The duration of immunity in cattle following inoculation of rinderpest cell culture vaccine

By W. PLOWRIGHT*

*A.F.R.C. Institute for Research on Animal Diseases, Compton, Newbury,
Berkshire, RG16 0NN, U.K.*

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SUMMARY

The duration of immunity following a single administration of rinderpest cell culture vaccine, of 90 or more monolayer passages, was studied in E. African zebu (Boran) and grade (cross-bred European) cattle. All animals were kept for periods of 6–11 years in rinderpest-free environments; groups of them (in all 23 Borans and 10 grades) were then challenged by parenteral or intranasal inoculation of virulent virus or by contact exposure to reacting cattle. Nasal excretion of virus was studied daily over the 10- to 14-day period following challenge, and simultaneous attempts were made to detect viraemia. The neutralizing antibody response was followed at 6-month intervals over the whole post-vaccination period and then daily for 10 days and at longer intervals to 3 weeks after challenge.

All 33 animals which were exposed by various routes failed to react clinically and a rinderpest viraemia was never detected. No transmission of virus from the vaccinates to susceptible in-contact controls occurred within 14 or more days, from the 20 animals which could be so tested. Clearcut serological responses to challenge were seen in six cattle (four Borans and two grades) which were challenged after 7 years or more; these reactions were all delayed to the 9th or 10th days, i.e. they were not typically 'anamnestic'.

These results are discussed in relation to mass vaccination campaigns for the control of rinderpest and from the comparative viewpoint of measles vaccination in man.

INTRODUCTION

The recent resurgence of rinderpest in many countries of Africa and the Near East has led to renewed suggestions for the establishment of long-term, international campaigns for its control and, hopefully, its eradication (Nawathe & Lamorde, 1982; Nawathe, Lamorde & Kumar, 1983; Rossiter *et al.* 1983). A crucial prerequisite for such endeavours is a cheap and stable vaccine which confers a solid, enduring and preferably lifelong immunity. In 1970 the World Health Organization Expert Committee on Biological Standardisation published, in their twenty-second report, internationally agreed requirements for the manufacture and control of rinderpest vaccine, including Rinderpest Cell Culture Vaccine (Live), which is now

* The work reported here was all carried out at the (then) East African Veterinary Research Organisation, P.O. Box 32, Kikuyu, Kenya, E. Africa.

the product most commonly employed. These requirements include (part A, section 1.4 and 3.1.1) mandatory proof that the cell-propagated virus should be 'safe and confer an immunity of at least 5 years duration' (WHO, 1970).

Evidence for the duration of immunity conferred by one such vaccine, the Muguga modification of the Kabete 'O' strain of virus after 90 or more passages in primary calf kidney culture, has been provided at intervals (Plowright, 1962*b*; Plowright & Ferris, 1962*b*; Plowright & Taylor, 1967; Plowright, 1972*a* and *b*; Rweyemamu, Reid & Okuna, 1974). Details of the serological responses to the end of the fourth year post-vaccination and of the behaviour of vaccinated animals following experimental challenge with virulent strains of virus were provided for East African zebus (Boran) and crossbred (grade) cattle. In addition, antibody responses were recorded for Ugandan Ankole (longhorn) cattle over a period of 2 years (Plowright & Taylor, 1967). Clinical resistance to challenge was later found to last up to and even beyond 10 years after vaccination and was absolute. Nevertheless, a small proportion of animals, in many of which neutralizing antibody had declined to low or negligible levels, supported a transitory multiplication of virulent virus and/or showed serological reactions; in only one case did transfer of rinderpest virus take place to a susceptible steer housed with a challenged heifer (Plowright & Taylor, 1967; Plowright, 1972*b*).

The unequivocal nature of these observations was unfortunately thrown into question by Provost, Maurice & Boredon (1969) who used two different rinderpest cell-culture vaccines in zebus (Bororos) of the Central African Republic. The first of these was the Muguga modification of the Kabete 'O' strain after only 35–36 passages in secondary calf kidney cells; the second was an American culture-passaged strain (De Boer & Barber, 1964) which had been 'cloned' by the end-point dilution technique. Both strains produced serological responses, which in a third of the animals waned to 'non-protective' or undetectable levels by 2 years post-vaccination, whilst five of 16 cattle died of rinderpest following contact exposure at 35 months.

As a result, Provost, Maurice & Borredon (1969) suggested that it was necessary to revaccinate cattle, in Central Africa at least, every two years and, if possible, annually. They also hypothesized, from restricted observations on inapparent re-infection of some vaccinated cattle, that hopes for rinderpest eradication would be in vain unless vaccination were accompanied by quarantine and slaughter of affected herds. The cost of these proposed measures demands a more critical appraisal of wider evidence, and in this paper we give a detailed presentation of our findings in East Africa. Although a brief outline of these has been published already (Plowright, 1972*b*) it is considered necessary to provide convincing evidence at a time when international immunization campaigns, to rival the previous JP-15 programmes (Lepissier & Macfarlane, 1967), are again under consideration. In addition, by drawing attention to the serological reactions to challenge in clinically resistant animals we may stimulate work on the immunological mechanisms responsible for preventing re-infection by rinderpest virus, as opposed to protecting against clinical reaction.

MATERIALS AND METHODS

Cattle employed

As already described, these included the following groups (Plowright, 1962*a*; Plowright & Taylor, 1967).

(a) *East African zebu (Borans)*. 50 seronegative female animals, 10–13 months old, were inoculated in April 1961 at the Government Experimental Station, Naivasha, Kenya. These cattle were later transferred to Government ranches and eventually to the Muguga laboratory estate, all of which were reliably free of natural rinderpest. The test cattle were not revaccinated, so far as is known, to the end of the 11th year of the trial.

(b) *Grade dairy cattle*. These were high-grade Jersey or Friesian crosses, all females inoculated at 8–12 months of age whilst on the dairy farm of origin; they were excluded from subsequent vaccinations which were carried out by the laboratory staff and they were not exposed to virulent rinderpest virus. At the 47th month of the experiment 10 animals were sold whilst the survivors were transferred to the laboratory estate and added to a milking herd, which was never exposed to natural rinderpest infection up to the 123rd month.

Vaccination

The Borans received $10^{1.9}$ TCD 50 of reconstituted, freeze-dried culture vaccine, which consisted of the 91st passage of the Kabete 'O' (RBOK) strain in primary calf kidney cells; the grade animals were inoculated subcutaneously with $10^{2.6}$ TCD50 of similar 90th-passage virus.

Virulent rinderpest virus

Two strains were used for challenge of immunity, i.e. the RGK/1 isolate, from a reticulated giraffe in Kenya (Liess & Plowright, 1964), and the Malakal isolate, derived from a severe outbreak of the disease in Sudanese cattle and received as freeze-dried spleen powder.* The RGK/1 strain was stored at -70°C as fragments of spleen pulp, which were thawed when required to prepare 10% (w/v) suspensions in culture maintenance medium. These were always titrated at the time of use by inoculation into pre-formed monolayers of primary calf kidney cells in roller tubes, five or 10 cultures per tenfold dilution. One spleen, which was titrated 12 times over a period of 3 years after freezing, showed no fall in infectivity during this time (mean titre $10^{5.5}$ TCD50/g).

Challenge procedures

Vaccinated cattle were housed singly in isolation units, which were provided with individual showers and facilities for a complete change of clothing on entry and exit. They received 2, 5 or 10 ml subcutaneously (s/c) or 2 or 10 ml intranasally (i/n) of RGK/1 spleen suspension (Plowright, 1964). After 24–48 h a rinderpest-susceptible steer was introduced as a 'sentinel' to detect natural transmission of virus. Contact was maintained for periods of 14–27 days (Tables 1, 2) when the vaccinated animals were removed. The sentinels were challenged 1–31 days later

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by s/c inoculation of virulent virus (RGK/1 or RBOK strains) or by exposing them to cattle reacting to infection with the RGK/1 strain.

Challenge by contact exposure was effected by inoculating one or two susceptible cattle in an isolation unit with either the RGK/1 or Malakal strains and introducing single vaccinated animals after the former had shown pyrexia for 2–3 days. When Borans were thus exposed to Malakal virus (Table 2) another susceptible animal was also put into each unit to check the capacity of the virus to spread by contact. A second susceptible ox was also added 15 days later to acquire infection and extend the duration of exposure of the vaccinates. Similar controls were also employed for the RGK/1 virus used to challenge grade vaccinates at 123 months (Table 1). Whatever the route of exposure the vaccinates were examined clinically every day for 10 days and thereafter whenever blood was collected for serum (see below). Contact controls or virus donors were also examined at the same times and rectal temperatures were recorded daily, with precautions to avoid mechanical transfer of virus.

Recovery of virus from vaccinated cattle

Nasal secretions were collected from 25 vaccinates, six grades and 19 Borans, every day for 10 or 14 days following challenge (see Tables 1 and 2); the nostrils were cleaned with cotton wool and a cotton-tipped swab was inserted about 7–8 cm into both sides; the swab was then broken off immediately into a screw-cap bottle with 3 ml of culture maintenance medium, containing three times the normal concentration of antibiotics (see below). In the laboratory the fluid was expressed from the swab with forceps and pipetted vigorously to disperse mucus, etc. prior to inoculating 0.2 ml into each of five primary calf kidney (BK) cultures. In many cases a 10^{-1} dilution of the swab extract was also inoculated into a similar group of cultures.

Tests for viraemia were also carried out daily for 10 or 14 days after challenge. Approximately 20 ml quantities of blood in EDTA (2:1) were collected and used to prepare buffy-coat samples which were inoculated into five BK tube cultures (Plowright & Ferris, 1962*a*); for a few animals the quantity of blood processed and the numbers of cultures inoculated were doubled.

On the day following inoculation BK cultures were washed 2–3 times with 2 ml of phosphate-buffered saline (PBS) and the maintenance medium (LYE:OS5 with twice the usual quantities of antibiotics; Plowright, Herniman and Rampton, 1969) was replaced and changed every 2 days. Final microscopic examination for the cytopathic effects of rinderpest virus was carried out on the 9th to 12th days.

Neutralizing antibody

Cattle were bled for serum at approximately 6-month intervals from the time of vaccination in 1960/61. Serum was again collected immediately prior to challenge and daily thereafter for 10 days; further samples were taken at 14 days and, usually, at 17 and 21 days. All samples were stored at -20°C until required. In the case of animals challenged at 7 years and later, 30 or more sera were available and all were titrated simultaneously for neutralizing antibody, using the same batch of trypsin-dispersed cells, according to the technique already described (Plowright & Ferris, 1961; Plowright, Herniman & Rampton, 1969).

Up to the end of the 6th year of the trial 10-fold dilutions of heat-inactivated (56 °C, 30 min) serum were employed, with five tube cultures for each dilution; the test dose of virus was required to fall between $10^{1.8}$ and $10^{2.8}$ /culture. Later assays employed twofold dilutions of serum and virus, in order to reduce within-test variations; 0.1 % bovine plasma albumin in PBS (pH 7.2) was used as a diluent, and virus was prepared on each occasion from a pool of ampoules of a large freeze-dried stock. Serum:virus mixtures were held overnight at 5 ± 3 °C and a standard immune serum was included in each test, to monitor between-test variations in sensitivity. The observed dose of virus in these tests ranged between $10^{1.65}$ and $10^{2.67}$ TCD₅₀.

Cytopathic changes were recorded at intervals up to 10–11 days, when virus and serum ($\log_{10}$ VN50) titres were calculated by the Spearman–Kärber method (Dougherty, 1964).

RESULTS

Boran cattle

Table 1 gives details for 24 animals which were challenged in groups of four at intervals of between 67 and 132 months after vaccination. The last group were reported on by Rweyemamu, Reid & Okuna (1974), and unfortunately included one animal (No. 340) which had already been used for contact exposure at 116 months. It is clear that all 23 animals challenged for the first time were completely resistant both clinically and virologically; rinderpest virus was never recovered from blood or nasal swabs. In one case (No. 384) a virus, identified serologically as that of infectious bovine rhinotracheitis (IBRV) was isolated continuously from nasal swabs from days 3 to 10 inclusive but no pathological sign was observed.

In 14 cases susceptible animals housed in contact with vaccinates failed to react following an exposure of 14–27 days, i.e. significant excretion was not detected. When exposure to virus-donor animals was used to test immunity at 116–18 months, it was not possible to use such controls, but the disease did spread predictably in three of four boxes from the donors to sentinel cattle within 7–12 days.

Serologically the results were less clearcut. Four animals showed what were probably significant increases of neutralizing antibody beginning 9 to 10 days after challenge. These were nos. 387, 384, 394 and 367 exposed to virulent virus after 67, 87, 87 and 132 months respectively (Table 1); antibody titres ($\log_{10}$ VN50) prior to challenge were in the range 1.11 (no. 384) to 2.2 (no. 367), but three were towards the lower end of the scale ($\leq 10^{1.87}$). The route of challenge was subcutaneous in two of these cases and intranasal in the other two, including one already described by Rweyemamu, Reid & Okuna (1974). The increases varied from approximately 0.8 to 1.7 $\log_{10}$ units (no. 384) by the 14th day after challenge (see Fig. 1 for no. 384).

Grade cattle

Details for 10 animals challenged at intervals between 72 and 123 months are given in Table 2. Not one of these animals reacted clinically or yielded virus from buffy coat preparations. Nasal swabs were negative in six cases but a suspicious cytopathology was noted in one culture inoculated with blood from cow no. 802 on the sixth day following challenge; it could not be passaged. Contact controls,

Table 1. *Long-term immunity studies on East African Zebu (Boran) cattle immunized with rinderpest culture vaccine*

Animal no.	Months after vaccination	Route of challenge/dose*	Recovery of virus from		Clinical/serological response	Infection of contacts†
			Blood†	Nasal swabs†		
385	67	sc/3·2	Nil	NT	Nil/—	—(27 days)
387	67	sc/3·2	Nil	NT	Nil/+	—(27 days)
391	67	sc/3·2	Nil	NT	Nil/—	—(27 days)
396	67	sc/3·2	Nil	NT	Nil/—	—(27 days)
372	87	sc/4·5	Nil	Nil	Nil/—	—(14 days)
384	87	in/4·5	Nil	Nil§	Nil/+	—(14 days)
390	87	in/4·5	Nil	Nil	Nil/—	—(14 days)
394	87	sc/4·5	Nil	Nil	Nil/+	—(14 days)
339	109	in/6·0	Nil	Nil	Nil/—	—(19 days)
356	109	sc/6·0	Nil	Nil	Nil/—	—(19 days)
364	109	in/6·0	Nil	Nil	Nil/—	—(19 days)
377	109	sc/6·0	Nil	Nil	Nil/—	—(19 days)
340	116	Contact	Nil	Nil	Nil/—	NA
344	116	Contact	Nil	Nil	Nil/—	NA
353	116	Contact	Nil	Nil	Nil/—	NA
366	116	Contact	Nil	Nil	Nil/—	NA
350	118	Contact	Nil	Nil	Nil/—	NA
354	118	Contact	Nil	Nil	Nil/—	NA
357	118	Contact	Nil	Nil	Nil/—	NA
363	118	Contact	Nil	Nil	Nil/—	NA
349	132‡+	in/5·7	Nil	Nil	Nil/—	—(14 days)
362	132‡+	in/5·7	Nil	Nil	Nil/—	—(6 days)
367	132‡+	in/5·7	Nil	Nil	Nil/+	—(14 days)
340	132‡+	in/5·7	NA	NA	NA	NA

(repeat)

* Challenge virus strain was RGK/1 except for contact challenges (strain Malakal). sc = subcutaneous; in = intranasal. Dose is expressed in $\log_{10}$ TCD₅₀ units of RGK/1 spleen tissue virus.

† Observation was for 10 days except for group at 132 months (6 or 14 days) — leucocyte fractions from 14 ml blood examined.

‡ Figures in parentheses refer to period of exposure.

§ IBR virus recovered continuously, days 3–10 inclusive.

+ From Rweyemamu, Reid & Okuna, 1974.

NT = not tested; NA = not applicable.

present in eight cases for 15–25 days, did not acquire the disease or develop an immunity to rinderpest. In the challenge by contact exposure at 123 months after vaccination, three non-vaccinated cattle reacted after 5–7 days and two of these succumbed to rinderpest, i.e. transmission occurred in the expected manner.

Serologically, two vaccinated cattle developed delayed responses to challenge, namely no. 770 at 7 years and no. 794 at 87 months. In the first case the increase in $\log_{10}$ VN₅₀ titre was 0·54 from a base of 1·59 and in the second 1·56 (no. 794) from a base of 1·29 (Fig. 2). No. 770 was inoculated subcutaneously, no. 794 by the intranasal route. In these cases the increase in antibody titre did not reach fourfold (0·6 $\log_{10}$ units) until the 9th or 10th days and the maximum was not reached until 14–21 days after challenge.

Fig. 1. The neutralizing antibody response of two Boran cattle vaccinated with rinderpest cell culture vaccine and challenged 87 months later.

Table 2. Long-term immunity studies on high-grade East African cattle immunized with rinderpest culture vaccine

Animal no.	Months after vaccination	Route of challenge/dose*	Recovery of virus† from		Clinical/serological response	Infection of contacts
			Blood	Nasal swabs		
780	72	sc/3.9	Nil	NT	Nil/-	-(25 days)
800F	72	sc/3.9	Nil	NT	Nil/-	-(25 days)
770	84	sc/2.8	Nil	NT	Nil/+	-(20 days)
802	84	sc/2.8	Nil	NT	Nil/-	-(20 days)
421	87	sc/4.9	Nil	Nil	Nil/-	-(15 days)
794	87	sc/4.9	Nil	Nil	Nil/+	-(15 days)
144	91	in/3.9	Nil	Nil	Nil/-	-(22 days)
731	121	sc/5.6	Nil	Nil	Nil/-	-(18 days)
790	123	Contact exposure	Nil	Nil	Nil/-	NA
800M	123	Contact exposure	Nil	Nil	Nil/-	NA

* sc, subcutaneous; in, intranasal.

Dose is expressed in $\log_{10}$ TCD₅₀ units of RGK/1 spleen tissue virus.

NT, not tested; NA, not applicable.

† Leucocyte fractions from 14 or 28 ml blood or nasal swabs collected for 10 or 14 days following challenge exposure.

Fig. 2. The neutralizing antibody response of two grade cattle vaccinated with rinderpest cell culture vaccine and challenged 99 months later.

DISCUSSION

The results presented here, together with those of Plowright & Taylor (1967) and Rweyemamu, Reid & Okuna (1974) confirm beyond any reasonable doubt that the duration of clinical immunity conferred on East African cattle by rinderpest cell-culture vaccine was at least 10 years and probably lifelong. Both zebu (Boran) and high-grade (European) cattle were involved, whilst the serological results previously obtained for Ankole longhorns, up to 2 years post-vaccination, were consistent with a similar prolonged duration of immunity (Plowright & Taylor, 1967). It did not appear, therefore, that breed had an effect on the efficacy of vaccination such as was suggested by Provost, Maurice & Borredon (1969).

The failure, 6 years or more after vaccination, to detect proliferation of virulent challenge virus in cattle exposed by the subcutaneous or intranasal routes or by contact (i.e. presumably through the respiratory tract) contrasts with previously reported results, which demonstrated a subclinical viraemia in two of eight grade cattle which were challenged at 27–50 months after vaccination (Plowright & Taylor, 1967); contact transmission to a susceptible control was also recorded in one additional case. Rweyemamu, Reid & Okuna (1974) reported the presence of minimal amounts of infectious virus in the tonsillar tissue of one of six grade animals challenged 6 days previously by intranasal instillation of virulent RGK/1 virus, 6 years after immunization with cell-culture vaccine. However, no clinico-pathological or serological response was observed, and nasal excretion of virus was not detected up to the time of slaughter at 6 days after challenge.

The serological responses to challenge of 'immune' grade cattle in the experiments

reported previously (Plowright & Taylor, 1967) and in this paper appeared to fall into four categories. In the first there were two animals, exposed parenterally at 27 and 37 months (nos. 829 and 738); both had trace or undetectable levels of antibody prior to inoculation but showed significant increases at 5–6 days, with high peak titres at 10–11 days. These animals had minimal amounts of maternal antibody at vaccination and developed a viraemia following challenge. In the second category were two cattle (nos. 411 and 596) which showed very rapid 'anamnestic' responses within 48 h, from low pre-challenge titres ($10^{0.6}$ and $10^{1.2}$ respectively). In the third group there were three animals, nos. 721, 770 and 794, challenged 50, 84 and 87 months after vaccination, which showed a secondary response on the 9th–10th days, with pre-challenge titres in the range $10^{1.0}$ to $10^{1.59}$. The fourth category consisted of 10 animals which did not respond serologically, their serum titres at the time of exposure being in the range $10^{1.8}$ – $10^{2.7}$. The Borans contributed four and 19 animals to the third and fourth groups respectively.

Only the first group showed serological responses resembling those of unprimed cattle. In the latter, neutralizing antibody first appears 6–7 days after inoculation of $\geq 10^4$ ID₅₀ of virulent virus, more than 7 days after administration of partially attenuated caprinised virus (Plowright, 1962*a*) and 7–10 days after inoculation of avirulent cell culture vaccine in a dose of $10^{4.0}$ – $10^{4.6}$ TCD₅₀ (Taylor & Plowright, 1965) or 10^6 TCD₅₀ (Anderson *et al.* 1982). The second category of grade animals was, therefore, the only one with typical 'anamnestic' responses, whilst those in the third group showed somewhat delayed responses, resembling those seen also in 4/23 Boran zebus, i.e. at 9–10 days.

The retarded serological responses could have been attributable to appreciable pre-challenge titres which masked relatively small early increases. This interpretation would not be supported, however, by the results of an experiment in which 10 cattle, immunized 15 months previously with lapinized rinderpest vaccine, were inoculated with formalin-inactivated bovine spleen virus; nine of these had initial neutralizing titres of $10^{1.0}$ to $10^{2.2}$ but nevertheless showed significant increases ($\geq 0.6 \log_{10}$ units) within 6 days of receiving the killed antigens (Plowright, 1962*a*). The occurrence of serological responses in three cattle (nos. 794, 384 and 387) challenged by the intranasal route showed that the stimulus for group 3 animals was probably proliferating virus, rather than viral antigens present in the inoculum; virus replication was not detected, however, possibly because it was limited to a restricted tissue site, such as the tonsil.

The association between circulating levels of neutralizing antibody and resistance to natural or experimental challenge following vaccination is one which is often emphasized in morbillivirus infections, including canine distemper (see Appel & Gillespie, 1972) and human measles (e.g. Anon, 1971; Kalis, Quie & Balfour, 1975). The most extreme form of exclusive attention to circulating antibody in rinderpest immunization is provided by papers such as that of Iyigören, Ünlü & Yonguc (1976), who considered that animals with titres less than $10^{2.0}$ after inoculation of cell-culture vaccine should be considered susceptible and, on challenge, would develop either clinical signs or inapparent infection. However, we have shown that the association of antibody with resistance is not invariable, since some animals which have become negative or weakly positive after cell-culture vaccine are still clinically, if not virologically, resistant. Similarly, animals which have been

vaccinated with caprinised virus and have then been challenged with virulent virus can become serologically negative within 6 months, although they are undoubtedly immune (Plowright, 1962*a*, 1968).

Not unexpectedly, there are very close parallels between vaccination against human measles with attenuated virus and rinderpest immunization using cell-culture vaccines. Thus the great majority of reports show a comparably enduring serum antibody response (see Krugman, 1977; Weibel *et al.* 1980), even in the absence of re-infection by 'wild' measles virus (Brown, Gajdusek & Tsai, 1969). It is also clear that residual, passively acquired antibody can interfere with the degree of antibody response to vaccination (e.g. Kalis, Quie & Balfour, 1975), and it is widely acknowledged that subclinical re-infection does occur in both 'immune' children and adults (Linneman *et al.* 1972; Weibel *et al.* 1980). Nevertheless, Krugman (1971) reported that measles had never been seen in a child who had been shown to have an antibody response after live measles vaccine. All these observations show a remarkably close analogy with our data for cultured rinderpest vaccine.

Few investigations have been made of the role of local antibody in rinderpest immunity, but Provost (1970) found that specific IgM and IgA appeared in the nasal secretions of cattle only after local infection of the respiratory tract and not usually after ingestion of antibody-containing colostrum or parenteral live virus vaccination. He therefore hypothesized that, on natural exposure, vaccinated animals could support inapparent local replication of virus and thus contribute to its maintenance. The results presented here do not support Provost's suggestion; the only 'immune' animals able to support virus growth, adequate for transmission, are calves with residual maternal immunity, producing a poor antibody response to vaccination, which in some instances may fade rapidly to minimal or zero values. Such animals cannot be frequent in field conditions, as was shown in Tanzania where 89.5% of 351 randomly selected cattle, inoculated twice as calves and yearlings, still had antibody 4–5 years later, in the absence of naturally circulating virus (Plowright, 1972*b*).

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