										
26.4.35	Horse 20987, bled 21.1.35.....	172	00	00	00	000x	4000	4000	*45*	44	45	44
26.4.35	Horse 20991, bled 21.1.35.....	172	00	00	00	0000	0000	4000	*44*	40	44	44
17.5.35	Horse 20968, bled 27.3.35.....	175	00	00	00	00	0000	0000	0000	000x	*4450*	56
17.5.35	Horse 20985, bled 27.3.35.....	175	00	00	00	00	0000	4000	*4500*	*3456*	45	46
7.6.35	Horse 20941, bled 8.5.35.....	180	—	—	—	00	0000	0000	4000	*4555*	3450	4446

NOTE.—Conc. of Antigen = Serum saline dilution of stock emulsion.
In the tables the numbers indicate the day after injection on which the mice died.
0 = Survival for more than 9 days.
x = Death due to some cause other than horsesickness encephalitis usually injury at time of injection.
Thus, 450x means of 4 mice injected 1 died day 4, 1 died day 5, 1 died as a direct result of injection and 1 survived.

Discussion.

It is not possible to generalize from the results obtained from so small a number of animals but it would appear that the simultaneous injection of 3 different strains of neurotropic attenuated virus results in the production of a solid immunity against each. In other words, there is no antagonistic action of one strain against another. This finding is the justification for issuing a vaccine which consists merely of a mixture of the different available strains diluted in such a way that the final product will contain not less than 100 infecting doses of each strain per dose of vaccine, and obviates the necessity of repeated injections in the field. Whether there will be a similar antigenic response when a greater number than 3 strains are included is a point which is being investigated at the moment and will be reported in due course.

Summary.

By in vitro and in vivo methods it has been shown that the simultaneous injection of 3 strains of neurotropic horsesickness virus results in the production of a solid immunity against each.

REFERENCE.

ALEXANDER, R. A. (1935). Studies on the Neurotropic Virus of Horsesickness III. The Intracerebral Protection Test and its Application to the Study of Immunity. *Onderstepoort J. of Vet. Sci. and An Ind.*, Vol. 4, No. 2, pp. 349-377.