

# Experimental challenge trial with a “very virulent” strain of Infectious Bursal Disease virus (vIBDV) in commercial pullets vaccinated with an IBD vectored vaccine or with three different modified live vaccines

By courtesy of



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In order to evaluate the level of active immunity stimulated by different Infectious Bursal Disease (IBD) vaccines and vaccination schedules, an experimental challenge trial with a “very virulent” strain of the IBD virus (vIBDV) was carried out in commercial pullets with maternally derived antibodies (MDA) to IBD and reared in isolation units. In particular, the aim of the study was to compare the protection induced by the IBD vector vaccine vHVT13, in which the herpes virus of turkeys (HVT) is used as the vector of the IBDV VP2 gene, with the protection induced by different conventional modified live vaccines (MLVs) (“intermediate” and “intermediate plus”). vHVT13 was administered once by subcutaneous injection in 1-day-old pullets, when MDA are at their highest levels, whereas the MLVs were administered by oral drop between 17 and 25 days of age. The development of an active IBD antibody response to vaccination and to the challenge was evaluated using two commercially available IBD ELISA kits, one using an antigen derived from a classical strain grown in tissue culture and detecting mainly the antibodies produced against the VP3 protein of IBDV following a natural infection or vaccination with MLV, the other using a native bursal derived strain antigen which allows an accurate detection of IBDV protective anti-VP2 antibodies.

A gap between protection conferred by MDA and then immunity conferred by vaccine induced antibodies was observed in all of the groups vaccinated with MLVs. In contrast, complete protection was recorded in the group vaccinated with vHVT13, confirming that MDA do not interfere with the mechanism of action of this vaccine. Furthermore, the combined use of both ELISA kits enabled differentiation between chickens vaccinated with vHVT13 from birds challenged with vIBDV. In fact, after 3 to 4 weeks of age vHVT13 vaccinated chickens had low antibody titres using the IBD Ab test based on an antigen derived from a classical strain grown in tissue culture, but high antibody titres (normally >6000) using a native bursal derived antigen. On the contrary, chickens infected by vIBDV had high antibody titres detected with both tests 10 days after challenge.

## Introduction

Infectious Bursal Disease (IBD) or Gumboro disease is caused by a small, non-enveloped double-stranded RNA virus, belonging to the Birnaviridae family. There are two serotypes of Infectious Bursal Disease virus IBDV, designated 1 and 2. Viruses of both serotypes naturally



infect chickens and turkeys, but the disease is recognised only in chickens and only serotype 1 viruses are pathogenic. VP2 and VP3 are the major structural proteins, forming the outer and inner capsid of the virus, respectively (Böttcher et al., 1997; reviewed by Saif, 1998; Van den Berg, 2000; Müller et al., 2003). The antigenic site responsible for the induction of neutralising antibodies is within a minimal region, called the variable domain of VP2, and is highly conformation-dependent (Becht et al., 1988; Schnitzler et al., 1993). This site is also responsible for serotype specificity. Conversely, VP3 is a group-specific antigen that is recognized by non-neutralising antibodies, which may cross-react with both serotypes (Oppling et al., 1991).

IBDV is a highly contagious disease of young chickens, especially between 3 and 6 weeks of age, which can develop into an acute syndrome clinically characterised by depression, ruffled feathers and high mortality rate. Gross lesions can be represented by swelling, oedema and haemorrhages of the bursa of Fabricius (the main target of IBDV), haemorrhages in the muscles and kidney changes in the advanced stages of the disease.

In addition to the direct economic losses of the clinical disease, the damage caused to the immune system results in lowered resistance to other infectious agents (i.e., *E. coli*, *Clostridia*, *Coccidia*) and a poor immune response to commonly used vaccines.

The impact of IBDV depends upon the IBDV strain, the type of chickens (commercial pullets are more susceptible than broilers), as well as management factors.

At the end of the 1980s, very virulent (vv) IBDV strains in Europe (antigenically similar to the “classical strains”) emerged in vaccinated flocks and rapidly spread all over the world. Infection with classical virulent strains results in high morbidity and usually low mortality, whereas vvIBDV strains can cause up to 90%

of mortality in layer-type birds (reviewed by Saif, 1998; van den Berg, 2000; Müller *et al.*, 2003).



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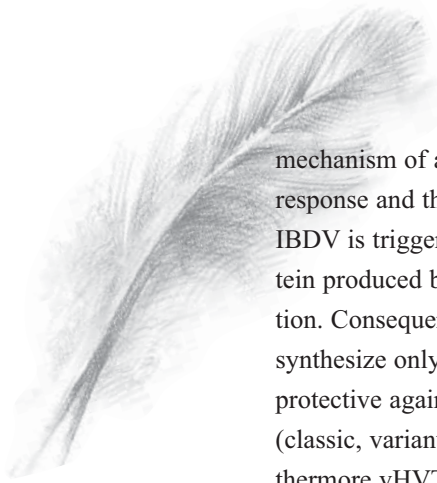
Since IBDV is very resistant in the environment in farm conditions, the sanitary measures commonly applied on poultry farms are not sufficient to control this infection and vaccination is an essential tool for the protection against IBD. Different modified live vaccines (MLVs) have been developed and classified as “mild”, “intermediate”, “intermediate plus” IBD vaccines, depending upon their ability to break through maternally derived antibodies (MDA) that can neutralise the vaccine virus. MLV sometimes are not completely efficacious against vvIBDV, when they are applied in presence of significant MDA titres. Furthermore they can induce moderate to severe bursal lesions and immunosuppression that can impair a chicken’s response to other vaccinations.

Therefore, a new vaccine combining excellent safety and efficacy in the presence of high maternally derived IBDV titres was required (Bublöt *et al.*, 2007). vHVT13 is a vector vaccine in which the herpes virus of turkeys (HVT) is used as the vector expressing the protective IBDV VP2 gene inserted into its genome, to achieve protection against IBDV. This HVT vector vaccine can be administered by the *in ovo* route 3 days before hatching or by the subcutaneous route in 1-day-old chicks. Due to the

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mechanism of action of vHVT13, the immune response and the consequent protection against IBDV is triggered only by the IBDV-VP2 protein produced by the vector during the replication. Consequently the chickens vaccinated synthesize only anti-VP2 antibodies that are protective against all types of IBDV challenges (classic, variant and very virulent strains). Furthermore vHVT13 stimulates a level of protection against Marek Disease equivalent to the HVT parental vaccine strain.

The aim of this study was to compare, in commercial pullets, the protection against a vvIBDV challenge, induced by vHVT13, with the one induced by three different conventional MLVs, two of them classified as “intermediate” and one as “intermediate plus”.

## Materials and methods

### Chickens and experimental design

The trial was carried out at the animal room facilities of the Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna - Sezione Diagnostica di Forlì - Italy, where 170 brown commercial pullets (Hy Line® Brown) were utilized. At 1 day of age, 20 pullets were blood sampled to evaluate the MDA titres. The remaining 150 day-old pullets were split into six groups (G.1 to G.6) each containing 25 birds and placed in isolators (Montair Andersen HM® 1500 ) as summarized in *Table 1*.

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**Table 1- Summary of the study groups and treatments**

Group	Vaccination	Vaccine	Doses	Vaccination timing (days)	vvIBDV challenge at 42 days of age
G.1	Yes	A - “Intermediate”	2	17 and 25	Yes
G.2	Yes	B - “Intermediate”	2	17 and 25	Yes
G.3	Yes	C - “Intermediate plus”	1	17	Yes
G.4	Yes	D - vHVT13	1	1	Yes
G.5	No	Unvaccinated, challenged	-	-	Yes
G.6	No	Unvaccinated, unchallenged	-	-	No

### Vaccines and vaccinations

The vaccines reported as A and B were both commercially available “Intermediate” IBDV vaccines.

A commercial dose of the vaccines A and B was administered to birds of group 1 and 2, respectively, by oral drop at day 17 and 25. The vaccine reported as C was a commercially available “Intermediate plus” IBDV vaccine. Birds of group 3 were vaccinated once with a commercial dose of the vaccine C at 17 days of age by oral drop. Vaccination timing was chosen in order to simulate the IBD vaccination schemes commonly applied in field conditions, without estimating the age of vaccination according to the Deventer formula.

The vaccine reported as D was the cell-associated IBD vector vaccine vHVT13 (currently registered under the name VAXXITEK® HVT+IBD), generated by inserting an IBDV VP2 gene into the HVT genome used as vector (Bublott *et al.*, 1999). Chicks of group 4 was injected subcutaneously at 1 day of age with a commercial dose of vHVT13 according to manufacturers’ recommendations.

Birds from group 5 and 6 were not vaccinated.

### Blood sampling and serology

Blood samples were taken from 20 1-day-old pullets to assess the level of IBD MDA. Afterwards, all the groups were blood sampled at the ages of 17, 31, 42 (challenge day) and 53 days (11 days after challenge, i.e. the end of the trial), respectively, to monitor, by means of two different commercially available ELISA kits, the decay of IBD MDA and the antibody response to the vaccinations and/or to the challenge.

The IBD antibody titres were evaluated using two ELISA kits, namely PROFLOK® IBD Ab test, Synbiotics, USA, and an “improved” kit, PROFLOK Plus IBD Ab test, Synbiotics, USA. Both kits are indirect ELISAs and the principle of both tests is similar but the difference

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between them lies in the nature of the IBDV antigen coated on the plates. PROFLOK IBD Ab test uses an antigen derived from a classical strain grown in tissue culture and is thought to detect mainly the antibodies produced against the VP3 protein of IBDV after a natural infection or vaccination with a MLV. PROFLOK Plus IBD Ab test uses a native bursal-derived classical strain antigen which allows a better detection of anti-VP2 antibodies; data suggest that when these antibodies are detectable at significant levels, birds show to be protected. With the combined use of the two IBD ELISA tests, the field situation can be more efficiently monitored by discriminating between vHVT13 induced antibodies (anti-VP2 only, detected by the IBD Plus ELISA test) and IBDV antibodies induced by a natural field infection or by vaccination with MLV (including anti-VP3 antibodies detected by the classical IBD test) (Prandini et al., 2008). The threshold of positivity with these kits was set at a titre of 554 for the PROFLOK IBD Ab test and at 1002 for the PROFLOK Plus IBD Ab test.

### Bursa sampling and histology

In order to evaluate the impact of vaccination on the bursa of Fabricius, whole bursas were randomly taken from 10 birds per group at 31 days of age, i.e. 6 days after the second vaccination with MLV in groups 1 and 2, and 15 and 31 days after vaccination in groups 3 and 4, respectively.

At 53 days of age, i.e. 11 days after challenge, whole bursas were randomly taken from all the remaining pullets to evaluate the effects of the challenge in the different groups. Evaluation was based upon size and weight of bursas in relation to the body weight (bursa to body weight ratio = bursa weight (g) /body weight (kg)), as well as on histological lesion score,

ranging from 0 to 5, assessed according to the method described in the European Pharmacopoeia 5.0 (2005).

### Experimental challenge

All the groups, except group 6, were challenged at the age of 42 days with a very virulent strain of IBDV (ref: 77165) obtained from Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (Martin *et al.*, 2007), at a challenge dose of  $5.2 \log_{10} \text{EID}_{50}$  in 0.5 ml of distilled water per bird, administered by an oral drop.

### Parameters of evaluation

The following parameters were monitored to assess the effects of the vaccination and of the vvIBDV challenge: presence of IBD clinical signs, mortality rate, gross lesions, size and histological lesions of bursa of Fabricius, and IBD serology.

## Results

### Clinical signs and mortality after vvIBDV challenge

Presence of clinical signs and mortality rate after vvIBDV challenge are summarized in *Table 2*.

**Table 2 - Summary of IBD clinical signs and mortality rate after the challenge**

Group	Vaccine	Challenge	Clinical signs of IBD	Mortality rate
G.1	A - "Intermediate"	Yes	2/15	2/15
G.2	B - "Intermediate"	Yes	2/15	2/15
G.3	C - "Intermediate plus"	Yes	2/15	1/15
G.4	D - vHVT13	Yes	0/15	0/15
G.5	Unvaccinated, challenged	Yes	15/15	2/15
G.6	Unvaccinated, unchallenged	No	0/15	0/15

In the group of pullets unvaccinated and challenged (G.5), severe clinical signs of IBD such as depression, ruffled feathers and watery diarrhoea were observed in all of the birds and two out of fifteen died.

In both groups vaccinated with Intermediate MLVs (G.1 and G.2), two out of fifteen birds showed clinical signs of IBD and died.



In the group vaccinated with the Intermediate Plus MLV (G.3) two out of fifteen birds showed clinical signs of IBD and one of them died. Neither clinical signs of IBD nor mortality were observed in the group vaccinated with vHVT13 (G.4) and in the control group unvaccinated and unchallenged (G.6).

### Gross lesions after vvIBDV challenge

Gross lesions typical of IBD (oedema of bursa of Fabricius, nephritis, haemorrhages in the muscles and on the mucosa of proventriculus) were observed only in the birds of groups 1, 2, 3 and 5 which died (see *Table 2*) following vvIBDV challenge (see pictures in *Figure 1*).



Figure 1 - Type of lesions observed in birds that died following challenge with vvIBDV

No lesions were recorded in the group vaccinated with vHVT13 (G.4) and in the group unvaccinated and unchallenged (G.6).

### Size and histological lesions of bursa of Fabricius before and after vvIBDV challenge

The mean weight of bursas and the bursa/body weight ratio at 31 and 53 days of age, are reported in *Tables 3* and *4*, respectively.

At 53 days (table 4) there is a statistically significant difference within the groups (variance analysis,  $p < 0.01$ ); G.1, 2, 3 and 5 are not different ( $p = 0.52$ ); G.4 and G.6 are significantly different ( $p = 0.01$ )  $G.6 > G.4$ . This is indicative of some susceptibility to the challenge in G.4 although far less than in the other groups.



Figure 2 shows the mean bursa/body weight ratios 11 days before and 11 days after challenge.

Table 3 - Mean bursa weights and bursa/body weight ratios at 31 days

Group	Vaccine	Mean bursa weight (g.) at 31 days (11 days pre-challenge)	Mean bursa/body weight ratio % at 31 days (11 days pre-challenge)
G.1	A - "Intermediate"	1.24	4.06
G.2	B - "Intermediate"	1.55	5.03
G.3	C - "Intermediate plus"	1.40	4.89
G.4	D - vHVT13	1.34	4.46
G.5	Not vaccinated, challenged	1.55	5.32
G.6	Not vaccinated, not challenged	1.40	4.65

No statistical differences on B/BW criteria (variance analysis on all groups,  $p = 0.10$ )

Table 4 - Mean bursa weights and bursa/body weight ratios at 53 days

Group	Vaccine	Mean bursa weights (g.) at 53 days (11 days post-challenge)	Mean bursa/body weight ratio % at 53 days (11 days post-challenge)
G.1	A - "Intermediate"	0.66	1.07
G.2	B - "Intermediate"	0.76	1.18
G.3	C - "Intermediate plus"	0.75	1.19
G.4	D - vHVT13	2.13	3.71
G.5	Not vaccinated, challenged	0.75	1.13
G.6	Not vaccinated, not challenged	3.72	5.26

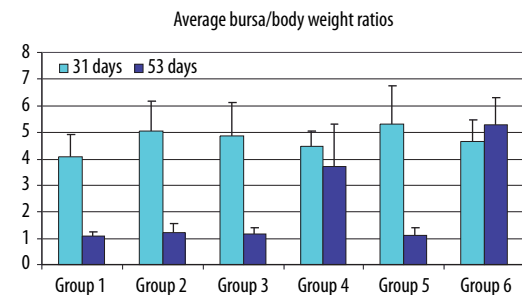


Figure 2 - Mean bursa/body weight ratios at 31 and 53 days of age

No significant differences between the groups were observed before vvIBDV challenge, whereas after challenge the bursa weight and bursa/body weight ratio decreased dramatically in the group unvaccinated (G.5) and in all the groups vaccinated with MLVs (G.1, 2 and 3). On the contrary bursa size was not negatively affected in the unchallenged group (G.6) and only slightly impacted in the vHVT13 group (G.4).

Table 5 reports the average histological bursa lesion scores as assessed at 31 and 53 days of age, respectively.

Table 6 reports the respective number of birds of each group showing the different bursa lesion scores 11 days after challenge.

**Table 6 - Bursa lesion scores 11 days after challenge**

Group	No and mild lesions: scores 0-1	Intermediate lesions: scores 2-3	Severe lesions: scores 4-5	Total number of birds
G.1	0	2	13	15
G.2	0	4	11	15
G.3	1	4	10	15
G.4	11	3	1	15
G.5	0	0	10	10
G.6	10	0	0	10

There was no difference within groups 1, 2 and 3 according to the proportion of severe lesions (Chi square test;  $p > 0.5$ ); these 3 groups all together do not differ on the same criteria from G.5 (positive control; fisher's exact test  $p = 0.21$ ), but differ very significantly from G.6 (negative control;  $p < 0.01$ ) and G.4 (vHVT13;  $p < 0.01$ ). G.4 shows a smaller proportion of severe lesions than G.5 (Fisher's exact test,  $p < 0.01$ ) but also a smaller proportion of mild to absent lesions than G.6 ( $p < 0.01$ ).

These results are coherent with the macroscopic observations.

### IBD serology: antibody response kinetics

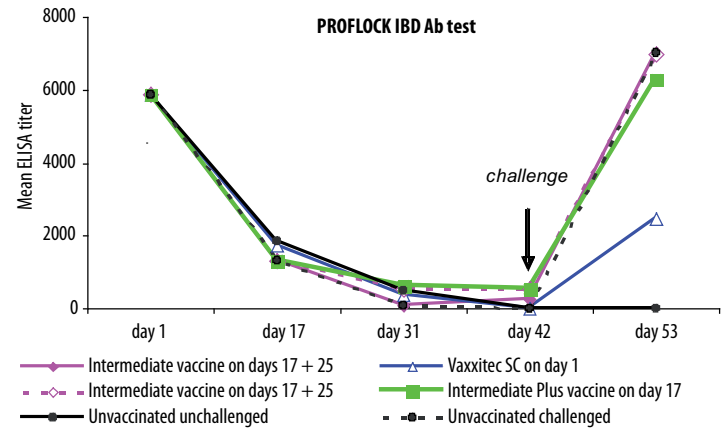
The pattern of antibody responses after vaccination with the different vaccines and after challenge, assessed using two ELISA kits, PROFLOK IBD Ab test and PROFLOK Plus IBD Ab test, is shown in Figure 3 and 4, respectively.

Before challenge, a decline of antibody titres was observed in all groups, using both the classical and IBD Plus ELISA tests, except in the vHVT13 vaccinated group where antibody titres assessed by IBD Plus ELISA test remained high ( $>6000$ ) and stable. The lack of detectable antibody response in MLVs groups on day 42 was probably due to the interference of MDA at the time of vaccination which hampered and/or delayed a correct vaccine take. At day of age, MDA were very high with either ELISA kits as a consequence of the IBD vaccination of parents using inactivated oil emulsified vaccines. As a matter of fact mean titres measured at 17 days with the classical ELISA test were about 1300 i.e. much higher than the break through titres estimated for MLVs; even

**Table 5- Average histological bursa lesion scores at 31 and 53 days of age**

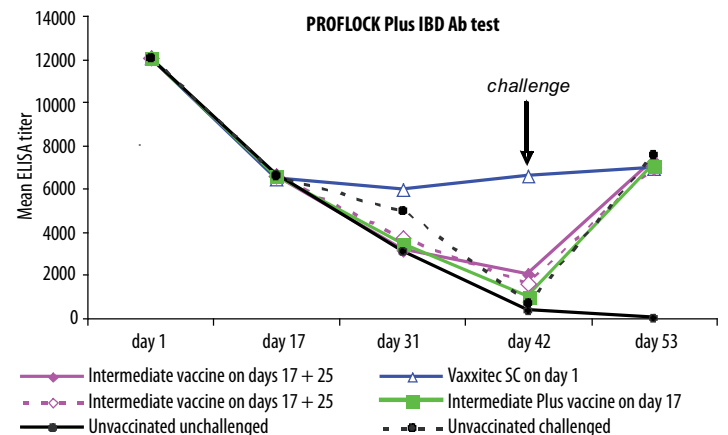
Group	Vaccine	Average bursa lesion score at 31 days (11 days pre-challenge)	Average bursa lesion score at 53 days (11 days post-challenge)
G.1	A - "Intermediate"	0.6	4.0
G.2	B - "Intermediate"	0.3	3.7
G.3	C - "Intermediate plus"	0.1	3.7
G.4	D - vHVT13	0.3	1.5
G.5	Not vaccinated, challenged	0.0	4.1
G.6	Not vaccinated, not challenged	0.1	0.5

at 31 days some individual titres were still above 500 and could have caused a delay in the vaccine take and an impairment in the immune response to the MLVs. After challenge, with both ELISA tests, a marked increase in anti-



**Figure 3 - IBDV mean antibody titres detected using the PROFLOK IBD Ab test.**

The mean Elisa titres showed a statistically significant difference within G.1, 2, 3, 4 and 5 on day 53 (variance analysis;  $p < 0,01$ ); G.4 (vHVT13) showed indeed a titre lower than G.1, 2 and 3 that were comparable each other.



**Figure 4 - IBDV mean antibody titres detected using the PROFLOK Plus IBD Ab test.**

There was a statistically significant difference on D31 and D42 within all groups (variance analysis,  $p < 0.01$ ). On D31, G.4 was higher than G.5 ( $p = 0.045$ ) and G.5 higher than the comparable groups G.1, 2, 3 and G.6 ( $p = 0.01$ ). On D42, G.4 was higher than the groups G.1, 2 and 3 ( $p < 0.01$ ), themselves higher than G.5 and 6 ( $p < 0.01$ ).

body titres was observed in all groups, except in the vHVT13 vaccinated group which had a less pronounced sero-conversion, more evident when tested with the Proflok IBD test.

## Discussion

Based upon clinical signs and mortality following challenge with a vvIBDV, full protection was observed in the vHVT13 vaccinated group and a partial protection in birds vaccinated with intermediate or intermediate plus vaccines. After vvIBDV challenge, gross lesions of IBD were observed in all birds of the unvaccinated group and in the few non-protected birds of the groups vaccinated with MLVs only.

The size of bursas and the bursa/body weight ratios were dramatically reduced only in the groups vaccinated with MLVs and in the unvaccinated controls following challenge, whereas they were not negatively affected in the unchallenged group and only slightly reduced in the vHVT13 group (Figure 2). Consistently, histological lesions were as severe in the MLVs groups as in the unvaccinated challenged control group, and were negligible in the remaining groups (Table 5).

The presence of IBDV MDA in young chickens represents a major problem because they may interfere with classical IBD MLVs; therefore, in presence of MDA the timing of active immunisation with classical vaccines remains complicated. Considering the variability of individual titres, even the estimation of optimal time of vaccination by the Deventer formula does not solve this problem definitely.

Thus after the decline of MDA, birds which do not respond to vaccination become susceptible to field IBDV infection. In the presents study, in all groups vaccinated with MLV, ELISA results show the lack of antibody response observed from day 31 up to the challenge day; after the challenge a rapid seroconversion was

detected with both ELISA kits. On the contrary, in chickens vaccinated with vHVT13, before MDA decay, by means of PROFLOK Plus IBD Ab test was observed an active and strong anti-VP2 response which showed to correlate to protection against the challenge. Hence no protection gap occurred in this group. This earlier response can be obtained because vHVT13 efficacy is not affected by the presence of high levels of MDA, and this vaccine can thus be administered either *in ovo* or at 1 day of age, removing the question of timing of vaccination seen with all MLVs. The cell-associated nature of vHVT13, the lack of expression of VP2 on the surface of infected cells or of HVT vector virus, and the mode of replication of the HVT vector, probably all contribute to the ability of this vaccine to overcome MDA (Bublout *et al.*, 2007). Anti-VP2 antibodies induced by vHVT13 are protective as already described previously (Bublout *et al.*, 2007) and as confirmed by vaccination/challenge experiments in both pullets and in broilers.

Overall, complete protection against a vvIBDV challenge was observed in the vHVT13 group when it was administered in day-old pullets with high levels of MDA. These high levels of MDA may explain a partial protection failure observed in the birds individually vaccinated with MLVs by oral drop at 17 and 25 days when MDAs were found to be still at significant levels.

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