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Daniel Nzingu Awa, André Ngagnou, Eléonore Tefiang, Daouda Yaya, Aboubakar Njoya. Post vaccination and colostral Peste des petits ruminats antibody dynamics in research flocks of Kirdi goats and Fulbe sheep of North Cameroon. 2003, 6 p. hal-00137968

HAL Id: hal-00137968

<https://hal.science/hal-00137968>

Submitted on 22 Mar 2007

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Post vaccination and colostral *Peste des petits ruminants* antibody dynamics in research flocks of Kirdi goats and Fulbe sheep of North Cameroon

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Abstract — A study was carried out with Kirdi goats and Foulbe sheep kept on-station at the Institute of Agricultural Research for Development, IRAD, Garoua, to look at their immunological response following vaccination with a specific peste des petits ruminant (PPR) virus vaccine. Pre-vaccination PPR antibody seroprevalences were 44% and 29% in goats and sheep, respectively. Seroprevalences following vaccination were 100% in both species. Titres remained above the protection threshold level (1:10) in both species at 12 months. Maternal antibodies in young animals were detectable to up to 6 months of age but fell below the protection threshold level at 3.5 months and 4.5 months in lambs and kids respectively. Lambs and kids from immunised or exposed dams should be vaccinated at 4 months and 5 months of age, respectively.

Résumé — **Dynamique des anti-corps post-vaccinaux et colostraux de la peste des petits ruminants chez les chèvres kirdi et les moutons foulbé au Nord-Cameroun.** Une étude a été menée en station, à l'Institut de recherche agricole pour le développement de Garoua au Cameroun, avec des chèvres Kirdi et des moutons Foulbe pour évaluer la réponse immunitaire après la vaccination avec un vaccin homologué du virus de la PPR. La séroprévalence d'anticorps du virus de la PPR avant la vaccination était de 44 % chez les chèvres et 29 % chez les moutons. Après la vaccination, la séroconversion était à 100 % pour les deux espèces. Le niveau d'anticorps est resté au-dessus du seuil de protection (1 : 10) 12 mois après la vaccination. Chez les jeunes nés de mères vaccinées, les anticorps maternels sont restés détectables jusqu'à 6 mois, mais sont passés au-dessous du seuil de protection à partir de 3,5 mois chez les agneaux et 4,5 mois chez les cabris. Les agneaux et les cabris nés de mères immunisées doivent donc être vaccinés respectivement à partir de 4 et 5 mois.

Introduction

"Peste des petits ruminants" (PPR) is an important disease of small ruminants, and is widely distributed in the sub-Saharan belt of Africa (Roeder *et al.*, 1994). PPR is considered one of the primary causes of small-ruminant mortality in Cameroon (Awa and Ngo Tama, 1997; Awa *et al.*, 2000). Epidemics occur annually during particular periods of the year that vary slightly from one region to another. Flock mortality rates of > 80% have been recorded in north Cameroon (Awa and Ngo Tama, 1997). Mortality in small ruminants can be reduced by more than half when animals are vaccinated using the bovine tissue-culture rinderpest vaccine – TCRV - (Njoya *et al.*, 1997). Extensive studies on the economics of

vaccination of small ruminants against PPR with the TCRV in the sub-Saharan belt of west Africa have been carried out (Awa et al., 2000; Reynolds & Francis, 1988; Tillard *et al.*, 1992), and have shown the economic advantages of vaccination. A specific PPR vaccine has been produced and is efficacious even at very low doses (Diallo *et al.*, 1989 ; Martrenchar *et al.*, 1997, 1999). Because this vaccine is being introduced for replacement of the TCRV, it is necessary to look at the response to vaccination so as to propose an efficient vaccination programme. Our aim was to determine the proportion of animals that had protective titres after vaccination, the duration of protective titres in adults, and the age at which maternal antibodies probably would no longer interfere with vaccination of kids and lambs.

Materials and methods

The animals

Small-ruminant flocks of the Institute of Agricultural Research for Development (IRAD) Garoua Station were used for the study. The animals were Kirdi (Djallonke) goats ($n \approx 60$) and Foulbe sheep ($n \approx 100$). The most recent vaccination of the flocks was with the TCRV (tissue-culture rinderpest vaccine), 3 years previously. The animals were kept under a semi-intensive management system where they grazed the same pastures during the day but were housed separately at night. The flocks were treated against gastrointestinal helminths with albendazole (VALBAZEN[®]) produced by CEVA - Santé Animale at the beginning of the experiment which ran from August 1999 to September 2000.

Sampling

All the animals were initially identified using ear tags. All the goats in the flock > 1 year old were retained for sampling and the same number was randomly selected from the sheep using a hand calculator random number generator. Blood was collected through jugular vein puncture for PPR serological antibody assay. A total of 80 samples were therefore collected from a population of about 160. One day after blood sampling, the entire flocks were vaccinated with an attenuated PPR virus vaccine, CAPRIPESTOVAX[®] (PPRV75/LK6078 vaccine strain) produced by LANAVET at the recommended dose of $10^{2.5}$ TCID₅₀. Monthly serum sampling continued in the 80 adult animals for 12 months.

To monitor maternal antibodies in kids and lambs, monthly sampling was also done in the young animals born from immunised dams, from 1 month through 6 months of age. Because synchronised breeding was not done, young animals were included as they were born ; the first inclusions took place 2 weeks after the vaccination of the flock. This ensured a considerably high level of antibodies in the dams prior to kidding or lambing. All kids and lambs born during this period were sampled, and a total of 27 kids and 28 lambs were included.

Sample analysis

The blood samples were centrifuged 24 hours after collection. Sera were aspirated into vials and stored at -20°C until analysis was done. The serum neutralisation test (sensitivity = 98%; specificity = 99%) was used (World Organisation for Animal Health, 2000). Vero cells were used as the indicator system to show the effective (absence of a cytopathic effect) neutralisation of the virus.

Serum samples were incubated at 56°C for 30 minutes to deactivate complement. 80ul of minimum essential medium (MEM) supplemented with 10% fetal-calf serum, 100 IU/ml penicillin G and 0.05 mg/ml streptomycin was dispensed in the first row of a cell culture microplate and 50ul into the rest of the wells. 20ul of test sera were added to the first row of wells in pairs to obtain 6 samples in a row of twelve wells. 50ul then were pipetted from the first into the second row, from the second to the third row and down until the final serum dilution of 1:1280 was obtained in the last row of wells. 50ul of 100TCID_{50} of virus solution (PPRV75/LK6078 vaccine strain) were added to all the wells and incubated at 37°C in 5% CO_2 for 1 hour. Trypsinised vero cells were washed by centrifuging in MEM at 4000 rpm for 10 minutes. They were resuspended in MEM and counted to obtain a concentration of 4×10^5 cells/ml. 50ul of the cell suspension were distributed in each well and the plates were incubated at 37°C under 5% CO_2 and cytopathic effect read after 8 days.

Analysis and interpretation of serological results

To compare baseline prevalences, the chi-square test was used. The serum neutralisation titres (reciprocal of the dilution factor) were log-transformed before use in plotting curves or in other parametric tests. The mean logs of monthly titres were plotted in graphs to illustrate the dynamics of vaccine antibodies in adults and maternal antibodies in young animals. Pearson's correlation was used to test the relationship between dam antibody titre and that of offspring at 1 month of age.

Results

Data analysis used 35 adult goats, 39 adult sheep, 23 kids and 26 lambs because animals that died early in the experiment (before 6 months for adults and before 3 months for young animals) were eliminated from the analysis. About 60% of the mortalities were due to attacks by stray dogs that frequent the station pastures.

Baseline uncorrected seroprevalences were 43% and 28% and when corrected using the formula of Toma *et al.* (1998) which states:

$$P = \frac{P_0 + (Sp - 1)}{Se + Sp - 1}$$

P = true prevalence ; P_0 = measured prevalence ; Sp = specificity of the test ; Se = sensitivity of the test

and calculating the 95% confidence interval (CI) were 44 ± 14 and 29 ± 9 in goats and sheep respectively (table 1). There was no significant difference between them. The \log_{10} -flock means prior to vaccination correspond to antibody titres of 1:4 and 1:2 in goats and sheep, respectively (figure 1). Maximum titres were detected one month after vaccination in goats (mean titre = 1:1175) and at 4 months in sheep (mean = 1:102). Minimum antibody titres were obtained at 9 months after vaccination in both species, followed by a rise which maintained an upward trend even up to 12 months when sampling stopped. The rise did not seem to be significant, especially in goats where the variation in antibody titres in the later months was very wide (figure 1).

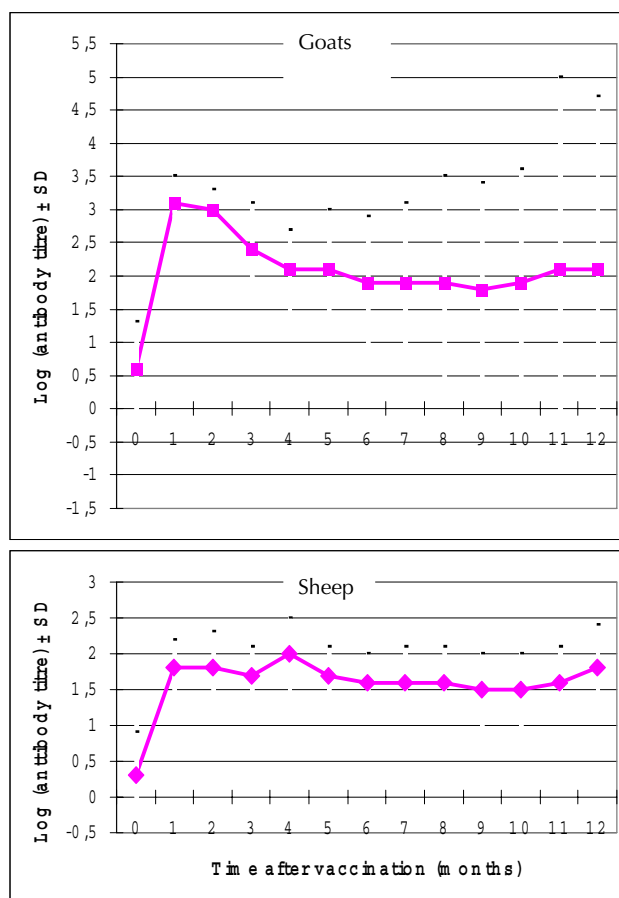


Figure 1. Peste des petits ruminants antibody dynamics in 35 adult Djallonké goats and 39 adult Foulbé sheep following vaccination.

Table 1. Serological results of PPR antibodies in on-station sheep and goat flocks, Garoua, North Cameroon.

Species	Sample size	Prevalence (%)	95% Confidence interval
Goats (N=59)	40	44	14
Sheep (N=103)	40	29	9

N = population size

Maternal antibody decay in kids and lambs from immunised dams is presented in figure 2. The correlation between the antibody titres in dams and their offsprings was insignificant.

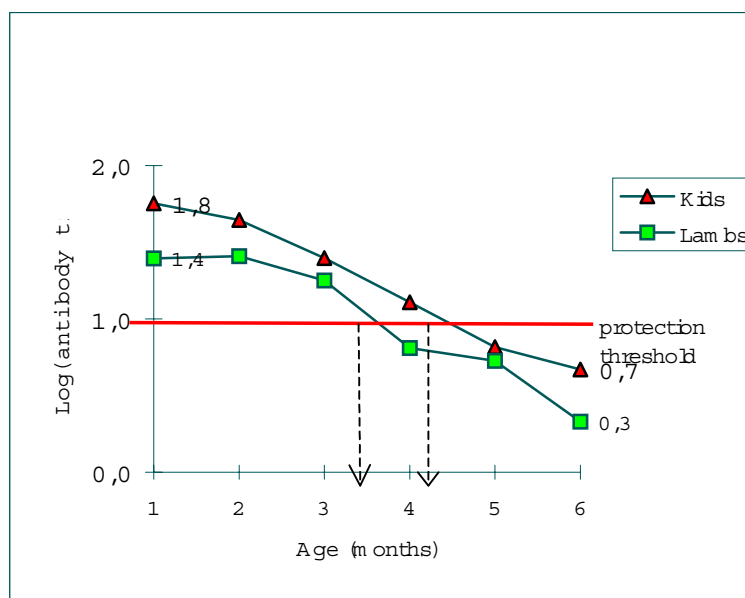


Figure 2. PPR antibody decay in kids and lambs from immunised dams.

Discussion

The prevalence of PPR antibodies of 44% in goats and 29% in sheep differs from the values obtained by (Martrenchar *et al.* 1995) of 8% and 37% in goats and sheep respectively from field samples in the same region. (Ekue *et al.* 1992) found an overall prevalence of 45.6% in small ruminants from a near nation-wide sample in Cameroon. More-recent field results in north Cameroon (Hayatou, 1999) showed prevalences of 46% in goats and 98% in sheep. Lower seroprevalences we obtained are probably because on-station animals are less exposed to the virus because they have very limited contact with other animals. On the other hand, the low seroprevalence also signifies the vulnerability of the flock in the event of an outbreak.

All animals sampled tested positive for PPR antibodies 1 month after vaccination. In both species, titres stayed well above the protection threshold level of 1 : 10 as determined by (Diallo *et al.* 1989). No explanation is given for the rise in titres between the ninth and tenth months following vaccination. It is possible that the animals were exposed to the field virus and since they had been vaccinated, the exposure instead had a booster effect on the immune response. This hypothesis however needs to be investigated. Our results for the young animals (Figure 2) are intermediate to those of other authors that write of 3 to 4 months (Ata *et al.*, quoted by (Bidjeh *et al.*, 1999) ; Obi, quoted by (Reynolds 1988), and 4 to 5 months (Bidjeh *et al.*, 1999). The principal difference between the present results and others is the distinction made between kids and lambs from which one can conclude that lambs could be vaccinated at 4 months and kids at 5 months, instead of 3 months for both species as has been the usual practice.

The lack of correlation between dam and offspring antibody titres was an important observation. These results would have been more authentic if sampling was done in the first two weeks of life since antibodies are acquired through colostrum. However, the low variation in the early months of the offspring antibody decay graph (Figure 2) suggests that extrapolation to the first two weeks of life will not alter the relationship.

Conclusion

The PPRV75/LK6078 vaccine strain elicits protective serotitres in Kirdi goats and Foulbe sheep. The flock mean titre remained above the protection threshold 12 months after vaccination. Lambs could be vaccinated as late as 4 months and kids at 5 months of age from recently vaccinated flocks.

Acknowledgements

The authors thankfully acknowledge that this study was financed by the Pôle Régional de Recherche Appliquée au Développement des Savanes d'Afrique Centrale (PRASAC). Special thanks goes to Mr. Kammegni Jacob and Miss Vivian Awahmukalah for their technical assistance in monitoring the flocks and sample collection. The collaboration of the National Veterinary Laboratory of Cameroon (LANAVET) Garoua, in the analysis of samples almost cost-free is well appreciated. Finally we are thankful for the useful contributions of Dr. Hubert Guerin in the conception of the research protocol.

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