Serological evidence for the susceptibility of the hippopotamus (*Hippopotamus amphibius* Linnaeus) to natural infection with rinderpest virus

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It is well known that a very large number of species of the order Artiodactyla may contract natural infection with rinderpest virus. As noted by Curasson (1932) they include members of the families Bovidae, Giraffidae, Tragulidae and Cervidae, representing the suborder Ruminantia; the same author regarded camels (family Camelidae, suborder Tylopoda) as of proven susceptibility. In the suborder Suiformes many wild species of the family Suidae are certainly frequent victims of the disease and peccaries of the family Tayassuidae (Burton, 1962) were said to have been affected during the 1871 rinderpest outbreak at the *Jardin d'acclimatisation de Paris* (Curasson, 1932). However, we know of no record suggesting that the hippopotamus (*Hippopotamus amphibius* Linnaeus), which belongs to the same suborder, Suiformes (family Hippopotamidae), has ever been seen to be clinically affected by rinderpest. As a result it is generally regarded as insusceptible.

This communication records the finding of rinderpest-neutralizing antibody in serum samples collected from the hippopotamus population of the Queen Elizabeth Park in Uganda. The distribution of this antibody between various age groups supports the contention that it was acquired as a result of infection with rinderpest virus during the course of the recorded epizootics of the disease which occurred in this area during the last 30 or 40 years. The probability that rinderpest-neutralizing antibody persisted in moderately high titre for periods of more than 30 years is an outstanding instance of the stability of such antibodies in an animal species other than man.

MATERIALS AND METHODS

Collection and storage of sera

Hippopotamuses were killed twice weekly, by shooting, during the course of a systematic 'game-cropping' operation to relieve overstocking on the shores of Lake Edward and the Kazinga Channel (see Fig. 1). The animals were towed to land after they had floated to the surface, 1–2 hr. after shooting, under the influence of gases accumulating in their gastro-intestinal tract; they were butchered for meat and also served for general ecological and physiological studies by staff members of the Nuffield Unit of Tropical Animal Ecology.

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They were all weighed and assigned to relative age groups numbered from 3 to 20 (Table 1). In determining these age groups the criteria defined by Longhurst (1958), based on tooth replacement and wear, were adopted with slight modifications. These relative age groups were later transformed to estimated real age groups with a span of $x \pm 1$ or 2 years, ranging from $x = 1$ to $x = 41$; these are the values given
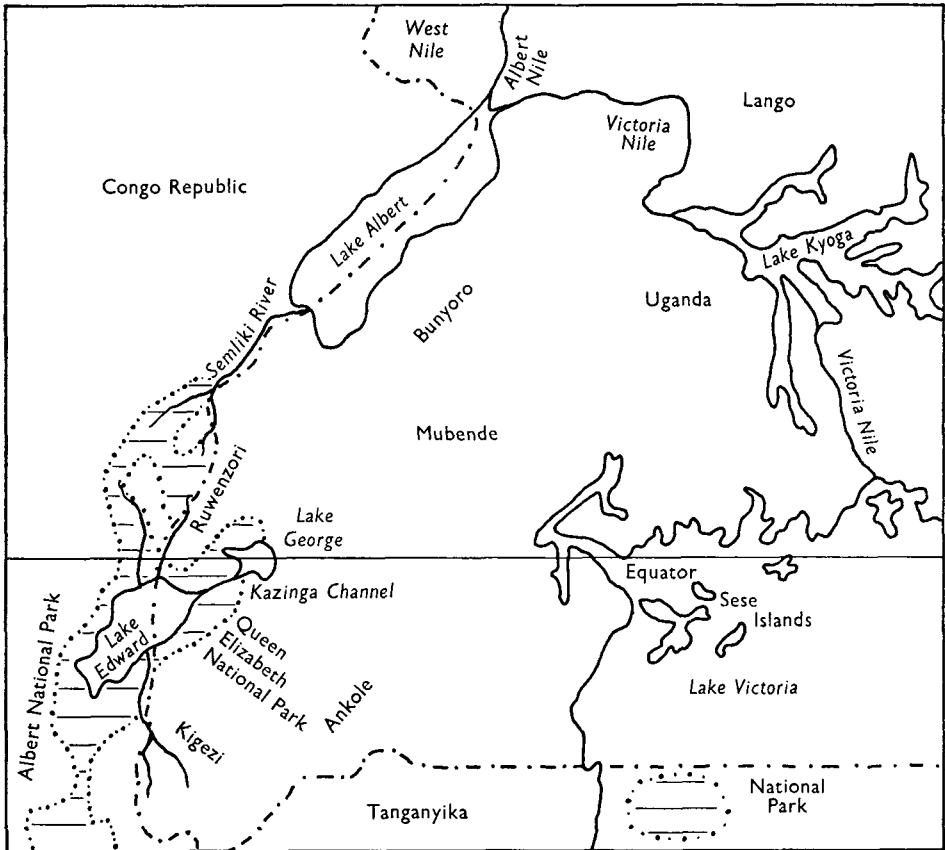

Fig. 1

in Table 1. In assigning real ages arbitrary estimates of the elapsed time between successive relative age groups were made, taking into account tooth growth and wear. The transformation was found to agree reasonably well with the observed tooth replacement and wear in six known-age captive hippopotamuses (aged 15–41 years). The oldest captive animal recorded was over 49 years at death (Burton, 1962), which does not conflict with a maximum longevity of 41 ± 2 years in the present wild sample of 315 animals. A full account of the age criteria and procedure will be given elsewhere.

There were no signs of a generalized disease process in any of the animals. Free-flowing blood was collected from large vessels during the course of the slaughter process; it was allowed to clot in sterile screw-cap bottles and serum was separated

on the following day. All samples were stored at 4° C. until they could be returned to the laboratory—generally speaking by air and within 2–4 days. They were then reclarified and stored at − 20° C.

Table 1. *The occurrence of rinderpest-neutralizing antibody in the sera of hippopotamuses shot in the Queen Elizabeth Park, Uganda*

Age group	Estimated age (years)	No. of sera tested	No. of sera positive	Proportion positive (%)	Class figures
3	1	3			
4	3	9			
5	4	11			
6	7	7	Nil	Nil	0/70 positive
7	8	21			
8	11	29			
9	13	35	1	2·9	
10	15	26	1	3·9	
11	18	13	1	7·7	5/120 positive
12	20	9	0	Nil	(4%)
13	22	18	2	11·1	
14	25	19	0	Nil	
15	28	50	13	26·0	
16	31	42	16	38·1	
17	33	16	8	50·0	41/115 positive
18	36	1	0	—	(36%)
19	38	4	3	75·0	
20	41	2	1	—	
Totals		315	46	—	46/315 positive

Virus neutralization tests

Sera were inactivated at 56° C. for 30 min., immediately before setting up the tests. Rinderpest virus neutralization tests were all performed in primary calf kidney tissue cultures using techniques which have already been described in detail (Plowright & Ferris, 1961; Plowright, 1962). Sera were first used undiluted in 'screening' tests, to eliminate all those which had no significant neutralizing activity; the dose of virus employed commonly fell between $10^{2\cdot0}$ and $10^{3\cdot4}$ TCD 50 per tube and only two tubes were used per sample. The test dose of virus was thus somewhat higher and more variable than the $10^{1\cdot8}$ to $10^{2\cdot8}$ TCD 50 previously advocated (Plowright, 1962) and may have resulted in a failure to detect antibody in a few sera with very low titres.

Quantitative neutralization tests were carried out on all except one of the sera which were positive in the screening tests. For this purpose undiluted serum and 10-fold serum dilutions were mixed with an equal volume of one of two virus stocks. After holding overnight at 4° C., each mixture was inoculated into five tubes of calf kidney cells. Final readings, for cytopathic end-points, were always carried out on the 11th or 12th days following, at which time the dose of virus in different series of tests varied from $10^{2\cdot0}$ to $10^{3\cdot4}$ TCD 50 per tube. Log_{10} SN 50 titres were calculated by the method of Thompson (1947). When less than half of the five tubes

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inoculated with serum at a final dilution of 1/2 were protected, the antibody content was recorded as a 'trace'.

A standard immune cattle serum was included for comparison with each batch of sera from hippopotamuses.

RESULTS

As shown in Table 1, a total of 315 sera were screened for antibody, these being derived from hippopotamuses of eighteen groups, varying in estimated age from 1 to 41 years. The numbers of samples in each group varied considerably, from one to fifty, but it was possible to subdivide all the donor animals into three classes of six age groups each. These classes ranged from 1 to 11, 13 to 25, and 28 to 41 years old, respectively. When this was done it was immediately obvious that marked differences in antibody status characterized the aggregate figures for the three classes (Table 1).

Table 2. *Titrations of rinderpest-neutralizing antibody in the sera of hippopotamuses*

Age group(s)	Number of sera titrated	Titre range ($\log_{10}$ SN 50)	Mean titre ($\log_{10}$ SN 50)	Number with titre $\geqslant 1\cdot 0$
9–14	5	0·4–1·2	0·76	2
15	12	0·3–1·8	1·32	9
16	16	Trace–1·8	1·18	9
17–20	12	Trace–2·0	1·11	6

There were no positives in 70 animals constituting the age class 1–11 years; 4 % (5/120) had antibody in the 13–25 years class, the positives being distributed in small numbers in age groups from nos. 9–13. In the age class 28 years and upwards 36 % of all animals tested possessed antibody and there was some indication of a rising 'immunity' rate between age groups 15–17 inclusive, with the percentage of positives almost doubling from 26 to 50 %.

The overall distribution of positives as between males and females was uneven (13:33) but this was accounted for by a predominance of females in the higher age groups, probably due to the fact that many of the latter were shot from schools of elderly matrons.

The results of quantitative neutralization tests on forty-five positive sera in screening tests are given in Table 2. There was a considerable range of titre in all the larger series, with figures varying from a trace to $10^{1\cdot 8}$ or $10^{2\cdot 0}$ (final serum dilutions of 1/50 or 1/100). Half to three-quarters of the animals in the age groups 15–20 had titres of 1/10 or above.

DISCUSSION AND INTERPRETATION OF RESULTS

The finding of a heat-stable virus-neutralizing substance in the sera of hippopotamuses immediately raised the question as to whether this appeared as a non-specific concomitant of the ageing processes, as a result of infection with an agent immunologically related to rinderpest virus or as a result of naturally acquired rinderpest infection.

The first possibility would be supported by the figures for a rising percentage of positives in animals of increasing age. However, there were some anomalies, such as absence of positives in age group 14 and the abrupt increase in groups 15–17 inclusive. Such an interpretation would also not be consistent with the results of the quantitative neutralization tests, which showed a slowly decreasing mean titre between groups 15 and 20 (see Table 2).

There were twenty-six animals with titres of $\geqslant 1/10$, and 16 with titres of 1/40 to 1/100, the dose of virus varying, as noted in the section on Materials and Methods, from $10^{2 \cdot 0}$ to as high as $10^{3 \cdot 4}$ TCD 50 per tube. These figures would hardly suggest that a non-specific virus inhibitor was involved. Further, no evidence for such an age-associated factor has come to light in the serum of other animal species which have been investigated (Plowright, unpublished).

The second possibility cannot be dismissed, since it is known that at least two other agents do have immunological relationships with rinderpest virus. These are the viruses of human measles and canine distemper (Warren, 1960; Imagawa, Goret & Adams, 1960; Plowright, 1963), but neither of them is known to infect any species of the order Artiodactyla and the opportunities for them to gain access to populations of hippopotamuses would appear to be somewhat remote.

The third hypothesis, that the hippopotamus is susceptible to natural infection with rinderpest virus, would be made more tenable by evidence that this agent was prevalent in the area under consideration up to 28 years ago, i.e. until 1934, and thereafter recurred in a more restricted form at intervals up to 1950. In the calculations we may ignore the effect of maternally derived antibody, since it is most unlikely that protection due to this factor would last for more than a year and the young born of immune dams in 1949 would have become susceptible by one year later; such animals would still fall in the age group number 9.

The Annual Report of the Uganda Veterinary Department for 1931 described numerous rinderpest deaths in buffalo to the south and west of Lake George in October and November of that year (Poulton, 1932). In 1932 it was noted that 'buffalo were dying along the shores of Lakes George and Edward for a long period' (Poulton, 1933). Rinderpest persisted in game and cattle of the neighbouring Kigezi and Ankole districts at least through 1933 and 1934 but there was no specific report of the disease from the shores of the lakes (Poulton, 1934, 1935).

After 1934 the Annual Reports of the Uganda Veterinary Department do not mention the occurrence of rinderpest in the Western lacustrine region, until the infection was again introduced via the Sudan border in 1942 (Simmons, 1943), spreading southwards to reach the Ankole district, bordering Lakes George and Edward, in 1943/44. Similar, limited incursions occurred in 1948 (Cronly, 1949) and 1955 (Randall, 1956); however, these did not reach the Western Province of Uganda, although the later outbreak was reported by the Belgian Congo authorities to have affected game animals to the West of Lake Albert.

Pitman (1942) in discussing the Uganda Kob, stated that it was 'very susceptible to the ravages of rinderpest, and some 12 years ago suffered disastrously in the Western Rift around Lake George and Lake Edward'. Hubert (1947) in a monograph on the Parc National Albert, to the south of Lake Edward, presented records

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of a rinderpest epizootic in 1932, affecting particularly warthogs (phacochères) but possibly also giant forest hogs (hylochères) and topi. In addition, he mentioned a previous well-known and catastrophic outbreak of the disease, in 1920.

According to Bourlière & Verschuren (1960) rinderpest decimated the buffaloes in the area of the Parc National Albert in the epizootic of 1921, and reached them again in April 1932, having arrived from Uganda via the district to the south of Lake George. It reappeared, once more from the vicinity of Lake George, in August 1944 and spread slowly among buffaloes, reaching the plain to the south of Lake Edward in April 1945. Guyaux (1951), who gave an account of the same outbreak, proved the identity of the buffalo disease agent and mentioned that bushpigs, giant forest hogs and bushbuck were also found dead.

In conclusion, from the available records, it can be said with some degree of assurance that the hippopotamus population in the region of Lakes George and Edward was exposed to rinderpest during the epizootics of 1920/21, 1931/33 and 1944/45, but no reliable evidence exists that it has since had contact with the infection. Hence it is possible to account for all the antibody-positive animals with the exception of one each in groups 9 and 10. Apart from the possibilities of confusion in labelling of specimens or minor errors in the estimation of age, it is difficult to explain the possession of antibody by these two individuals.

The much higher 'immunity' rate in animals 28 years of age and over suggests that the intensity of exposure in the earlier epizootics was greater than in 1944/45. The lower rate of serological conversion in the latter episode may also imply that the particular strain of virus involved did not spread so readily among susceptible hippopotamuses, since it would be expected that opportunities for transfer would be optimal in such a gregarious and semi-aquatic species, which is continually fouling its own living medium. The grazing habits of hippopotamuses, including their continual use of the same lake-shore paths, would also appear to favour dissemination of the virus. The same behavioural factors would undoubtedly facilitate the acquisition by the hippopotamus of virus from other species, such as buffalo and waterbuck. These, when suffering from rinderpest, tend to remain near water in order to slake their abnormal thirst; in addition they often die in or near drinking points.

There is apparently no record of disease in the hippopotamus which has been attributed to rinderpest virus. It is, however, well known that a significant local mortality occurs periodically in areas where the species is abundant. Multiple deaths have been observed at intervals over many years and a series of such incidents has been summarized in tabular form by Bourlière & Verschuren (1960). The general opinion is that the mortality results from anthrax (see, for example, Randall, 1951, 1957, 1958 and Cronly, 1952) although Bourlière & Verschuren (1960) make reference only to 'charbon symptomatique', a clostridial infection commonly known as 'blackquarter' in English. The deaths have not ceased in the last 10 years, during which time rinderpest has been absent from the region; nevertheless it is possible that some of the earlier episodes, as in 1930 (Kazinga Channel) or 1933 (Lake George) may have been due, at least partially, to rinderpest infection.

So far as the persistence of rinderpest-neutralizing antibody is concerned, Brown & Raschid (1957) reported that cattle which had received caprinized rinderpest vaccine were still immune and possessed circulating antibody 13 years later, although they had never been re-exposed to virus; quantitative data were not presented. Neutralizing antibody in man, to the closely related measles virus, persists throughout life and the proportion of serological positives among individuals in endemic areas does not change appreciably after the age of 8–9 years (Black, 1959). The mean titre, however, falls gradually in adult life in spite of the fact that there are probably periods of re-exposure, particularly in the 13–14 and 20–40 years age groups (Black, 1959). Black & Rosen (1962) studied measles antibody patterns in residents of Tahiti, where epidemics occurred in the years 1929, 1951 and 1960. There was essentially no change between 1951 and 1959 in the neutralizing-antibody titre of sera from patients infected in the former year, although no re-exposure to measles occurred during this time. Individuals who were infected in 1929 and presumably exposed again in 1951 showed only a very slow decline in serum antibody over a 30-year period. The degree of stability of antibody was regarded as 'almost without precedent'.

The rinderpest titres of sera from hippopotamuses (Table 2) were not appreciably different from those which have been found in other rinderpest-susceptible game species in the Serengeti area of North Tanganyika, where the virus has produced epizootics during the last 3–4 years. Thus forty-two recently-infected yearling wildebeest (*Gorgon taurinus taurinus*) showed log SN 50 titres which ranged from a 'trace' to $10^{2 \cdot 8}$ but the mean was only $10^{1 \cdot 15}$, against a dose of virus varying from $10^{1 \cdot 6}$ to $10^{2 \cdot 6}$ TCD 50. The wildebeest is a species which reacts clinically, sometimes severely, to rinderpest virus infection and it was all the more surprising, therefore, to find that the mean antibody level in hippopotamuses first infected about 30 years ago ($10^{1 \cdot 11}$ to $10^{1 \cdot 32}$) was at least as high as that in yearling wildebeest, infected only a few weeks or months before (Plowright, to be published).

SUMMARY

A survey of rinderpest-neutralizing antibody was carried out on the sera of 315 hippopotamuses which were shot in the Queen Elizabeth Park, Uganda. No serological positives were found in animals aged 11 years or less but about 4 % were detected in the age class 13–25 years. In the 28–41 years age class the proportion of positives rose to about 36 %. All except one of the positive sera were titrated, the figures obtained being comparable to those in other recently infected species of wild ungulates.

It was concluded that the heat-stable antibody in hippopotamuses resulted from inapparent infections with rinderpest virus during epizootics which passed through the areas of Lake George and Lake Albert in the years 1920/21, 1931/33 and 1944/45. No record was available of clinically recognizable rinderpest in the hippopotamus population but deaths due to other causes were mentioned briefly.

Comment was made on the stability of rinderpest-neutralizing antibody over periods of about 30 years.

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