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Research Application Summary

Sero-epidemiology of Peste des petits ruminants infection and the associated risk factors in South Kivu, DR. Congo

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Abstract

Peste des petits ruminants (PPR) are an acute viral disease of small ruminants caused by Peste des petits ruminants virus (PPRV). The PPRV has high morbidities and mortality rates in domestic small ruminants causing high socio-economic losses to the farmers. This study determined the Sero-prevalence of PPR associated with transmission risk factors in unvaccinated sheep and goats in South-Kivu province in DR. Congo. The competitive enzyme-linked immunosorbent assay (cELISA) and a semi-structured questionnaire were used in data collection. The results showed an overall seroprevalence of 28.5% with a 11.3% and 32.7% seropositivity in sheep and goats, respectively. Peste des petits ruminants seroprevalence was higher in the territories that recorded high rainfall at 34.5% and 29.4% in Shabunda and Mwenga, respectively. Ruminant's age, grazing and farming system, geographic location of the territory, and animal's origin were found to be the most significant risk factors. Further, PPR seroprevalence was higher in small ruminants kept under communal grazing system. Considering that this was a snap shot study from an assessment perspective, there is need to undertake more robust studies with a focus on molecular characterization and isolation of PPR virus in the DR. Congo.

Key words: Seroprevalence, Risk factors, Peste des petits ruminants, South-Kivu (DR. Congo)

Résumé

Peste des petits ruminants (PPR) est une maladie virale aiguë des petits ruminants causée par le virus la peste des petits ruminants (PPRV). Le PPRV a une morbidité élevée et un taux de mortalité chez les petits ruminants domestiques qui entraînant des pertes socio-économiques élevées pour les petits éleveurs. Cette étude consistait à déterminer la séroprévalence de PPR associée à des facteurs de risque de transmission chez les moutons et les chèvres non vaccinés dans la province du Sud-Kivu en RD. Congo. La technique du dosage compétitif immuno-enzymatique (cELISA) et un questionnaire semi-structuré ont été utilisés. Les résultats ont montrés une séroprévalence

globale de 28,5% avec une séropositivité de 11,3% et 32,7% chez les ovins et les caprins, respectivement. La séroprévalence de Peste des petits ruminants était plus élevée dans les territoires qui ont enregistrés de fortes précipitations à 34,5% et 29,4% à Shabunda et Mwenga respectivement. L'âge des animaux, le pâturage et le système d'élevage et d'exploitation des petits ruminants, la zone agro-écologique du territoire, et l'origine des animaux se sont révélés être des facteurs de risque les plus importants pour la PPR. La PPR séroprévalence était plus élevée chez les petits ruminants gardés dans le système de pâturage communal comparé aux animaux gardés en stabulation permanente ou semi-stabulation. Considérant que cette étude avait un point de vue de l'évaluation, il est nécessaire d'entreprendre des études plus approfondies avec un accent sur la caractérisation moléculaire et l'isolement du virus de la PPR dans le DR. Congo.

Mots-clés: séroprévalence, les facteurs de risque, peste des petits ruminants, Sud-Kivu (RD Congo)

Background

Peste des petits ruminant (PPR) is a trans boundary animal disease (TAD) and is attracting attention especially in the Southern African Development Community (SADC) due to its occurrence in explosive form, high mortality and rapid spread across national borders or even across continents (Kayunze et al., 2012). According to TADs Steering Committee for Africa during the years 2008-11 a total of 4,079 outbreaks, 431 258 cases and 56 663 deaths due to PPR were reported in 25-30 (56%) countries in Africa. It is believed that PPR might have been introduced to African countries through the movement of live infected animals (Kaukarbayevich et al., 2009). The SADC report indicates that the DRC indicated that since its emergence in 2010 to June 2012, PPR had caused the death of almost 120 000 small ruminants. It is estimated that the direct loss, i.e., value of dead sheep and goats, to be US\$5.3 million (SADC, 2012). This estimate does not take into account the socio-economic impact and other benefits of goats and sheep to the smallholder farmers. Trans-border movements of cattle, goats and sheep for breeding in neighboring countries, farming systems, trade and other socio-economic functions could be the main source for introduction, transmission and maintenance of PPRV in several countries.

The South-Kivu region of the DR.Congo is believed to have been infected since 2010, when outbreaks based on clinical signs were reported, but no laboratory diagnosis (molecular or serological) has been done to confirm the cases. Indeed there is a fear that without coordinated action it will spread to neighboring countries such as Rwanda, Burundi, Uganda and further south, such as Angola, Botswana and Zambia. In severe outbreaks affected goats may die within ten days of exposure to the virus. An estimated one million goats and 600 000 sheep are at risk of contracting PPR, representing one-quarter of goats and two-thirds of sheep throughout the DR. Congo (IPAPEL, 2012; FAO and OIE, 2013).

Literature summary

Peste des petits ruminants (PPR) is one of the acute contagious and viral epizootic diseases of domestic (sheep, goats) and wild (springbuck, gazelles and impala) small ruminants caused by PPR virus (PPRV). The virus has a linear negative-stranded RNA genome consisting of 15,948 nucleotides and six genes that encode eight proteins which belongs to the family Paramyxoviridae (Luka, 2012). It has been reported by Banyard *et al.* (2010) that PPR is highly infectious and causes high mortality. Abubakar *et al.* (2011) found that cattle are able to seroconvert in case of a high prevalence of PPR in small ruminants, although they are not susceptible to the disease.

The transmission of PPR is from infected to susceptible animals by close contact or through respiratory and oral routes (Muse *et al.*, 2012a). Clinically, the disease is characterized by proliferative and self-resolving lesions around the muzzle and lips of animals, serous nasal and ocular discharge which become mucopurulent (Zhaok *et al.*, 2010). Of the four known lineages of PPR virus, lineages I and II have been found exclusively in West Africa, but the virus from an outbreak in Burkina Faso in 1999 fell into the lineage I group. Viruses of lineage III have been found in East Africa, where an outbreak in Ethiopia in 1996 was of this type. Lineage IV is found in south Asia, Middle East and China (Dhar *et al.*, 2002). To-date it has not been established which lineage of the PPRV is circulating in the South Kivu region, East of Democratic Republic of Congo.

Study design

This study was conducted in South Kivu region located in the East of DRC to determine the PPRV antibody prevalence in small ruminants (goats and sheep) population. The information would help in epidemiologic control and identify risk factors associated with PPR transmission in reported outbreak zones in South Kivu Region of DRC with a view to support control and surveillance strategies. This study employed a cross-sectional study design with purposive sampling of animals within the PPR reported areas. A total of 319 sera samples for serology analysis were collected randomly from villages in targeted regions. The age groups of sampled animals were in the range of 1-4 months to rule out maternal antibody (from 5-12 months) and to discover recent infection (>12 months) and only unvaccinated animals (sheep and/or goat) were considered for serological analysis. Territories and villages were selected purposely in clusters based on outbreak report and then a random selection of animals within the selected villages was conducted. For sera-collection, an estimated sample size based on priori prevalence from the Rapid Epidemiological Assessment study described by Kgotlele et al. (2014) with p = 22.1%according to the formula: $n = z_a^2 \times [p \times (1-p)/L^2]$ was used. A large number of goats were considered due to their high susceptibility to the disease compared to sheep.

Sample collection and preparation

Blood was collected by jugular vein puncture using sterile vacutainer tubes of 5 ml (BD Biosciences, Franklin Lakes, USA) and left to clot overnight at room temperature and left to clot overnight at room temperature for serological test. Serum was decanted into

sterile cryovials and kept on ice during transportation to the laboratory. In the laboratory, sera were stored at -80°C. Each tube was labelled using codes describing the village and/or district and the number of the questionnaire where several parameters for risks factors analysis were collected.

Sample analysis

The sera were analyzed using competitive enzyme linked immunosorbant assay (cELISA) kit (BDSL, Institute for Animal Heath, Pirbright, UK), according to Anderson et al.. (1991). Briefly the plates were coated with PPRV antigen and incubated with test sera, control sera and monoclonal antibody. Afterwards, horseradish peroxidase conjugated rabbit anti-mouse immunoglobulin was added followed by substrate and chromogen. Colour was allowed to develop for 30 minutes followed by reading of plates at 492 nm (MTX Lab Systems, Vienna, USA). The ELISA micro-plates were read using ELISA DATA Interchange (EDI) software to give optical density (OD) values and percentage inhibition (PI) values were calculated using the following formula:

$$PI = 100 - (\frac{Replicate OD of each Control}{Median OD of Cm} \times 100)$$
. The formula for the percentage inhibition (PI)

which was used for acceptance of replicate values for test sera and diagnostic interpretation was: $PI=100 - (\frac{Replicate\ OD\ of\ each\ Test\ serum}{Median\ OD\ of\ Cm} \times 100)$.

Samples with a PI value of $\geq 50\%$ were considered positive for PPR antibodies, and test sera demonstrating mean PI value less than 50% were considered to be negative at CI 95% level for the prevalence of antibodies to PPRV.

Data collection for PPR risks factors

Laboratory results were completed with the risks factors found using structured questionnaire for every sample herd. The significant associated factors, found from univariate analysis using chi-square were further analyzed multivariably by logistic regression.

Data Analysis

Epi info and Microsoft Office Excel 2010 were used to calculate frequencies of PPR samples prevalence. Chi-Square test (X^2) was used to test the significance of proportions between animals tested negative and those tested positive. F-test for multiple regressions (Logistic model) at 95% CI was used to see the correlation between the serological statues of animal with the risk factors associated with PPR transmission.

Results

Prevalence of antibodies against PPRV

The results showing seroprevalence of anti-PPRV antibodies according to territory, species and animal's age are shown in Table I. A total of 319 sera samples from goats and sheep were collected from the three territories, where PPR outbreak had been

previously reported in South-Kivu region.

Table I. Seroprevalence of antibodies against PPRV in small ruminants in Shabunda, Mwenga and Fizi territories of South-Kivu region using cELISA.

Location			PPR seroprevalence	
	Species	Total samples	cELISA positive	Seroprevalence
		tested	samples	(%)
Shabunda Territory	Goats	112	45	40.2
	Sheep	30	4	13.3
	Overall	142	49	34.5
Mwenga Territory	Goats	65	21	32.3
	Sheep	14	2	14.3
	Overall	79	23	29.1
Fizi Territory	Goats	80	18	22.5
•	Sheep	18	1	5.6
	Overall	98	19	19.4
South-Kivu Province	Goats	257	84	32.7
	Sheep	62	7	11.3
	Overall	319	91	28.5

The overall PPR seropositivity in goats and sheep was 28.5% (n = 319) out of which 11.3% (n = 62) and 32.7% (n = 257) seropositivity was found in sheep and goats, respectively. Considering different regions, the highest percentages of sero-positivity was found in serum samples from Shabunda (34.5%) and Mwenga (29.4%) while the lowest sero-positivity was found in Fizi territory (19.4%). As mentioned earlier, the seroprevalence was higher in goats (32.7%) than in sheep population (11.3%).

Risks factors associated with PPR

Table II presents the summary of the risk factors that are associated with the seroprevalence of PPR. All factors with p-value of less than 0.05 with a positive coefficient indicate the strong association between the risk factors for PPR transmission and the likelihood of finding antibodies against PPRV.

Table II. Logistic regression of factors associated with the presence of antibodies agains PPRV in goats and sheep samples from South-Kivu in DRC.

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Variables	Coef.	Std. Err.	\mathbf{Z}	p > z	[95% Co	onf. Interval]
New animals	-0.762191	1.021829	-0.75	0.456	-2.764939	1.240557
introduction						
Grazing system	1.274841	1.078373	1.08	0.036	-3.388412	0.838731
Veterinary service	-0.427702	2.992279	-0.14	0.886	-6.292462	5.437057
Use of vaccine	2.090301	1.553719	1.35	0.179	-0.954932	5.135535
Bio safety	0.216319	1.628277	0.13	0.894	-2.975045	3.407684
Animal sex	2.187659	1.910057	1.15	0.252	-5.931302	1.555984
Animal age	0.106806	0.795910	0.34	0.041	-2.62802	0.491892
Animal origin	-1.276695	0.475360	-2.69	0.007	-2.208384	-0.345006
Farming system	0.264545	1.245253	0.21	0.022	-2.176105	2.705197
_Cons	7.337694	3.118044	2.35	0.019	1.22644	13.44895

From Table 2 results, the animal aged >12 months had a significantly higher seroprevalence and were more likely to be sero-positive compared to those aged less than 12 months in any outbreak occurrence (Coef=0.1068064 and p-value<0.05). More seropositive cases were likely to be observed in animals kept in communal grazing system (coef.=1.274841, p-value=0.036) and free-ranging farming system (Coef. = 0.2645458, p-value=0.022) compared to the animals kept in zero-grazing farming system. In contrast, grazing and farming systems, vaccination of small ruminants, age and sex of animal affected positively the likelihood of animals getting the disease during any outbreak's occurrence (log likelihood = -21.706265; LR chi² (9) = 21.24; Prob>chi²= 0.0116; pseudo R²= 0.3286).

Table 3. Univariate analysis for risk factors associated with PPR seropositivity using Chi-square $(\chi 2)$ test.

Risk factor	parameters	tested animals	+ve samples	Prevalence (%)	χ2	p- value
Animal age (mont	th) 1-4	61	11	18.1	24.793	0.041*
	5-12	110	28	25.5		
	<12	148	52	35.1		
Grazing system	Communal	271	83	30.6	31.23	0.036*
	Zero-grazing	48	8	16.7		
Farming system	Free ranging	268	84	31.4	38.12	0.022*
	Zero-grazing	51	7	13.7		

From these findings, it is speculated that the prevalence of PPR was affected significantly by age, grazing system and farming (p-value>0.05) among the visited territories in South-Kivu region. Sheep and goats of >12 months had a significantly higher seroprevalence, followed by animals with age from 5 to 12 months, while the low prevalence was found in kids aging 1 to 4 months. Animals kept in communal grazing and free ranging are more affected by PPRV with respectively the seropositivity of 30.6% and 31.2% compare to animals kept in zero-grazing farming system (13.7%).

Table 4. Multiple regression analysis for risk factors associated with PPR seropositivity

Variables	Odds Ratio	Std. Err.	Z	p> z	[95% Conf	. Interval]
New animals introduction	0.4666428	0.476829	-0.75	0.456	0.0629799	3.457538
Grazing system	6.2794755	0.301378	1.08	0.037	0.0337622	2.31343
Veterinary service	0.6520055	1.950982	-0.14	0.886	0.0018502	229.765
Use of vaccine	8.087353	12.56548	1.34	0.189	0.3848382	169.9552
Bio safety measures	1.241499	2.021504	0.13	0.894	0.0510452	30.19522
Animal sex	5.1121791	0.214268	1.17	0.243	0.002655	4.739747
Animal age	9.3436732	0.273533	0.35	0.041	0.0722213	1.635408
Animal origin	0.2789577	0.132605	-2.37	0.017	0.1098781	0.708216
Farming Systems	11.302839	1.622364	0.21	0.022	0.1134826	14.95726
_Cons	1537.163	4792.942	2.35	0.019	3.409071	693112.6

The odds ratio involves the relationship between the probability of success and failure. Thus, the higher the odds ratio, the higher is the probability of an event to happen. The introduction of new animals from the same village was less likely to make an animal (goats and/or sheep) to be sero-positive during any infection (OR=0.46). Other variables such as presence of veterinary service in the region to diagnose, control and prevent PPR outbreak occurrence and spread, the origin of animal were all likely to affect the PPR sero-prevalence with OR=0.65 and OR=0.28. Likewise factors such as the grazing system, the usefulness of different vaccine, bio safety measures that is taken to prevent the disease at the farmer level, farming system and the age and sex of animals were also likely to influence the PPR sero-prevalence with respectively odds ratios of 6.28; 8.09; 1.24; 11.3; 9.34; 5.11. (Log likelihood = -21.706265, LR chi2 (9) = 21.24, Prob>chi2= 0.0116 and Pseudo $R^2=0.3286$).

Discussion

Seroprevalence data are useful because they give information on the distribution of PPR in different geographical areas and can help in predicting the level of protection in animals. This information is helpful in developing disease control strategies and understanding the patterns of PPRV infection. During this study, farms with small ruminants (goats and sheep) in South-Kivu region where there was a suspected outbreak of PPR were visited. The PPR suspected goats and sheep with clinical signs including oculonasal discharges, diarrhea and cutaneous nodules were sampled and tested. These clinical signs have been previously reported in sheep and goats confirmed with PPR (Chauhan et al., 2009; IPAPEL, 2013). There were no known documented reports on previous vaccination in South-Kivu region where this study was conducted; hence presence of PPR antibodies was directly attributed to natural PPR infection. The overall seroprevalence in small ruminants found in this study was 28.5% (n=319) from which 11.3% (n=62) was found in sheep and 32.7 % (n=257) in goats (Table I). Previous studies from northern and southern Tanzania showed a seroprevalence of 45.4% (Sreenivasa et al., 2009) and 31.0% (Luka et al., 2012). Different studies in Sudan have shown higher levels of seroprevalence including 54%; 50.6%; 62.8% and 61.8% (Abdalla et al., 2012). Other studies show that goats are more severely affected than sheep which generally undergo a mild form (Kaukarbayevich et al., 2009). The differences in the PPRV seroprevalence found in neighboring countries compared to DRC could be also attributed to differences in management systems of small ruminants, sampling procedures used or technical knowledge levels of natural immunity and variable natural PPRV infection rates in different geographical areas. Territories that registered higher seroprevalence of PPR were Shabunda (34.5%) and Mwenga (29.1%) while Fizi (19.4%) had the lowest PPR seroprevalence. The highest sero-positivity in Shabunda can be explained by intensive uncontrolled trans-border ruminants' movements between this territory with Kalima and Maniema where PPR outbreaks have been reported by FAO in 2012. In addition, poor management of animals and the use of communal grazing system could also contribute to this higher prevalence.

The interaction between sheep and goats in pastoralist system especially in the areas with high density of wild small ruminants like Shabunda and Mwenga could also have contributed to the higher PPR prevalence. The role of PPR transmission from wild to domestic ruminants has previously been reported by Swai et al. (2011). In addition, Shabunda territory has big animal markets that allow contact of many animals from different areas and enhance viral transmission and spread. Domenech et al. (2006), described that trade of live animals at markets was an important vehicle for transmission of infectious diseases. The highest PPR seroprevalence in Shabunda and Mwenga districts might be also related to the differences in climatic factors. These findings are in line with the reports of Saeed et al. (2010) and Elnoman et al. (2011), suggeting that high rainfall and cool weather can contribute to PPR spread. As shown in Table 3, sheep and goats aged above 12 months had a significantly higher seroprevalence (35.1%) compared to those aged less than 12 months. As no data is available in the DRC to compare with, these results can support the findings of Abubakar et al. (2009) who reported that highest PPR seroprevalence is likely to be seen in animals aged above two years old. Therefore, adult animals might be more vulnerable to PPR infections as compared to younger animals. However, Sarker and Islam (2011) reported the highest PPR seroprevalence was in young animals in North Bengal because of poor immunity and nutrition. Animals reared in communal system (pastoralist system or transhumance) and/or free ranging farming were likely to have high seroprevalence with respective seroprevalence levels of 30.6 and 31.3%, while low seroprevalence of 16.2% was found in the animals reared in zero grazing or intensive system. These results were similar to the findings of Muse et al. (2012b), who found that the source of the infection and spread of PPR was contact between animals during communal grazing and housing.

Conclusion

Based on the results found in this study it is concluded that antibodies against peste des petits ruminants virus are widely prevalent in small ruminants (sheep and goats) kept in South-Kivu region located in DRC. The PPRV antibodies could be detected by cELISA. An overall seroprevalence for PPR of 28.5% was found and was associated with some risks factors including, animal's age and sex, grazing, and farming system. Communal grazing (open grazing) and free ranging farming systems were most important factors associated with PPR transmission from an infected region to none infected. On the other hand, PPR was mostly prevalent in animals aged >12 months and goats were more affected than sheep. As the present survey provides the first preliminary information on PPRV seroprevalence in the Eastern part of DRC, more studies on the epidemiology of PPR are recommended to gather more data for effective control measures including characterizing the strains that can be used for development of vaccines using local strains.

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