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OBSERVATIONS ON THE OCCURRENCE OF AFRICAN HORSESICKNESS AMONGST IMMUNISED HORSES

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INTRODUCTION

The natural occurrence of African horsesickness in the endemic areas of southern Africa is distinctly seasonal. Particularly widespread epizootics have been described by Theiler (1921) and du Toit (1924). In most instances these outbreaks were preceded by abnormally high rainfall, which apparently created highly favourable conditions for transmission of the virus by insect vectors.

Following the introduction of the polyvalent mouse adapted vaccine in 1936 a recurrence of these devastating epizootics has not been observed. Within the recognised endemic areas, however, immunisation has not brought about the complete disappearance of the disease and from time to time minor enzootics have been reported within certain confined localities.

The experimental material investigated in this report was collected during one of these cyclic appearances of the disease amongst an immunised equine population. The opportunity was provided by the exposure, at range, of a group of equines on the Government farm Kaalplaats, adjoining the Onderstepoort Research Institute. This farm is in close proximity to the Bon Accord dam and its immediate surroundings have the reputation of a notoriously bad endemic area. The grazing camps are interspersed with numerous temporary streams and small vleis, which presumably provide breeding sites for the responsible insect vectors. In addition the summer of 1958 was preceded by abnormally high rainfall.

No special precautions to protect the animals against natural infection were taken, other than the customary annual immunisation during the latter portion of 1957, with a potent polyvalent mouse adapted vaccine, prepared by standard technique and containing attenuated strains representing 7 of the established antigenic types, namely Type 1 (A501), Type 2 (OD), Type 3 (L), Type 4 (VRY), Type 5 (VH), Type 6 (114) and Type 7 (Karen).

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MATERIALS AND METHODS

Virus strains

During the period 7 March to 22 April, 1958, a total of 33 specimens was collected for examination. The majority of these samples consisted of blood taken as soon as possible after an animal was reported ill. As the majority of the animals were at range and could not be examined daily, it was not possible to take all the samples at the height of the febrile reaction. In many cases the temperature had already subsided and well developed symptoms of the cardiac form of the disease were present. Blood was collected in equal quantity in Edington's oxalate-carbolic-acid-glycerine diluent (O.C.G.) (Theiler, 1921) which served as both anticoagulant and preservative. In fatal cases spleen was collected post-mortem and stored in 50 per cent buffered glycerine at pH 7·4. During the course of the investigation all the original samples were held at 4°C.

Isolation of virus

Strains of virus were isolated by standard techniques (McIntosh, 1958; Howell, 1962). Infective brain tissue suspensions of the third serial passage were prepared as 10 per cent suspensions in M/50 phosphate buffer, pH 7·2, containing 5 per cent lactose and 1 per cent peptone, dispensed into ampoules, freeze-dried and stored at —20°C for further experimental investigations.

Neutralisation tests

(*a*) Antigens for the serum-virus neutralisation test included the recently isolated strains, the type strains in current use for the preparation of polyvalent vaccine as well as two additional strains 18/60 and 7/60 representing the eighth and ninth immunological types respectively (Howell, 1962). A titration was conducted in adult mice by the intracerebral route of injection. From the specific mortality recorded, 50 per cent end points were calculated by the method of Reed & Muench (1937). That dilution was determined which would give a final 100 LD_{50} under the conditions of the test.

(*b*) Type specific sera against the established antigenic types were prepared by the hyperimmunisation of young adult rabbits, as described by McIntosh (1958).

From a group of 37 horses serum samples were collected two months after the annual routine immunisation of equines on the farm with polyvalent horsesickness vaccine and three months before the first recorded case of horsesickness in March of the following year.

From a number of animals which had not been previously bled, acute phase sera were taken at the same time as blood was collected in anticoagulant for virus isolation.

(*c*) Technique for the *in vitro* serum virus neutralisation test. The procedure followed for the setting up of this test was identical with that described in a previous publication (Howell, 1962) and embodied certain modifications over the methods used by Alexander (1935) and McIntosh (1958).

EXPERIMENTAL RESULTS

From the 33 samples examined, eleven strains of virus were recovered by the intracerebral injection of mice, of which eight were obtained from spleen tissue. All the specimens which failed to produce specific mortality in mice were then injected intracardially into young adult ferrets under general anaesthesia. Only one additional strain of virus (10/58), which was subsequently shown to be a Type 4, was recovered by this technique. This ferret had shown a mild febrile reaction on the seventh and eighth days after injection, whereupon blood collected at this stage produced specific mortality in suckling mice after intracerebral inoculation. These results confirmed the difficulties previously associated with the reisolation of virulent virus from the blood of immunised horses, as described by Mulligan (1938) and McIntosh (1953).

The results of the neutralisation tests conducted with the twelve strains of virus are given in Table 1. It will be observed that only a single strain of virus was isolated from each specimen. These strains represented four distinct antigenic types of virus namely Groups 1, 4, 6 and 7. Since strains of attenuated virus representing Groups 1 to 7 were included in the polyvalent vaccine, it is apparent, therefore, that the vaccine did not pıoduce a polyvalent immunity.

TABLE 1.—*Neutralisation of virus strains by type specific antisera*

Antigen		Type Antisera								
Isolate	LD_{50}	1	2	3	4	5	6	7	8	9
1/58	100	0	0	0	25*	0	0	0	0	0
9/58	40	0	0	0	0	0	75	0	0	0
10/58	20	0	0	0	56	0	0	0	0	0
11/58	30	0	0	0	40	0	0	0	0	0
13/58	100	0	0	0	40	0	0	0	0	0
14/58	10	386	0	0	0	0	0	0	0	0
23/58	178	0	0	0	30	0	0	0	0	0
25/58	100	0	0	0	25	0	0	0	0	0
27/58	80	0	0	0	45	0	0	0	0	0
35/58	40	10	0	0	0	0	0	625	0	0
36/58	100	0	0	0	30	0	0	0	0	0
45/58	100	0	0	0	0	0	0	458	0	0

* Indicates reciprocal of serum dilution giving 50 per cent protection.

Evaluation of the immune status of the virus donors

The acute phase sera of the animals, from which virus was isolated, were examined in order to determine the presence of specific antibodies to each of the strains incorporated in the polyvalent vaccine. These results are summarised in Table 2.

For the purpose of this analysis aged animals include all those in excess of twelve years. It will be observed from the data presented in the table that the age of animals from which virus was isolated, varied from six months to that stage where age could no longer be accurately assessed. The incidence of the disease on the farm was, therefore, not affected by the age of individual animals. However, the period of time which these animals had spent within the enzootic area was with only three exceptions less than two-and-a-half years.

TABLE 2.—*Origin and Identification of Virus Strains, with Immune Status of AffectedAnimals*

Donor						Virus isolated			Antibody titre of acute phase sera Antigenic type No.						
Identi-fica-tion	Breed	Age (years)	Sojourn on station (years)	Previously recorded imm.	Outcome	Specimen No.	Date of collection	Anti-genic type	1	2	3	4	5	6	7
3921	Percheron	3	1	1	Died	14/58	19 Mar. 58	1	*21	96	64	5	12	24	24
3402	Farm type	2⅓	2⅓	0	Died	1/58	17 Mar. 58	4	—	—	—	—	—	—	—
951	Farm type	12	10	12	Recovered	10/58	15 Mar. 58	4	>125	77	>125	0	>125	>125	>125
3862	Farm type	Aged	½	1+	Died	11/58	15 Mar. 58	4	>125	125	>125	56	>125	56	125
3345	Farm type	Aged	2¼	4	Died	13/58	7 Mar. 58	4	>125	125	125	56	125	>125	76
2613	Farm type	5	2½	6	Died	23/58	24 Mar. 58	4	>125	48	125	0	0	125	96
3032	Percheron	9	4	5	Recovered	25/58	26 Mar. 58	4	>125	125	>125	24	125	125	41
2755	Farm type	Aged	5	3	Died	27/58	28 Mar. 58	4	>125	125	>125	0	16	>125	85
3553	Farm type	3½	1½	2	Died	36/58	22 Apr. 58	4	—	—	—	—	—	—	—
3858	Mule	½	½	0	Died	9/58	13 Mar. 58	6	—	—	—	—	—	—	—
3922	Percheron	Aged	½	1+	Died	35/58	13 Apr. 58	7	>125	>125	>125	11	>125	125	11
3893	Mule	½	½	0	Died	45/58	23 Apr. 58	7	—	—	—	—	—	—	—

* Indicates reciprocal of serum end point dilution giving 50 per cent protection.
1+ Denotes 1 recorded and an unknown number of previous vaccinations.

It is unfortunately not possible to determine whether the antibodies detected in these acute phase sera are the result of an immunological response to immunisation only or whether antigenic stimulus has been provided by natural infection as well. If the latter is indeed the case these attacks must have been inapparent, since no record has been made of illness amongst these animals during their sojourn within the endemic area. It is, however, highly significant that the acute phase serum of an animal, showed either a complete lack of or otherwise a very low titre of antibodies against that immunological type of virus which was recovered from the circulation.

The presence of low titre antibodies in the sera of five of the animals, homologous with the antigenic type of the virulent virus isolated, may appear contradictory but can be the result of inhibition by heterologous antibody as shown by Alexander (1935) or alternatively due to the rapid rise in antibody following infection by a virulent strain of virus. In this respect the results of an experiment to demonstrate the rapid formation of homologous antibody are given in Table 3. Serum samples were collected at periodic intervals from a horse injected intravenously with 2 ml blood of specimen 45/58. From the serum end point titres presented in the table it is evident that high titre antibodies were present six days after the cessation of the febrile reaction, which started on the fifth day after infection and lasted ten days.

TABLE 3.—*Development of Homologous Antibodies to Specimen* 45/58 (*Type* 7 *Virus*)

Days after cessation of febrile reaction	Antibody titre
Prebleed	0
6	386*
15	453
24	453
33	8125
64	5625
85	3125
96	625

* Indicates reciprocal of serum end point dilution giving 50 per cent protection

Immune status of animals prior to the 1958 *epizootic*

Apart from the twelve equines from which strains of virus were isolated, serum samples from an additional group of 37 horses collected two months after vaccination were examined for specific neutralising antibodies against the components of the polyvalent vaccine, as well as two new antigenic types of horsesickness virus recently identified. These results are presented in Table 4.

Provided allowance is made for the presence of antibodies resulting from occasional natural infection in previous years and which it is believed will persist in detectable titre during the life of the animal, it is apparent that in spite of repeated immunisation, a large percentage of animals failed to produce antibodies against certain antigenic types and that the vaccine in its present composition did not produce a polyvalent immunity.

If the above group of animals can be considered as representative of the immune status of the equine population on the station, then the relatively greater frequency, with which antibodies to Types 4, 5, 6 and 7, but particularly Type 4 are absent or of low titre, supports the previous findings. There is, therefore, a direct correlation between the immunological types of virus isolated and the lack of antibodies in horses to these particular types.

TABLE 4.—*Immune Status of a Group of Horses prior to the 1958 Epizootic*

Donor		Sojourn in endemic area	Previous immunisation		Antibody titre to Antigenic Type No.								
Identi fica-tion	Age		No. of recorded innocu-lations	Age at first inno-culation	1	2	3	4	5	6	7	8 Strain 18/60	9 Strain 7/60
	(Years)	(Years)		(Months)									
3267	14	2	5	—	>625*	>625	>625	202	>625	66	>625	280	>625
3308	13	3	4	—	125	35	>625	125	90	56	56	<25	125
3387	13	2	5	—	386	<25	625	<25	<25	—	25	<25	202
3406	12	2	4	—	56	202	172	<25	<25	<25	40	<25	38
3739	11	1	2	—	625	625	625	<25	125	40	66	<25	—
1010	10	9	13	—	386	280	625	<25	280	125	<25	77	>625
1100	10	10	10	24	>625	280	>625	25	625	280	>625	453	>625
1110	10	10	11	24	<25	625	>625	70	66	625	25	90	>625
1543	10	9	10	—	>625	>625	>625	<25	125	280	66	625	>625
1939	10	7	10	—	>625	>625	>625	190	125	125	35	172	625
1612	8	8	11	10	280	172	>625	625	328	35	25	280	532
1679	8	8	10	21	56	77	>625	125	25	625	25	90	>625
2804	8	4	7	—	625	172	>625	125	56	25	25	34	300
1810	7	7	10	17	>625	625	>625	48	30	202	<25	<25	238
1829	7	7	9	13	>625	625	>625	202	386	25	66	386	>625
1870	7	7	9	11	280	280	>625	280	66	625	35	106	>625
2046	7	7	9	11	147	280	>625	<25	<25	90	<25	25	172
2558	7	4	8	—	>625	>625	>625	48	>625	280	>625	625	>625
2108	6	6	9	10	>625	125	>625	<25	625	625	77	202	>625
2311	6	6	7	11	147	25	280	<25	40	<25	56	125	386
2358	5	5	9	18	>625	328	625	<25	386	56	<25	125	386
2615	5	4	8	—	147	25	125	<25	<25	<25	25	<25	>625
3093	5	2	3	—	<25	<25	625	<25	25	66	<25	<25	280
3098	5	2	5	—	±625	>625	625	40	35	56	<25	77	280
3192	5	2	5	—	>625	>625	>625	145	90	<25	<25	40	>625
3200	5	2	5	—	386	>625	>625	56	280	>125	35	125	328
3208	5	2	5	—	>625	625	>625	<25	202	56	147	<25	625
3296	5	2	5	—	>625	>625	>625	>625	625	625	>625	452	>625
3298	5	2	4	—	386	125	625	>625	172	125	56	66	238
3300	5	2	5	—	>625	>625	625	125	280	125	—	125	>625
2594	4	4	7	7	147	280	>625	<25	<25	25	25	<25	>625
2896	3	3	6	8	625	125	386	<25	25	125	—	<25	125
2899	3	3	5	8	280	147	625	<25	280	280	125	328	386
2908	3	3	3	18	625	77	280	<25	25	<25	<25	<25	56
3104	2	2	3	16	280	90	625	125	625	386	>625	125	45
3421	2	2	3	9	56	125	—	<25	—	56	<25	<25	280
3435	2	2	4	1	56	<25	280	<25	56	<25	280	<25	416

* Reciprocal of the serum dilution end points giving 50 per cent protection.

Discussion

Prophylactic immunisation has been accepted as one of the indispensable aids in any programme designed to control the incidence of African horsesickness.

Initially a trivalent vaccine prepared from strains 449, 0 and 464B was used (Alexander, 1936). From time to time additional strains of virus were added to the vaccine, while others were withdrawn. The new strains were invariably isolated from immunised horses showing clinical manifestations of the disease. The result was that by 1949 the vaccine contained eight attenuated strains of virus. Following the classification of numerous strains by McIntosh (1958) into seven distinct immunological types, the composition of the vaccine was subsequently altered to contain seven heterologous strains of virus.

In spite of these alterations in the composition of the vaccine, breakdowns in immunity have continued to manifest themselves. Whereas the earliest investigations invariably revealed the presence of new immunological types, recent observations including those reported by McIntosh (1958) have shown that the strains of virus which are isolated from immunised horses, suffering from horsesickness, are homologous with the established antigenic types included in the polyvalent vaccine.

During the course of the 1953 epizootic in this area (McIntosh, 1958) the predominance of a Type 5 strain was observed, whereas in this study a Type 4 virus was most frequently isolated, although in both epizootics other immunological types of virus were active simultaneously.

From the data presented in this report it is apparent that there is a direct correlation between the frequency of isolation of a virus type and the susceptibility of the equine population to that particular type of virus. In spite of repeated immunisation with a vaccine in which Type 4 virus was incorporated, most of the animals included in this study have been shown to possess either low titre antibody to Type 4 or none at all. It is reasonable to conclude, therefore, that as a result of the poor antigenicity of the Type 4 strain included in the vaccine, the naturally occurring virulent strain was more frequently responsible for infection and symptoms amongst immunised horses. In view of the good antibody response in the majority of immunised horses and the failure to isolate Type 3 virus from reacting animals during several epizootics, it appears that the Type 3 strain incorporated in the vaccine is highly immunogenic.

In view of these observations, virus strains attenuated for prophylactic immunisation should be selected not only for type, but also for immunogenic properties. Since it is virtually impossible to obtain a sufficient number of fully susceptible horses in a country where the disease is endemic, it is imperative that a laboratory host should be sought which will permit an evaluation of the immunogenic properties of different strains of horsesickness virus before incorporation in a polyvalent vaccine.

Summary

During the course of an epizootic of African horsesickness it was possible to establish that:—

(1) strains of virus apparently homologous with components of the polyvalent vaccine could be reisolated from clinically affected equines previously immunised;

(2) infection by a specific immunological type of virus could be correlated with an absence of homologous antibody in the reacting animal;

(3) animals failed to develop specific antibodies to certain components of the polyvalent vaccine in spite of repeated immunisation.

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