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Horsesickness: Immunization of Horses and Mules in the Field during the Season 1934-1935 with a Description of the Technique of Preparation of Polyvalent Mouse Neurotropic Vaccine.

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RECOGNITION of the attenuation which accompanies neurotropic fixation of horsesickness virus strains in mice and guinea pigs (Alexander, 1933), has led to the development of a new method of immunization of horses and mules. The decision to introduce this method into general practice was taken after consideration of the promising results obtained under laboratory conditions (Alexander and Du Toit, 1934), and under field conditions on a small scale (Alexander and Van der Vijver, 1935). Up to the present time no detailed publication of the results obtained from the use of the commercial vaccine on large numbers of animals has appeared so that a review of the position after the horsesickness season 1934-35 will remedy this deficiency.

The results are being considered separately under two different headings, viz.:—

A. Immunization of remounts of the South African Police.

B. Immunization of other horses and mules.

This arbitrary subdivision has been made for several reasons. All the animals belonging to the Police were treated either by the Veterinary Officer of the Police personally (Major D. D. Morton, M.R.C.V.S.), or by his experienced farrier sergeants who could be relied upon faithfully to carry out all instructions and to use full aseptic precautions for all injections. In addition the conditions under which the Police animals are maintained made it possible to obtain in every instance a detailed report not only on the nature of the reaction to the vaccine but also on the subsequent history of the animal. This was essential as it was necessary to obtain reliable data on any possible adverse effect upon the performance of the horses as a result of immunization and to obtain an accurate diagnosis in the case of any reported breakdowns following exposure to natural infection. In the case of the remainder of the animals treated in spite of the fact that vaccine is issued to the veterinary profession

only, many of the injections were carried out by stock inspectors under conditions which did not always permit of the observance of aseptic precautions or of the rational handling of a vaccine containing a living infective agent. In addition it was realized that it would be exceedingly difficult to obtain reliable reports on the reactions, and that an estimation of the degree of immunity conferred could only be approximate, since, in only a small percentage of reported breakdowns could the diagnosis be relied upon. Moreover, it was realized that a complete return from every user of the vaccine could never be hoped for.

Preparation of the Vaccine.

The entire technique of vaccine preparation has been based upon that accumulated knowledge of the physical, chemical and biological properties of neurotropic virus contained in previous publications on the subject (Alexander, 1935).

Throughout the period under review the vaccine issued consisted of a mixture of 4 strains of virus which had been maintained " in pure culture " by separate serial passage in mice. The strains were those designated arbitrarily as O, 449, 464A and 464 B; the antigenic differences as determined by in vitro nutralization tests have been reported previously. In spite of some doubt as to the immunizing value of stain 464A it was included in the vaccine because repeated tests had shown it to have attained a safe level of attenuation for equines, and, as its injection apparently is innocuous, it was hoped that it might assist to immunize, at least partially, against some natural aberrant virus type at present not isolated; in other words it could do no harm but might be of value. For all practical purposes, however, it is considered that this vaccine was trivalent and not quadrivalent.

The 4 strains had been attenuated by the following number of intracerebral passages in mice: Strain O—143, strain 449—149, strains 464A and B—each 119.

The aim in vaccine production has been to turn out a bacteriologically sterile final product which would contain in 0·05 c.c. a minimum of 100 mouse infective doses of each of the strains. Since it has been shown that if 0·05 c.c. of a given emulsion contains approximately a single minimal infecting dose for mice then 10 c.c. will contain approximately 1 M.I.D. for horses, the routine dose was fixed at 10 c.c. given subcutaneously so that each dose of vaccine would contain not less than 100 M.I.D.'s of each strain. This was considered to be a perfectly safe margin and has been justified by the results of the vivo immunity tests and the in vitro neutralization tests published separately (Alexander, 1936, this journal).

In planning the production of a batch of vaccine the above-mentioned points have been borne in mind together with the knowledge that—

(1) the different strains of virus produce in mice a disease characterized by appreciably different periods of incubation and course;

(2) there is a rapid increase in the virus titre of the brains of infected mice in the later stages of the disease, i.e. in that short period from the appearance of symptoms up to the time of death;

(3) rapid and easy removal of infective brains may be assured only by using those mice which are destroyed in extremis by ether or other anaesthesia and not mice which are found lying dead in the boxes. Brains from the latter frequently show advanced autolysis, usually are removed intact with considerable difficulty, and may have been invaded by bacterial contaminants.

Consequently the following routine technique for commercial vaccine preparation has been adopted:—

Day 1—in the afternoon mice injected with standard emulsion* of virus strain O (about 30 mice).

Day 2—in the afternoon mice injected with strain 449 (about 15 mice).

Day 3—in the morning mice injected with strains 464A and B (about 15 mice each strain).

Day 6—on arrival in the morning 2 or 3 mice of each strain will be found dead and should be discarded. The majority of the remainder will be in extremis; these are etherized, the brains removed with aseptic precautions as quickly as possible and placed in sterile 50 c.c. centrifuge tubes fitted with corks, 3 entire brains being placed in each tube. In the afternoon the remainder of the mice will or should be in extremis and the brains are removed as before. All the tubes are stored upright in the freezing chamber of a refrigerator until required. This freezing facilitates disintegration and subsequent emulsification.

Day 7—approximately 50 c.c. of 10 per cent. normal serum—saline is added to 7 tubes of strain O, and 3 each of strains 449, 464A and B. A coarse emulsion of each is made by drawing the material into and forcing it out of sterile Agla syringes. The tubes are returned overnight to the freezing chamber of the refrigerator.

Day 8—the virus emulsions are rapidly thawed in an incubator at 37° C. and a fine emulsion prepared with sterile precautions as above. The tubes are centrifuged at about 3,500 revolutions per minute for 10 minutes and the supernatant fluid decanted into sterile containers of suitable capacity (120 c.c. bottles fitted with cotton wool stoppers were used) the strains being kept separate so as to make replacement simple should any accident occur. From each container 0·5 c.c. of emulsion is seeded onto large agar slants for bacterial sterility test by incubation for at least 48 hours.

Day 9—providing the sterility test is satisfactory the virus emulsions are added to 10 per cent. serum saline, the volume adjusted to 6 litres and 2 per cent. ether added as a preservative. After thorough shaking to ensure mixing of the virus strains as well as

* Standard virus emulsion constantly in use is a 5 per cent. emulsion of infective mouse brains in a 10 per cent. normal serum saline.

solution of the ether the vaccine is bottled, corked and sealed. The final bottled product is incubated overnight at 37° C., a precedure which greatly enhances the bactericidal action of the ether without affecting the virus titre and then stored in a refrigerator at 4° C. until required after a second sterility test has been carried out on 1 or 2 bottles selected at random. The virus titre does not decrease appreciably on storage at ±4° C. for up to 2 months, but does diminish fairly rapidly at room temperature. Consequently it is stipulated that the injections must be carried out within 14 days of issue from the laboratory. This has been shown to be an adequate and a safe margin.

It will be noted that the original virus emulsions of strains 449, 464A and B were diluted approximately 1:40. At this dilution 0·05 c.c. always contains at least 100 mouse infective doses; usually the titre more nearly approximates 250 M.I.D. Similarly prepared emulsions of neurotropic virus strain O invariably contain rather less than half these titres and this explains the use of 21 brains instead of 9. On this basis 48 mouse brains are required for the production of 600 doses of vaccine, i.e. 12·5 doses per brain.

At first sight the entire procedure may appear to be complicated but in actual practise that is not the case more particularly when the procedure is adapted to the preparation of at least one batch of vaccine every day. The quantities selected naturally may be varied to meet particular requirements but they were chosen as being most suitable to the equipment available. Moreover 6 litres of vaccine (i.e. 600 doses) per day was found to be sufficient to meet all demands.

In considering the technique described it may seem somewhat unnecessary to recommend that the injection of one strain of virus should be carried out in the afternoon while another strain should be injected in the morning, but the nature of the disease produced by a fixed neurotropic virus is so constant that a considerable experience with the production of some 90,000 doses of vaccine has shown the digerential treatment to be essential for maximum virus production together with economy in the numbers of mice used.

Mention must also be made of the bacterial sterility of the vaccine prepared. From the number of manipulations described it would not be unreasonable to expect that a number of vaccine batches would show a fairly heavy contamination, which could not be controlled by ether whose action in 2 per cent. concentration is bacteriostatic rather than bactericidal. Again experience has shown that this is not the case. Apparently any contaminants picked up during removal of the brains or introduced during any process requiring removal of the cork from the tubes become entangled with the fine suspended brain particles of the crude emulsions and are carried down mechanically during centrifugation. This is the only explanation that can be offered for the rarity of finding an infected emulsion. Out of about 150 batches of vaccine prepared only 2 have been discarded on account of infection; examination of records has shown that on both occasions the centrifuged emulsions had been left in the refrigerator overnight before being decanted, thus permitting diffusion of organisms from the deposit. Incidentally consideration of the results of immunization will show that abscessation at the site of injection has not been a complicating factor.

At this point it is desirable to emphasize the necessity for particular attention to uniformity of technique if a final product of constant potency is to be obtained regularly. Mice of the same strain, age, and weight should be used (for this work mice about 12 weeks old have been found most satisfactory) injections should be carried out at the same time each day, and the infectivity and dose of inoculum should not vary. If strict uniformity of technique is adopted it will be found that the virus titre of the final emulsions will vary within exceedingly narrow limits and this will minimize the number of potency tests necessary.

The actual production of the vaccine would be simplified by the discovery of some substance to replace the serum of the serum saline vehicle to counteract the virucidal action of saline alone. Preferably such a substance should be heat stable so as to enable sterilization by autoclaving to be carried out. Furthermore extensive research is necessary to bring to light some preservative more efficient than ether, but it should not have the selective bacterial action of many of the dyes and of course must be non-irritant and non-virucidal over a wide range of temperature.

A. The Immunization of Police Remounts.

A total of 1,815 horses were immunized over an area comprising practically the entire Union excluding the south-west Cape. In the larger centres the horses were maintained in the regular depots. In the outlying districts they were concentrated at suitable points for injection and then returned immediately to their posts. The routine procedure was to rest horses for 21 days after injection, then to put them on light patrol duty for 14 days before gradually bringing them back to full work. During the period of rest rations were reduced to the requirements for maintenance only, green feed being augmented where possible, and exercise was limited to a minimum.

Reactions.—In approximately 100 cases temperature records were kept, in the remainder observations were recorded on the general habitus of the horses. It became apparent that there is a considerable variation in the reaction produced in different animals maintained under identical conditions. Some horses show no demonstrable reaction whatever but in the majority a febrile and in some cases a general systemic reaction commences on approximately the 6th day and lasts for 3-5 days. During the period of pyrexia the afternoon temperature may rise as high as 105-106° but the morning temperature is seldom above 101. This diurnal fluctuation in temperature may be considered typical but occasionally there is a period of continuous fever. Usually return to normal follows the decrease in the afternoon temperature exacerbations. A remarkable fact is the observation that in spite of a fairly severe fever there is little or no constitutional disturbance other than that directly associated with hyperthermia (e.g. slight pulse acceleration and polypnoea). As a rule horses do not go off their feed and only a careful examination and a record of the afternoon temperature will indicate any deviation from normal. In only 1 or 2 cases was the period of convalescence, following the most severe reactions, prolonged beyond the 21st day and the percentage of cases showing edema of the supraorbital

fossae (dikkop), noticeable on about the 12th day, was less than 0·5 per cent.; mortality as a direct result of immunization was nil. In fact the position may be summarized by saying that in approximately 75 per cent. of cases the reaction in fully susceptible animals is so mild as to be detectable only by an experienced and competent observer. Animals which had been immunized previously either by the serum virus method, or by the neurotropic virus method even by the injection of only a single attenuated strain showed no reaction whatever to subsequent treatment.

After Effects.

As stated above an accurate record of the subsequent history of every horse was kept, the record including the amount and nature of the work carried out. In almost every instance the allocation of mounts remained unchanged prior and subsequent to immunization so that the opinion of the rider on any change in the performance of his mount could be obtained. In only one instance did a report show that a horse apparently had become sluggish some months after return to work. Veterinary inspection failed to connect this with horsesickness immunization and the animal picked up in condition rapidly after anthelminthic treatment.

Again the position may be summarized by saying that no harmful after effects were encountered, observations being made on horses engaged in all types of work from light patrol duty to the strenuous demands of daily attendance in the school of equitation at the Depot. No cases of staggers (acute liver atrophy) were encountered.

Immunity.

On exposure to natural infection during the horsesickness season 12 immunized horses (0·66 per cent.) contracted the disease. Of these 10 (0·55 per cent.) died and 2 (0·11 per cent.) recovered. Full details of these cases will be found in the appendix at the end.

In the two cases which recovered a diagnosis was made from the appearance of a typical dikkop; it is uncertain whether other cases of horsesickness fever unaccompanied by dikkop and hence undiagnosed aetiologically occurred. In the fatal cases a diagnosis was made either from the isolation of virus from blood collected immediately prior to death, or from consideration of evidence submitted to a board of inquiry. There is no reason to believe that an accurate diagnosis was not made in each case and it is not possible for any death not to have been reported.

Consideration of the appendix will show that 6 of the breakdowns occurred in the eastern and north-eastern Transvaal, 3 in the Natal-Zululand area, 2 in the Vryburg area and 1 in the Pretoria District. From the Natal and Transvaal cases 3 strains of virus have been isolated. These have been fixed neurotropically in mice and preliminary work has shown that they differ antigenically from the strains incorporated in the vaccine. A detailed report on the antigenic inter-relationship will form the subject of a future publication. From the one Pretoria and two Vryburg breakdowns unfortunately no strain of virus was isolated.

It is of interest to note that 6 of the 12 horses under consideration had been immunized by the serum virus method some time prior to reimmunization by the neurotropic virus method and that 1 horse had received 2 injections of neurotropic vaccine. Further in almost every instance there is a history of the horse dying somewhat suddenly, either immediately after return from patrol duty or after being noticed sick for the first time when actually on the road. The significance of this observation is not quite clear but it indicates the harmful effect of work on an immune animal undergoing either a definite or abortive reaction as a result of natural infection. This conclusion is supported by the finding that the strains of virus isolated proved fatal to susceptible animals in the laboratory but produced merely severe febrile and systemic reactions in horses that had been immunized previously with the routine vaccine but were maintained at rest in the stable during the entire period of the immunity test.

B. Immunization of Horses and Mules, other than Police Remounts.

This group comprises all types of horses from nondescript hacks to schooled polo-ponies and from purebred representatives of the heavy draft breeds to valuable Thoroughbred stud stock. In addition a large number of mules were treated but unfortunately when the returns were submitted a differentiation between horses and mules was not made in every instance, so that it may be stated only, that the number of mules immunized was not less than 1,524. As far as it is possible to do so the results are tabulated in the accompanying Table I

[Note.—These figures were compiled from reports submitted by Government Veterinary Officers for the districts under their control. These districts do not correspond with the magisterial districts, so that in some cases the classification of the districts may appear to be at fault.]

It must be conceded at the outset that these figures cannot be regarded as being other than merely approximate. For instance, according to the reports received a total of 28,659 horses and mules were immunized, whereas examination of the record of issues made shows that 31,416 doses of vaccine were supplied to meet orders received. Thus there is no record of the results obtained from the use of 2,757 doses of vaccine. It has not been possible to ascertain exactly how the discrepancy has occurred, but it is in part made up by the omission of 1,057 animals in the Piet Retief area from which no details are available. This omission is regrettable because the district is situated in that portion of the Union where the percentage of breakdowns might be anticipated to be not lower than that reported from Vryheid, namely 2·60 per cent.

Reaction to Immunization.—On two occasions it was reported that severe abscessation and local phlegmosis had occurred at the site of injection. The total number of animals involved was 8, but in each case it was determined that of over 1,000 horses injected with the same batches of vaccine no others had shown any local reaction. It must be concluded therefore that pyogenic infection had gained entrance from the use of contaminated needles and syringes and that the vaccine itself was not the primary cause.

TABLE I.

Results of Horsesickness Immunization, 1934-35.

Horses and Mules other than Police Remounts.

Province and District.	Number of Animals Immunized.			Number of Breakdowns in Immunity.					
	Horses.	Mules.	Total.	Deaths.	Per Cent.	Re-covery.	Per Cent.	Total.	Per Cent.
A.—CAPE.									
Mafeking.....	—	—	2,653	24	0·90	26	0·98	50	1·88
Vryburg.....	—	—	1,193	1	0·09	0	0	1	0·09
Kimberley.....	734	10	744	0	0	0	0	0	0
De Aar.....	—	—	822	0	0	0	0	0	0
Middelburg.....	—	—	180	0	0	0	0	0	0
Aliwal North.....	—	—	12	0	0	0	0	0	0
Capetown.....	—	—	58	0	0	0	0	0	0
Port Elizabeth...	244	377	621	0	0	0	0	0	0
East London.....	—	—	209	0	0	0	0	0	0
Grahamstown....	—	—	343	0	0	0	0	0	0
Umtata.....	—	—	300	0	0	0	0	0	0
Flagstaff.....	—	—	129	0	0	0	0	0	0
Kokstad.....	—	—	394	0	0	0	0	0	0
B.—ORANGE FREE STATE.									
Kroonstad.....	1,030	252	1,282	0	0	0	0	0	0
Bloemfontein.....	295	36	331	0	0	0	0	0	0
C.—TRANSVAAL.									
Highveld.									
Ermelo.....	—	—	2,501	5	0·20	2	0·08	7	0·28
Potchefstroom..	1,890	51	1,941	5	0·26	1	0·05	6	0·31
Johannesburg..	376	8	384	0	0	0	0	0	0
Middelburg.....	2,094	12	2,106	16	0·76	2	0·09	18	0·85
Northern.									
Pretoria.....	1,977	357	2,334	24	1·03	10	0·43	34	1·46
Potgietersrust..	—	—	464	5	1·08	1	0·22	6	1·30
Pietersburg....	381	42	423	8	1·89	7	1·65	15	3·54
Louis Trichardt	—	—	237	5	2·11	0	0	5	2·11
Rustenburg....	—	—	237	0	0	1	0·42	1	0·42
Eastern.									
Barberton.....	—	—	171	8	4·68	1	0·59	9	5·27
D.—NATAL.									
Highveld and Middle Veld.									
Dundee.....	1,419	57	1,476	6	0·41	3	0·25	9	0·66
Estcourt.....	1,041	54	1,095	1	0·10	0	0	1	0·10
Ladysmith.....	955	16	971	1	0·1	0	0	1	0·10
Greytown.....	601	16	617	1	0·17	2	0·33	3	0·50
Pietermaritz-burg.....	203	48	251	0	0	0	0	0	0
Ixopo.....	208	44	252	0	0	0	0	0	0
Low Veld.									
Vryheid.....	1,926	116	2,042	32	1·57	10	0·49	42	2·06
Nongoma.....	—	—	57	4	7·02	9	12·28	11	19·30
Eshowe.....	109	28	137	10	7·30	5	3·65	15	10·95
Durban.....	—	—	203	0	0	0	0	0	0
Port Shepstone	—	—	82	1	1·22	0	0	1	1·22
E.—SOUTH WEST AFRICA	—	—	943	0	0	0	0	0	0
F.—SWAZILAND..	—	—	217	5	2·30	0	0	5	2·30
G.—S. RHODESIA	—	—	247	3	1·21	4	1·62	7	1·83
TOTAL.....	—	1,524	28,659	165	0·58	82	0·29	247	0·87

Several reports were received from farmers that individual animals had died within 48 hours of injection. Obviously the deaths could not be attributed to the action of the vaccine-virus to that it is believed that these reports represented coincidences unconnected with immunization. On the other hand it is necessary to detail the histories of 3 horses which died under circumstances which cannot exclude the vaccine from all blame with certainty.

1. District Estcourt, Natal—horse injected 29/11/34; noticed to be dull on 6th day; on 8th day fever and swelling of the supra-orbital fossa; died on 9th day. After post-mortem examination an aetiological diagnosis of Dikkop Horsesickness was made but this could not be confirmed by histological examination of specimens submitted and no virus was isolated from the blood.

2. District Klerksdorp, Transvaal—horse injected 5/12/34; noticed to be dull and sluggish on 9th day; died on 12th day after showing symptoms described by the owner as being typically those of dunkop horsesickness. No opportunity of confirming or refuting this diagnosis was available.

3. District Machadodorp, Transvaal—it was merely reported that a horse had died on the 13th day after injection after showing typical symptoms of horsesickness.

Although these reports are vague and the diagnosis in each case was unconfirmed it is considered that the reaction to the vaccine may have been a factor contributing to the death of the animal. In spite of this it is merely necessary to point out that the death of 3 horses out of a total of 28,659 represents a percentage mortality which may be disregarded.

One other adverse report merits attention. The Government Veterinary Officer in Kimberley was advised that out of a troop of 40 horses in the Spytfontein District 1 was noticed decidedly ill on the 5th day after injection but appeared to have recovered by the 8th day. On the 7th day 2 mares and a gelding suddenly "went mad". After carreering wildly about a paddock they damaged themselves by charging into fences or trees and either succumbed to their injuries or had to be destroyed. Veterinary inspection of the remainder on the 9th day showed only a single mare undergoing a typical but severe horsesickness reaction, and careful examination of the pasture for the presence of poisonous plants yielded negative results. This report would appear to indicate neurotropism of the vaccine virus with involvement of the central nervous system possibly in peculiarly susceptible animals, but in the absence of corroborative evidence it must remain for the present an isolated and unconfirmed occurrence.

For the rest a careful examination of the returns shows that a large number of owners were disappointed at the absence of any clinical reaction because they believed, that without at least a definite horsesickness fever, no immunity could result; a further large number reported a mild febrile reaction occurring some time between the 6th and 13th day after injection; less that 3 per cent. reported severe febrile reactions and only rarely is mention made of the incidence of supraorbital edema (dikkop).

The horses in question were maintained under a wide variety of conditions. In a limited number of cases the hygiene, feeding, and general management left nothing to be desired but it was a fairly general practise to turn out injected horses under every variety of climatic conditions to fend for themselves on poor quality grass until the completion of the prescribed period of 21 days rest. Many horses were worked for at least 5 days after injection and were returned to work within a fortnight, others were worked continuously and yet there appeared to be no accidents. These practises cannot be condemned too strongly but the attenuation of the vaccine virus has attained such a degree that apparently it may be used with impunity under all conditions of farming in South Africa.

It is necessary to make no differentiation with regard to the immunization of mules. But, from two separate sources, it has been reported that a total of 5 mules showed a complete or partial blindness which developed progressively from about the 28th day after injection. All attempts to procure these animals for the purpose of carrying out a detailed examination were unsuccessful so that the entire question of neurotropism of the virus in mules must remain an open one pending the collection of additional data on a larger number of cases. In the laboratory a similar condition has not been encountered.

After-effects.—In one or two instances owners expressed the opinion that individual animals appeared to be sluggish for some months after treatment. On the other hand the observations of experienced horsemen, and of the members of recognized polo clubs playing immunized ponies regularly, makes it clear that the general concensus of opinion is that unpleasant sequelae need not be feared.

Immunity.—At the end of the horsesickness season it was reported that the immunity of 247 (0·87 per cent.) of immunized horses and mules had been broken down on exposure to natural infection. Of this number 165 (66·7 per cent.) died and 82 (33·3 per cent) reacted severely but recovered. It will be noticed that these figures correspond fairly closely with hose obtained with the police horses.

The heaviest mortality was experienced in the Natal-Zululand, Northern and Eastern Transvaal areas. In Nongoma the breakdowns amounted to 19·30 per cent. out of a total of only 57 immunized, in Eshowe 10·95 per cent. out of 137. Vryheid, Barberton, Louis Trichardt and Pietersburg showed breakdowns varying from 2 to 5·27 per cent. Blood was collected from a number of these animals immediately before death and preliminary work by in vivo methods has shown that the virus strains isolated are antigenically similar to the aberrant strains isolated from the police horses.

In the Mafeking area 1·88 per cent. of 2,653 immunized animals proved to be inadequately protected. Unfortunately only a single virus sample was collected from these animals. The virus isolated produced no reaction in animals immunized against the vaccine plus aberrant police strains, but from this single strain it is not possible to express any opinion as to the possibility of other antigenically dissimilar strains occurring over this wide area. This point is being

borne in mind because of the great difference in climatic conditions between the eastern and western portions of the Union, a factor which might favour the existence of yet another virus type.

From a statistical point of view it is admitted that these figures have very little value. In the first place the diagnosis of horse-sickness usually was made by laymen who are prone to believe that every immunized horse which dies during the late summer months must have died of horsesickness. It is surmized however that the number of incorrect diagnoses approximately would counterbalance the number of breakdowns which were not reported. In the second place the incidence of horsesickness among non-immunized animals maintained under similar conditions could not be ascertained, thus from an experimental and comparative point of view the fate of the all important controls cannot be recorded.

Discussion.

Consideration of the results reported above immediately emphasizes one important point, namely, that the neurotropic virus method of immunization of horses and mules may be practised with perfect safety. Out of 1815 police animals treated there was not a single death attributable to the use of the vaccine. Out of at least 28,659 other animals in general practise there have been recorded 3 deaths for which there is some possibility of the vaccine itself being responsible. This percentage is negligible and marks a considerable advance over the results obtained with the serum virus method where a mortality of 10 per cent. as a direct result of immunization has been recorded on several occasions.

Every effort has been made to determine the possible danger of neurotropism of the attenuated virus strains for equines. If this danger does exist it is so remote as to be almost negligible, as indicated be the experience in the field with over 30,000 equines and by direct and indirect methods in the laboratory with a limited number of animals. An unqualified statement that there is no danger of serious nervous complications cannot be advanced owing to the reported incidence of 5 cases of blindness in mules. This entire question, together with the affinity of the neurotropic virus for the nervous system of mules and donkeys, is being investigated further and will be reported in due course. At least there appears to be no necessity to complicate the entire method of immunization by developing a technique which will include the simultaneous use of hyperimmune serum, as has been found to be essential in the analogous case of Yellow Fever in man.

With regard to any remote ill effect of immunization it may be stated that up to the present time no unpleasant sequelae have been encountered. Certainly no permanent physical disability such as myocardial weakness or persistent slugglishness need be feared. The incidence of staggers (acute liver atrophy) may be cited with some justification as a complicating factor of great importance in the serum-virus method, but up to the present time no cases have occurred which could incriminate the present vaccine. Since the aetiology of this condition remains obscure it is undesirable to do more than record this observation.

The figures indicating the degree of immunity to natural infection are not entirely satisfactory because the season under review must be regarded as a mild one from the point of view of natural incidence of the disease. A recorded percentage of breakdowns in the case of police horses of 0·66 per cent. and in the case of other equines of 0·87 per cent. quite probably errs on the side of flattering the vaccine. This statement is supported by the fact that in some areas such as Nongoma, Eshowe, Vryheid, where every season must be regarded as a bad horsesickness season the mortality was as high as 19·30 per cent though the number of horses immunized was small. (Nongoma 10·30 per cent. of 57. Eshowe 10·95 per cent. of 137. Vryheid 2·06 per cent. of 2,042.) However it must be remembered that from this geographically and climatically similar tract of country there was isolated at least one strain of virus which differed antigenically from those which were available for atteunation. Natural infection with this strain probably was responsible for the adverse figures. On the other hand it is known that in some parts of the country the mortality from horsesickness amongst susceptible horses was high in spite of the usual prophylactic measures to prevent infection and yet the percentage breakdowns amongst immunized animals was less than 0·5 per cent. These observations, regarded in the light of the laboratory experience which has shown definitely that injection of a certain infecting dose of attenuated virus is followed by a solid immunity to the homologous strain, encourage the belief that the problem has become one of the development of complete polyvalency. Whether this is possible is a question which cannot be anwersed at present from the limited data available.

A number of additional problems possibly of minor importance await solution. For instance from the breeders point of view it is necessary to determine the effect of immunization on fecundity; the effect on susceptible pregnant mares at various stages of pregnancy; whether foals from immune dams are born with a passive resistance and if so the duration of that resistance; the youngest age at which immunization is both safe and efficacious. Then, from a general point of view, the duration of polyvalent immunity is a matter of importance. With the exception of the latter these are problems which do not lend themselves readily to solution by direct experimental methods in the absence of unrestricted access to a stud of considerable magnitude.

In conclusion it may be stated that neurotropic virus vaccine method of immunization has been attended by the most gratifying results. It is admitted freely that the method still awaits perfection, but a sound foundation has been laid on which to build.

Summary.

1. Details of the method adopted for the preparation of the vaccine are given.

2. The results obtained in the field during the season 1934-35 with the immunization of 1,815 police remounts and 28,659 other horses and mules are discussed.

Of the police horses there was no mortality as a direct result of immunization, no adverse after effects were noted, and the immunity of 12 was broken down on exposure to natural infection. Of those 10 died and 2 recovered.

Of the other horses and mules there were 3 deaths for which the vaccine may have been responsible or to which it may have contributed. On the whole the reactions were exceedingly mild and there were no adverse after effects. The immunity of 247 (0·87 per cent.) was broken down on exposure to natural infection. Of these 165 (0·58 per cent.) died and 82 (0·29 per cent.) recovered.

3. From the police breakdowns 3 strains of virus were isolated and have been fixed neurotropically in mice. Their antigenic interrelationship is being worked out.

4. In mules 5 cases of blindness following immunization have been reported.

5. The problem of immunization by the neurotropic virus method is discussed.

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APPENDIX.

Details of Cases Representing Breakdown of Immunity amongst Police Horses.

1. Horse T.387, stationed at Nelspruit, E. Transvaal. 19.3.34, immunized with neurotropic vaccine in Pretoria Depot. 23.8.34, immunized Nelspruit with neurotropic vaccine after transfer to that district. 19.1.35 reported sick, showing symptoms of Dikkop horsesickness. Died 21.1.35. Aetiological diagnosis, horsesickness.

2. Horse E.166, stationed at Ruimte, E. Transvaal. 21.1.35, sent to Schildpadfontein on duty; on arrival noticed to be off colour and sluggish; from 22.1.35 to 27.1.35 showed symptoms of fever up to 105° F., diagonsed as horsesickness; recovery uninterrupted and returned to work on 2.2.35.

3. Horse H.199, stationed at Bushbickridge, Eastern Transvaal; purchased 1933, and immunized by serum virus method prior to purchase; 6.7.34, immunized by neurotropic vaccine. 25.1.35, returned from a 4-day patrol. 26.1.35, noticed sick. 28.1.35, died; aetiological diagnosis, horsesickness.

4. Horse T.459, stationed at Eshowe, Natal. 24.4.34, immunized neurotropic vaccine. 1.9.34, immunized neurotropic vaccine. 2.2.25, noticed sick. 23.2.35, died. Diagnosis, horsesickness. Blood sample collected before death.

5. Horse E.517, stationed at Nylstroom. 29.11.28, immunized by serum virus method. 7.8.34, immunized neurotropic vaccine. 4.3.35, on 25-mile patrol. 5.3.35, noticed sick and on examination showed dikkop. 7.3.35, died. Aetiological diagnosis, dikkop horsesickness. Blood sample collected before death.

6. Horse T.737, stationed at Schoonoord, District Middelburg, Transvaal. 20.7.34, immunized neurotropic vaccine. 1.4.35, ridden from Schoonoord to Pakwani, left at 7 a.m. and arrived at 3 p.m.; started return journey following afternoon; after travelling 3 miles, noticed sluggish and immediately led back to Pokwani. 3.4.35, died. Aetiological diagnosis, dikkop horsesickness.

7. Horse H.960, stationed at Brits. 4.4.32, purchased after immunization by serum virus. 28.8.34, immunized neurotropic vaccine. 8.4.35, started on patrol at 7 a.m.; at noon horse noticed sluggish, immediately off-saddled and allowed to return to camp at its own gait. 9.4.35, died. Diagnosis, dikkop horsesickness.

8. Horse H.927, stationed at Schwayane cordon, Vryburg District. 4.4.32, purchased after serum virus immunization. 29.4.34, immunized neurotropic vaccine. 21.5.35, noticed sick, showing dikkop. 22.5.35, general condition brighter but marked oedema of head and throat; during the day struggled to break loose, and after breaking the leather dropped dead.

9. Horse B.280, stationed at Ntambanana, District Eshowe, Natal. 29.8.34, immunized neurotropic vaccine. 28.4.35, left at 2 a.m. for Empangeni, being led; when about 5 miles from Empangeni, commenced purging and appeared dull and distressed. Continued slowly on journey, arriving at 7.15; died 2 hours later. Horse did not feed or drink during the journey. Diagnosis, horsesickness.

10. Horse H.957, stationed at Matubatuba, District Eshowe, Natal. 27.6.33, immunized by serum virus method. 30.8.34, immunized neurotropic vaccine. 18.4.35, noticed sick when grazing in a paddock. 19.4.35, died. Diagnosis, horsesickness.

1. Horse H.913, stationed at Pietersburg, Transvaal. 4.4.32, immunized serum virus. 9.7.34, immunized neurotropic vaccine. 2.7.35, went on patrol and completed 30 miles, before return next day, being noticed sluggish before reaching camp. 4.7.35, died. Diagnosis, dikkop horsesickness.

12. Horse E.336, stationed at Honeyskop, District Taungs, Transvaal. 29.9.34, immunized neurotropic vaccine. 14.4.35, sick and subsequently showed dikkop. Recovered.