

Seroprevalence of *Peste des petits ruminants* Antibodies in Sheep and Goats after Vaccination in Karamoja, Uganda: Implication on Control

^{1,2}P.D. Luka, ¹J. Erume, ^{1,3}F.N. Mwiine and ³C. Ayebazibwe

¹Faculty of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda

²National Veterinary Research Institute, P.M.B. 1, Vom, Nigeria

³National Animal Disease Diagnostics and Epidemiology Centre, Ministry of Agriculture, Animal Industry and Fisheries, P.O. Box 513, Entebbe, Uganda

Abstract: A cross sectional survey was carried out to determine the prevalence of antibodies and seroconversion to *Peste des petits ruminants* virus (PPRV) in small ruminants in Karamoja region, Uganda. This region recently experienced serious outbreaks of PPR prompting vaccination interventions. A total of 316 sera samples (210 of goats and 106 of sheep) were collected from small ruminants with history of vaccination, no vaccination and unknown history in five districts of Karamoja region. Competitive enzyme linked immunosorbent assay (cELISA) was performed to detect the presence of antibodies against PPRV in serum. We found an overall PPRV specific antibody sero-conversion of 55.26% (84/152) (among Vaccinated), seroprevalence of 11.65% (2/17) (in Unvaccinated) and 53.33% (80/150) for shoats with unknown vaccination history. Seropositivity in sheep was 51.89% compared to goats with 57.62%. Among various age groups, 1-2 year olds showed higher sero-positivity (68.57%). Kaabong and Moroto district had highest sero-positivity of animals (78.33%) while Abim district had the lowest sero-positive animals (20%). From this study, it can be concluded that the animals in the region are not fully protected and this is particularly so in districts like Abim. There is need to find out the reasons for the poor protection levels and come up with measures to address these problems in order to improve protection against PPR.

Key words: cELISA, control, Karamoja, PPR, sero-conversion, seroprevalence, Uganda, vaccination

INTRODUCTION

Pestes des petits ruminants (PPR) is an acute, highly contagious viral disease of sheep and goats belonging to the genus *Morbillivirus* and family *Paramyxoviridae*. It is characterized by fever, anorexia, ulcerative necrotic stomatitis, diarrhea, purulent ocular and nasal discharges, pneumonia and death (Lefevre and Diallo, 1990). PPR was first reported in West Africa in 1942 (Gargadennec and Lalanne, 1942) and has since spread to other parts of the world. Morbidity and mortality vary considerably and can be as high as 90 to 100% depending on the susceptibility of the small ruminants' population in an area, animal husbandry, breed and age (Ezeokoli *et al.*, 1986).

Clinically, PPR is similar to Rinderpest (RPV). Initial diagnoses were made using Agar Gel Immunodiffusion (AGID), Counter-Immunoelectrophoresis (CIEP), Enzyme Linked Immunosorbent Assays (ELISA) and Virus Neutralization assays (VNT). These assays were time consuming and laborious, rapid, sensitive molecular techniques were subsequently developed (Couacy-Hymann *et al.*, 2002; Forsyth and Barrett, 1995).

In order to control the disease, various options had been identified as control measures including; efficient disease reporting, emergency response to outbreaks,

restriction of animal movement, quarantine and vaccination. Vaccination has remained the only feasible option because of the inability to afford the zoo-sanitary control measures. Attenuated rinderpest tissue culture vaccine has been used to confer immune protection of up to one year to susceptible small ruminant's population because of its cross reactivity with other members of the genus *Morbillivirus* (Taylor, 1979). This heterologous vaccine was used up to the 1990s when it was replaced by the homologous PPR vaccine developed from the Nig 75/1 strain after several passages (Diallo, 2004; Diallo *et al.*, 1989).

Antibodies to PPR were first reported in Uganda in the mid 1980s by Wamwayi *et al.* (1995) but the first confirmed PPR outbreak occurred in 2007 (MAAIF, 2009a) imposing a severe threat to small ruminants due to the heavy economic losses to farmers as well as the small ruminant industry. Animals that survive from disease are reported to be protected for life (Anderson and McKay, 1994). To control the disease, vaccination was conducted between 2008 and 2009 by the Ministry of Agriculture Animal Industry and Fisheries (MAAIF) after the outbreak in 2007.

However, the effectiveness of PPR vaccination depends on the level of vaccine coverage. Among small ruminant populations, recent studies suggest that coverage

may be as low as 5% which is inadequate for effective disease control (Diallo, 2004). Herd immunity has been estimated to be a minimum of 75-80% to control rinderpest (Rossiter and James, 1989), a related disease.

Therefore, this study was undertaken to determine the sero-positivity of small ruminants population to PPRV in North Eastern (Karamoja region) Uganda following vaccination.

MATERIALS AND METHODS

Study area and sample collection: A Cross-sectional study was conducted in Karamoja region of Uganda, which is bordering Kenya to the East and Sudan to the North. This region represents a locus where the first outbreak of PPR in Uganda was confirmed in MAAIF 2007. The region is made up of five districts of Nakapiripirit, Moroto, Kotido, Kaabong and Abim. Herd population within the region is 1.9 million goats and 763,243 sheep (MAAIF, 2009b). The sample size was calculated as 380 samples based on the prevalence of 57.6% (Mulindwa, 2009) using the statistical formula by Magnani, (1997). Of the 380 target sera samples, only 316 sera were collected from the small ruminants' population (210 Goats and 106 sheep) in the five districts of the Karamoja region. Of these, a total of 152 sera were from small ruminants with a history of vaccination, 17 from unvaccinated while 150 were from animals with unknown vaccination history. The samples were collected from animals of different ages and during the sampling period in November 2009. Collected samples were frozen at -20°C at the serum bank of the National Disease Diagnostic and Epidemiology Centre (NADDEC) of MAAIF, Entebbe prior to testing by competitive ELISA (cELISA).

Competitive ELISA: The PPRV antibody detection was carried out using PPR c-ELISA kit obtained from the Institute of Animal Health (Pirbright Laboratory, Surrey, UK). The kit contained user manual with fact sheets, distilled water (30 mL), PBS powder (Sigma, IL), Tween-20 (100 mL), ELISA plate (Nunc, Maxisorp) anti mouse HRPO conjugate (2 mL) substrate, H₂O₂, OPD tablet (30 mg), antigen (1 mL), strong positive serum (1 mL), weak positive serum (1 mL), negative serum (1 mL) and monoclonal antibody. The c-ELISA test was conducted according to the kit protocol. Briefly, PPR antigen was diluted in coating buffer (PBS-0.01M, pH 7.4), and each well of the microtiter plate was charged with 50 µL of diluted antigen followed by 1 h incubation at 37°C on orbital shaker. The plates were washed for 3 times with washing buffer, blot dried and 45 µL of blocking buffer (PBS+0.05% tween 20+0.5 µL of negative lamb serum) was added to all the wells. Subsequently, 5 µL of blocking buffer was added to monoclonal control wells,

Table 1: Origin of sera screened for PPRV antibody by c-ELISA

District	No. of sera		Total
	Sheep	Goats	
Abim	0	50	50
Kaabong	25	35	60
Kotido	45	60	105
Moroto	20	25	45
Nakapiripirit	16	40	56
Total	106	210	316

55 µL of blocking buffer to the conjugate control wells and 5 µL of test, strong positive, weak positive and negative control sera were added to the corresponding wells. Then 50 µL of mAb (diluted 1:100 in blocking buffer) was added to all the wells except the conjugate control ones, followed by incubation of the plates for 1 h at 37°C on orbital shaker. After three washings and blot drying, 50 µL of anti mouse HRPO conjugate were added to all wells. After 1 h incubation and 3 washings, 50 µL of the chromogen/substrate mixture (OPD/H₂O₂) were added to all wells. After 10 min incubation at room temperature, color development was stopped by adding 50 µL stop solution (H₂SO₄, 1M) to all wells. Optical Density (OD) values were read at 492 nm with ELISA plate reader (ImmunoScan BDSL, Thermo Lab. Systems, Finland). The absorbance was converted to Percentage Inhibition (PI) using the formula below with help of ELISA Data Interchanges (EDI) software manufactured by FAO/IAEA.

$$PI = \frac{\text{Absorbance of the test wells}}{\text{Absorbance of the mAb control wells}} \times 100$$

The test serum samples showing PI values of 50 or above were taken as positive for PPR antibodies.

RESULTS

Prevalence of PPRV antibodies in small ruminants with different vaccination status: A total of 316 serum samples collected from the five districts of Karamoja Region (Abim, Kaabong, Kotido, Moroto and Nakapiripirit) were screened for specific antibodies against PPRV using competitive-ELISA (c-ELISA) kit (Table 1). Of these samples, 152(48.10%), 17(3.8%) and 150(47.47%) were from small ruminants with vaccinated, non-vaccinated and of unknown vaccination history, respectively.

Our findings are as shown in Fig. 1. Out of the 152 samples with vaccination history, 84 (55.26%) showed vaccination sero-positivity in the small ruminant's population of the Karamoja region indicating a 55.26% level of sero-conversion or success of the vaccine administered. Among the unvaccinated only 2(11.76%) of 17 samples were sero-positive which may indicate that the PPRV virus had been in circulation. Of the 150 shoats

Table 2: Prevalence of PPRV antibodies in small ruminants district

District	Total	Vaccinated (no. of positive, %)	Not vaccinated (no. of positive, %)	Unknown (no. of positive, %)	Overall % seropositivity
Abim	50	none	none	50 (10, 20)	20
Kaabong	60	none	none	60 (47, 78.33)	78.33
Kotido	105	73 (28, 38.36)	5 (0, 0)	32 (27, 84.38)	52.38
Moroto	45	42 (35, 83.33)	3 (0, 0)	none	77.78
Nakapiripirit	56	47 (27, 57.45)	9 (2, 22.22)	none	51.79

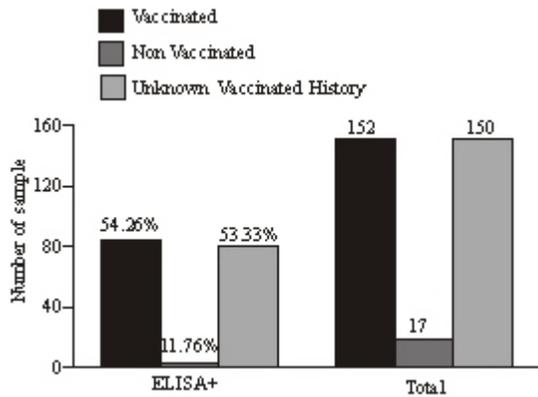


Fig. 1: PPRV antibody prevalence in small ruminants with different vaccination history

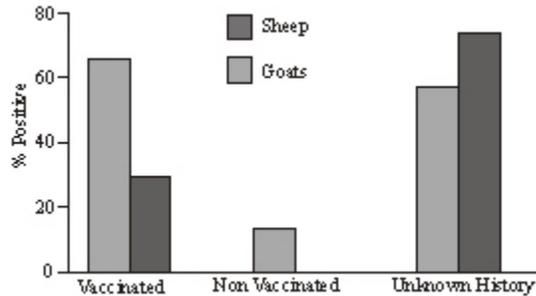


Fig. 2: Species seroprevalence of PPRV antibodies in Karamoja

with unknown vaccination history, 80(53.33%) were found to be sero-positive.

PPRV antibody status by species tested: We investigated the influence of species on antibody seropositivity in the 210 and 106, goats and sheep, respectively. Amongst the goats, 85 sera were from goats with a history of vaccination, 15 with no vaccination history, while 110 were from unknown vaccination status. Of these, 56 (65.88%), 2 (13.33%) and 63 (57.27%), were positive for PPRV antibodies by c-ELISA (Fig. 2), respectively. Overall sero-positivity for the goat's population in this study was 57.62 %. Of the 106 sheep that were tested for the presence of antibody, 69 had a history of vaccination, 2 sheep were not vaccinated and 35 were of unknown status. The overall sero-positivity for sheep was 51.89%.

PPRV antibody status by age of animal: The 316 sera of small ruminants that were sampled were within the ages; below 1 year (n = 29), 1-2 years (n = 140), 2-3 years (n = 93), 3-4 years (n = 50) and above 4 years (n = 4), respectively. The PPRV seropositivity was; <1year 5 (17.24%), 1-2 years 96 (68.57%), 2-3 years 47(50.54%), 3-4 years 18 (36.0%) and >4 years 0 (0%). The above results show that sero-positivity rose with age up to 2 years and thereafter decline.

PPRV antibody status by district studied: At the district level, 50, 60, 105, 45, and 56 sera were collected from Abim, Kaabong, Kotido, Moroto and Nakapiripirit, respectively. A total of 10 (20%), 47 (78.33%), 55 (52.38%), 35 (77.77%) and 29 (51.79%) sera samples were positive for PPRV antibody in Abim, Kaabong, Kotidi, Moroto and Nakapiripirit districts, respectively (Table 2). The data indicated that Kaabong and Moroto districts had the highest numbers of animals positive for PPRV antibodies. Additionally, Moroto districts showed the highest percentage of animals that seroconverted following PPRV vaccination.

DISCUSSION

This study found that 55.26% of the vaccinated small ruminant population were protected against PPRV, this is quite low compared to the minimum of 75-80% herd immunity required to control rinderpest (Rossiter and James, 1989). This low level of PPRV sero-positivity found in this study was unexpected since the PPR vaccine has been reported to confer protection for up to three years (Diallo *et al.*, 2007). However, the immunogenicity of PPR vaccine has been reported to vary. In a comparative study in Pakistan, Intizer *et al.* (2009) demonstrated that the Geometric Mean Titre (GMT) of a local PPR vaccine were higher (207.9) than the imported Pestvac® (73.3) from Jordan. Arguably, the vaccine used in Uganda may not be the appropriate or suitable one to be used or there is need to give a booster dose to induce a higher immunologic response.

Among the unvaccinated small ruminants, 11.76% had antibodies to PPRV which means that those animals could have been exposed to the field virus or got in contact with those that shed the vaccine virus. Following Wamwayi *et al.* (1995) first report of seroprevalence of 0.8%, PPRV could have been circulating in Uganda but misdiagnosed or undetected. The sero-positive

unvaccinated animals as detected by this study could perpetuate the dissemination of the virus among susceptible sheep and goats (Ezeibe *et al.*, 2008). Therefore, surveillance activities are needed to determine the importance of these shedders to PPRV prevention and control efforts (Anderson and McKay, 1994).

The level of sero-positivity among vaccinated sheep and goats and those with unknown vaccination history were similar. It is possible that the latter were also vaccinated but the herdsmen could not report their vaccination status. In the Karamoja region animals are sometimes taken out for days for grazing and vaccination may have occurred without the knowledge of other herdsmen responsible for these animals. Also, animal rustling is common in the region and vaccinated animals may have been rustled and taken to unvaccinated flock or vice versa. Our findings seem to agree with those of a previous study conducted by Mulindwa (2009) who reported a PPRV seroprevalence of 57.7% among small ruminants in Karamoja. In a survey in Ethiopia, Waret-Szkuta *et al.* (2008) reported a heterogeneous seroprevalence of 57.7% similar to Uganda among the small ruminants population. On the other hand, in PPR endemic Pakistan, Abubakar *et al.* (2009) reported a seroprevalence of 54.09%, also similar to the data from the present study. In the present study, the overall sero-positivity to PPRV in sheep was found to be a little lower (51.89%) than in goats (57.62%). These findings suggest that there was no difference in vaccination coverage among the small ruminant species. Nonetheless, Abubakar *et al.* (2008) reported vice versa. Similarly, in Saudi Arabia using neutralization test which is known for its low sensitivity (Al-Afaleg *et al.*, 2004) reported a higher prevalence to PPRV in sheep as opposed to goats, 3.1 and 0.6%, respectively. In contrast, Swai *et al.* (2009) in Tanzania reported a prevalence of 39.8 and 49.5% in sheep and goats, respectively. The latter results seem to concur with our findings.

The findings of this study also suggest that animals that were 1-2 years old had a better sero-positivity to PPR than any other age groups. Our data suggested that animals younger than 1 year and older than 2 years had lower chances of being sero-positive to PPR. These findings are in agreement with previous reports by (Abubakar *et al.*, 2009; Ozkul *et al.*, 2002; Singh *et al.*, 2004) who found that younger animals were more susceptible to PPRV. It has been documented that sheep and goats exposed to PPRV at a very young age may carry antibodies for 1-2 year following exposure (Dhar *et al.*, 2002; Ozkul *et al.*, 2002; Singh *et al.*, 2004). In the study area, the demographics showed that the majority of the animals tested were in the age bracket of 2 years. It is most probable that our findings are related to the practice of selling older animals and leaving the young ones as replacement stock.

District per district status in terms of vaccination success showed that Kaabong animals were more protected followed by Moroto, Nakapiripirit, Kotido district and Abim with the least sero-positivity. This is somewhat in agreement with earlier study by Mulindwa (2009), who reported a seroprevalence of 63.2% in Moroto, 72.0% in Nakapiripirit, 85.0% in Kotido and 1.6% in Abim. The high seroprevalence in Moroto could be due to the small sample size compared to other districts while the difference in Abim could be either due to the sample size or movement of animals and spread of the disease. The findings of PPRV antibodies in unvaccinated animals in the different districts suggested that disease could be spread by movement of animals and the sero-status suggest different level of vaccination coverage in the districts which has implication on the control of the disease. Control and prevention of PPR in Karamoja region is a very difficult task, largely because of the nomadic animal husbandry practices, and security threats due to rustling which has led to keeping animals in kraals and frequent long distance movement and criss-cross mixing of animal populations, within and between districts and across borders. Unless these huddles are eliminated, the disease will continue to spread, pausing control challenges.

CONCLUSION

PPR is a disease of economic importance because of its impact on the livelihood of the rural poor. The outbreak in Karamoja region prompted mass vaccination to control the disease. This study showed varying antibody levels in the affected districts reflecting the infection and vaccination profiles of the herds. There was serological evidence of seroconversion to the vaccine and seroprevalence to the circulating virus suggesting the level of vaccine coverage which is not enough to achieve herd immunity should the disease strike again. We recommend pastoralist awareness to enhance participation in disease surveillance and control program to better control the disease. Booster dose should be administered to get a higher immunologic response targeting wide vaccination coverage.

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