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Short Communication

Large mass vaccination of small ruminants against Peste des Petits Ruminants and Sheepox using a combined live attenuated vaccine

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- Sheep pox
- Vaccination
- Combined vaccine

Abstract

Peste des Petits Ruminants (PPR) and Sheepox (SP) are highly contagious diseases of small ruminants causing huge economic losses. Vaccination is the most efficient tool to control both diseases. Morocco conducted in 2015 a large vaccination program with a combined vaccine against PPR and SP, recently developed. The study was carried out on 1079 randomly selected sera, sampled 6 and 9 months after vaccination, tested by competitive ELISA (c-ELISA) for PPR and Virus Neutralization Test (VNT) for SP. Significant level of antibodies among sheep population was detected on 84% for PPR and 77.5% for SP. No statistically significant difference found regarding antibody response of young animals (≤ 12 months) and adult (> 12 months), breeding system (extensive vs intensive) and post-vaccination sampling time ($P > 0.05$). However, response to vaccination was different between breed ($P < 0.01$). The high seroconversion rate reflected a strong immunity status against both diseases, which indicates that the combined vaccine can be used for economic vaccination strategy.

ABBREVIATIONS

PPR: Peste des Petits Ruminants; SP: Sheep pox; SPV: Sheepox Virus; VNT: Virus Neutralization Test; ELISA: Enzyme-Linked Immunosorbent Assay; cELISA: Competitive Enzyme-Linked Immunosorbent Assay

INTRODUCTION

Small ruminants farming in many developing countries in Africa and Asia represent a major, if not the only source of nutrition for a large part of the low income population. Peste des Petits Ruminants (PPR) and Sheepox (SP) are highly contagious and frequently fatal viral diseases, leading to huge economical losses and have to be notified to the World Animal Health Organization (OIE) [1,2].

Vaccination is a key tool to fight these devastating diseases of goats and sheep overlapping in many regions worldwide. It was successfully applied using monovalent live vaccines in many countries [3-5]. However, despite the efficacy of such monovalent vaccine for corresponding infections, PPR and SP continue to spread in new geographical areas in Africa and Asia, it mainly because of vaccination coverage which is insufficient to cut the virus cycle.

Indeed, in many endemic areas, vaccination is a costly act because of poor infrastructure and large distances, making access to the small ruminant flocks difficult. A combined vaccine

effective against both PPR and SP has been developed and tested experimentally in many countries. In Cameroon, a combined vaccine based on PPR Nigeria N75 strain and SP RM 65 was tested experimentally but failed to procure efficient immunity against a challenge with environment strain of goat poxvirus [6]. However, in India, the combination of PPR Sungri strain and an indigenous goat pox vaccine and association of PPR Izatnagar 1994 and SP Jaipur strains, showed a satisfactory results in protection against goat and sheep infections [7,8]. Similar results were reported experimentally using PPR Nigeria N75 and SP Romania strains [9]. Nevertheless, to the best of our knowledge, none of those vaccines have been used in the field for large vaccination campaign.

In the present study, we reported Morocco experience in mass vaccination using PPR/SP combined vaccine in 2015 after the reemergence of PPR in the country. The vaccine was locally produced using PPR-Nigeria N75 and SP-Romania attenuated strains [9]. We presented results of the vaccination monitoring conducted six and nine months after the campaign.

MATERIALS AND METHODS**Background information on animal population and vaccination procedure**

The general vaccination campaign of 2015 against PPR and SPV included all sheep population of more than three months of

age throughout the national territory. In total, more than 16.2 Million sheep heads were vaccinated, corresponding to 86% of the total sheep population. The campaign duration was about 2 months from beginning of October to the end of November. Sheep were vaccinated by one dose of 0.5 ml injected subcutaneously using an automatic multi dose syringe.

The present serological survey study was conducted on randomly selected 1079 sheep from 26 vaccinated flocks located within four major regions in Morocco during March and June of 2016. Inclusion criteria for flock selection were both proven vaccination and absence of any suspicion of PPR or SP between vaccination and sampling date. Specific data was recorded during sample collection, this included information about herd size, localization of the farm, vaccination date, animal identity, age and breed. Samples were divided in adult (>12 months) and young ≤ (12 months), feeding pattern (intensive vs extensive), post-vaccination time (6 vs 9 months), breeds and geographical location (Table 1).

Sera screening

All sheep were blood sampled by veterinarians in separate sterilized tubes with no anti-coagulants. Samples were then transferred to the laboratory under sterile conditions in ice bags. Sera were separated by centrifugation at 2000 rpm for 15

minutes, aliquoted and stored at -20°C until laboratory testing was performed.

SPV and PPR monitoring vaccination response was serologically assessed respectively, using virus neutralization test (VNT) as described in the OIE Terrestrial Manual (Chapter 2.7.14) and a competitive ELISA (c-ELISA) (ID Screen®PPRCompetition, IDvet, Montpellier, France). The OD values were converted to Percentage of Inhibition (PI) and the samples with PI>60% were considered as positive.

Statistical analysis

Statistical analyses were performed by using SPSS 16.0 software. The overall prevalence of SPV and PPR markers was expressed as the percentage of seropositive samples. A level of $p < 0.05$ was used to indicate statistical significance.

RESULTS AND DISCUSSION

Results

In all, 1079 serum samples were tested for anti-PPR and anti-SPV antibodies presence. The global results indicate that 84% of sheep population presents antibodies at a significant level against PPR and 77.5% are positive to SP. Serology likely to reflect vaccination antibody response since all selected flocks were free

Table 1: Characteristics of the sampled animals, seroprevalence and outcomes of univariate analyses ($P \leq 0.1$). The distribution of seropositivity results according to characteristics of sampled animals are reported here.

Parameters	Frequency	PPR		P value	SP		P value
		Seropositive (n)	% Sero-prevalence		Seropositive (n)	% Sero-prevalence	
Age				0,3			0,2
Young	388	329	84,79		295	76	
Adult	691	576	83,36		542	78,5	
Feeding pattern				0,1			0,4
Extensive	985	830	84,3		735	74,6	
Intensive	94	75	79,8		69	73,4	
Date of vaccination				0,4			0,2
6 months	779	649	83,3		537	68,9	
9 months	300	256	86,6		199	66,2	
Breed				<0,001			<0,001
Ouladjellal	104	104	100,0		48	46,5	
Noire de velay	30	28	93,3		23	76,7	
Beniguil	230	200	87,0		179	77,9	
Timahdit	300	256	85,6		224	74,8	
Merinos	94	75	79,8		69	73,4	
Sardi	201	156	77,6		164	81,5	
Bjaad	120	86	71,7		97	80,5	
Regions				<0,001			0,5
Oriental	364	332	91,2		286	78,7	
Chaouia	260	189	72,7		208	80	
Gharb	155	128	82,6		122	78,4	
Moyen Atlas	300	256	85,3		225	74,9	

Abbreviations: PPR: Peste des Petits Ruminants; SP: Sheeppox

from any clinical signs in the period between vaccination and sampling.

The distribution of seropositivity results according to characteristics of sampled animals are reported in Table 1. For both PPR and SP, there were no statistically significant difference in seropositivity regarding age, breeding systems and vaccination date ($P>0.05$). However, PPR and SPV antibodies sero-prevalence were significantly different between breeds ($P<0.01$); the seropositivity rate is the highest with Ouladjellal breed for PPR and the lowest for SP with only 46.5%. For the other breeds, PPR rate ranged between 71.7 and 93.3% while SP rate varied from 73.4 to 81.5%.

Discussion

PPR and SP are of concern in the Global Eradication Program implemented by international organizations, World Organization for Animal Health and the Food and Agriculture Organization with the objective of disease eradication by 2030 [10]. These transboundary viral diseases affecting mainly domestic small ruminants are widespread in Africa, Middle East and Asia, leading to heavy economic losses due to clinical manifestations and trade restriction. PPR is characterized by fever, anorexia, necrotic stomatitis, diarrhea, ocular and nasal purulent discharge, and respiratory distress, while SPV infection leads to pyrexia, ocular-nasal discharge and pock lesions ranging from erythema to scab on the body with occasional pulmonary nodules [11,12].

In Morocco, in spite of control strategies adopted and the high protection afforded by live vaccines used, SP outbreaks are regularly reported with a peak in 2010 and PPR reemerged in 2015 after seven years of country freedom (last outbreak in November 2008). The reason behind these outbreaks may be the re-introduction of the viruses through illegal movement of live animals from neighbouring countries where PPR and SP are endemic highlighting thus, the need for a regional approach for viral disease control [13,14].

In Morocco, the adopted strategy in 2015 was the use of combined PPR/SP vaccine in large vaccination campaign to control both diseases in small ruminants. This vaccination campaign targeted the sheep population with a coverage rate of 86%, but did not affect goat population, because of the abortion risk, as the campaign was conducted during pregnancy period. In the present study, we carried out post-vaccination monitoring based on sero-conversion rate against PPR and SP viruses. We also identify factors that could be associated with seropositivity, with the intention to generate baseline information necessary for the success of the eradication strategy. Results showed that the overall seroprevalence of PPR and SP antibodies were found to be 84 and 77.5% respectively, with no statistically significant difference in seropositivity distribution according to age, breeding system and date of vaccination. This high immunization level provided by the mass vaccination proves that the attenuated viruses did not interfere and can replicate independently to confer protection against the respective diseases. In support of the present observation, previous studies reported that PPR vaccine virus does not interfere with the immunogenicity to other antigens [8,15]. Besides, no side effect, local inflammation or abortions in pregnant ewes have been reported from the field,

which suggests that the combined vaccine is completely safe to be used in the target population.

Similar results were reported using the same vaccine in other African countries such, Mali, Tanzania and Uganda (Data not shown), these findings let predict that, the combined live attenuated vaccine based on PPR-Nigeria 75 and SP-Romania could represent an efficient tool to control and eradicate both diseases through mass vaccination.

On another hand, results of the present study, suggest that breed is a significant variable associated with PPRV and SPV seropositivity response; indeed, biological heterogeneity is evident between breeds, which consequently, influenced prevalence in different regions. In our study, PPR seropositivity in local breeds ranged, from 71.6 to 93.3%. This difference has also been reported during PPR outbreak evolution in Morocco in 2008, indicating difference in breed sensitivity (publication under press). For SP the seropositivity ranged between 74.8 and 81.5% which is more homogeneous, except for Ouledjellal breed which presented the lowest seropositivity for SP (46.5%).

Regarding analysis method, PPR seropositivity may be underestimated, using the cELISA, 6% of the vaccinated animals presented a doubtful result, which could be considered low positive. As reported, cELISA was described to have high specificity (99.4%) and satisfactory sensitivity (94.5%) [16]. For SP, since there is no ELISA kit commercially available, we used VNT which known to be less sensitive. For this reason, we could consider that the obtained SP rate is largely underestimated, which mean that large of negative animals are in fact protected. It is known that the protection against SP is mainly cell mediated in comparison to the humoral immune response, animals with no detected antibody after vaccination presented full protection after experimental infection [17-19].

CONCLUSION

In conclusion, given the data reported in the present study, including its efficiency and safety, the PPR/SP combined vaccine represent an proficient and a cost-effective tool to control in one campaign, the most devastating diseases of small ruminants particularly, where they share similar geographic distribution.

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