Study on Sero- prevalence and Risk factor of Peste des Petitis ruminant disease in Small Ruminant at Metekel zone of selected District in Benishangul Gumuz Regional State, Western Ethiopia

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Abstract: Across–sectional study was conducted from October to November 2017 in Mandura, Dangur and Debate districts of Benishangul Gumuz Regional State to determine the sero- prevalence of Pest des Petites Ruminants and associated risk factor in sheep and goat. A total of 452 serum samples were collected from 10 peasant association and the sera were tested for the presence of antibodies against PPR using competitive Enzyme Linked Immunosorbent Assay. The overall sero-prevalence of PPR was found to be 73.45% (332/452). The sero prevalence of the disease in the different study district was 72.08% (142/197), 73.28% (90/131), and 75.80% (94/124) in Mandura, Dangur and Debate respectively. There is no statistical significant difference in the different districts ($\chi 2=1.27$, $p>0.05$). At the same time the sero prevalence in <1year, 1-2 year and >3year age categories were 75.40% (92/122), 74.78% (175/234) and 67.70% (65/96) respectively, which is not statistical significant ($p>0.05$). Similarly; there is no statistical significant difference between male and female shoats ($p>0.05$), that is 67.14% (47/70) in male and 74.60% (285/382) in female. However, among species, body condition and vaccination status, was significant difference ($p<0.05$). The higher sero prevalence of PPR indicated a remarkable contagious nature of the disease. In conclusion, this study reveal a higher sero prevalence and subsequent endemic establishment of PPR in small ruminant in the selected area. Therefore, strict measures should be implemented for feasible prevention of the disease.

[Gebrehiwot Woldemichael, Asmamaw Aki and Kebede Gurmessa. **Study on Sero- prevalence and Risk factor of Peste des Petitis ruminant disease in Small Ruminant at Metekel zone of selected District in Benishangul Gumuz Regional State, Western Ethiopia.** *Rep Opinion* 2018;10(9):13-22]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). http://www.sciencepub.net/report. 3. doi:10.7537/marsroj100918.03.

Key words: *c-ELISA, PPR, risk factor, sero-prevalence, small ruminant*

1. Introduction

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants that is characterized by high fever, ocular and nasal discharge, pneumonia, necrosis, ulceration of the mucous membranes and inflammation of the gastro-intestinal tract leading to severe diarrhea (Radostits *et al.,* 2000). The disease caused by RNA virus belongs to order *Mononegavirales*, the genus *Morbillivirus* of the family *Paramyxoviridae* (Murphy *et al*., 1999; Olivier *et al*., 2011).

The disease is regarded as the most economically important viral disease of small ruminants particularly goats in areas where these animals are intensively reared. In the regions where PPR occurs in an epizootic form it may have dramatic consequences for animal owners due to high mortality rates. In endemic areas where sub-acute reactions is usually occurs, it opens the door to many other infections and its impact on animal production is certainly considerable. OIE classification: list (A). Latent infections may be activated and complicate the clinical picture (El Sawalhy, A. A. 1999).

Morbidity and mortality rates in small ruminants can be as high as 90-100% and 50-90% respectively (Ezeibe *et al*., 2008). Heavy losses can be seen, especially in goats; all of the affected animals in some herds may die. At one time, peste des petits ruminants was thought to be restricted to West Africa, but it has since been recognized from the equator to the Sahara desert, as well as in Asia and the Middle East. Other nearby areas, such as southern Africa and central Asia, are threatened. Although increased recognition of PPR is one reason for the expanded geographic range, it is also possible that this virus is spreading (Lefe`vre *et al*., 1991).

The host range of peste des petits ruminants in wild animals is still unknown, and it is possible that this disease could threaten the conservation of some wildlife species. Severe outbreaks were reported in susceptible buffalo in 1995 and in captive gazelles in 2002. Nearly all of the affected animals died. Other species, such as deer and wild relatives of domesticated sheep and goats, may also be affected (Bazarghani TT *et al.*, 2006).

PPR is an important disease in its own right, but it has also created problems because of apparent similarity to rinderpest - the clinical signs of PPR closely resemble those of rinderpest, making differential diagnosis difficult. It should, however, be borne in mind that clinical disease caused by

rinderpest in small ruminants is a relatively rare event, even in Asia (Dhar P *et al.*, 2002).

PPR was first suspected in Ethiopia in 1977 in afar region, East of the country (Pegram and Tereke, 1981). Clinical observations and serological evidence reported in1984 and confirmed in 1991 with cDNA probe in lymph node and spleen specimen collected from an outbreak in a holding near Addis Ababa (Roeder *et al*., 1994). An overall sero-prevalene of 1.7% in Oromia, 21.3% in Somalia was reported (Waret-Szkuta *et al*., 2008). Recently an overall sero-prevalence record of 30.9% from sheep and goat in pastoral and agro pastoral area of Afar and Gambella region of Ethiopia has been reported (Megersa *et al.,* 2011).

The virus is present in all body excretions and secretions such as tears, nasal discharge, sputum, and diarrheic feces. As in RP, PPR virus spreads by direct contact or close indirect contact and infection is mainly by inhalation of infective aerosols but could also occur through the conjunctiva and oral mucosa. As with RP, the transmission cycle of PPR virus is maintained through a regular supply of susceptible hosts plus sufficient animal movement to allow mixing of the population. As in RP, it is generally accepted that there is no carrier state in PPR (House, J.A. 1992). Even though pest des petites' ruminants was epidemic and priority disease in the study area Dangur**,** Debate and Mandura District at Metekel zone Benishagul Gumuz Regional State, there was no enough records and study on the importance PPR disease.

Therefore, the **objectives** of the study were:

- To estimate the sero-prevalence of peste des petits ruminants in small ruminant at selected districts.
- Assessment of the potential risk factors of the disease.

2. Materials & Methods

2.1. Study area

The study was conducted from October to November 2017 at three selected districts in Metekel zone of Benshangul Gumuz Regional State, namely Dangur, Mandura and Debate. Dangur district is found in Benishangule Gumuz Regional State, in Metekel zone. It is about 369 km from the capital city of Benishangul Gumuz Regional State, Asossa town. It has common boundaries with pawe, in the East, Madura in the South East, Wombera and Bullen in South, Guba in the west and Amhara in the North. The district is divided in to 29 peasant associations with total human Populations of 63,160. The district has minimum and maximum altitude of 910m and 3,300 m above sea level. The average annual rain fall is 1800mm with average temperature of 36^0c and the total land size of the area is about 838,700 hectar. The total Livestock population of the district is estimated as Cattle 36,624, Sheep2,656, Goat 33,892, Equines 5,574and 81,495 Poultry (According to Dangur district office of live- stock and fishery development, 2017).

Mandura district is located in Metekel zone. It is about 387km far from the capital city of Benishangul Gumuz Regional State, Asossa town which is found in north west part of the region at $11^0$03'24.4"N and $036^0$19'42.8''E with a minimum and maximum altitude of 1050m and 1400m above sea level. The District is divided in to20 peasant associations with total human populations of 46, 198. The average annual rain fall is 1000-1600 mm with average temperature of 28^0c and the total land size of the area is about 1100 km2. The total livestock population of the district is estimated as Cattle 67,053, Sheep 14,100, Goat 36108, Equines 4,655 and Poultry 84,317. (According to Mandura district office of live-stock and fishery development, 2017).

Debate district is located at54km far from the capital city of Metekel zone, Gelgel Beles town which is found in south part of the town at $10^0$46'00.3''N and $036^0$15'36.5''E with altitude of 1505m above sea level. The average annual rain fall is 1000-1600 mm with average temperature of 28^0c and the total land size of the area is about 368,289hr. The total Livestock population of the district is estimated as Cattle 116,687, Sheep 15,555, Goat 42,183, Equines 8439 and, 58,801 Poultry (According to Debate district office of live- stock and fishery development, 2017).

To represent the study area 3,4,3 ''PA''from Dangur, Mandura and Debate districts respectively were selected based on out- break disease report and office of livestock and fishery development office about the epidemiological distribution of PPR disease. However, clinical records show that there is wide distribution of the disease in the area. (fig. 1)

2.2 Study design

A cross- sectional study design was implemented to determine prevalence of Peste des petits ruminant's disease in small ruminant. The study animals was classified in different body conditions good, medium & poor (Nicholson & Butterworth, 1986), age groups (< 1 year and 1-2 year and >3year) and other factors including sex, species and vaccination would be used to determine prevalence of the PPR disease in small ruminant. The study animals were sheep and goat (shoat) under extensive traditional husbandry system.

2.2. Sample Size and Sampling methods

Samples was taken from 3, 4 and 3 selected ''PA''of Dangur, Manudra and Debate districts a respectively. From one peasant association 38-60 serum samples were collected. The required sample size in 10 PA (study site) was found to be 384 samples from the study district. This number was inflated to 452 samples for the effect of randomness and representativeness. The sample size was determined

by using 95% level of confidence interval & expected prevalence of 50% PPR disease with desired absolute precision of 5% & simple random sampling method will be used (Thrusfield, 2005).

$N= (1.96)^2 Pexp (1-Pexp)/ d^2$

= 384

Where,

N= required sample size for one strata

P exp= expected prevalence (in this case 50%)

d= desired absolute precision (in this case 5%)

Therefore; $1.96^2 x0.5 (1-0.5)/ (0.05)^2$= 384 sheep and goats

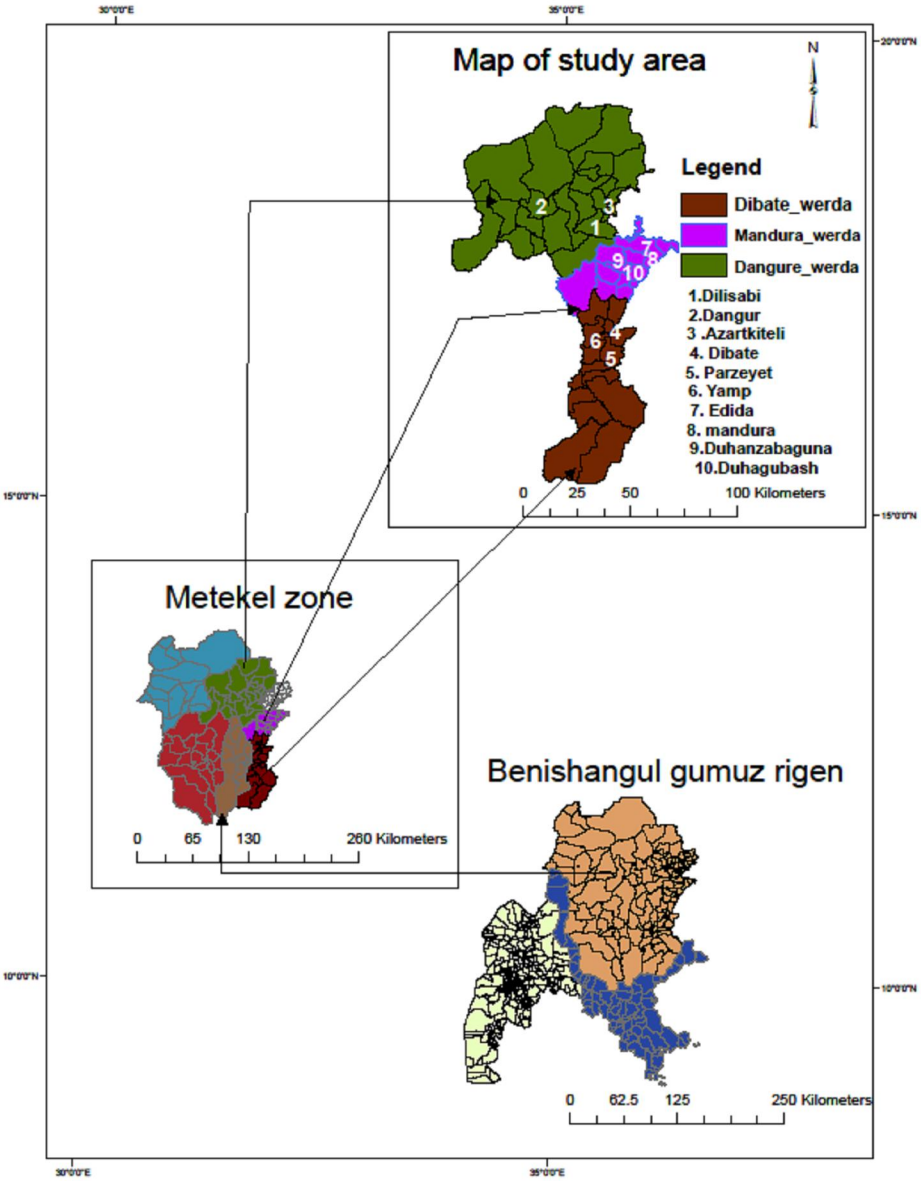


Fig. 1 Map of study area

3. Laboratory methods

3.1. Sample Collection

Blood samples were collected from the jugular-vein aseptically using disposable needles and vacutainer tubes. For collection of serum sample 5ml of blood from jugular-vein of shoat were collected using plan vacutainer tube and put at room temperature for about 24h in slant position after twenty four hour the serum was harvested using cryovials were kept on ice box and transported directly to Assosa Regional Veterinary Laboratory. The serum samples were stored at -20^0C until testing.

3.2 Laboratory Diagnosis of PPR

All collected serum samples were analyzed for the presence of PPR antibodies using an approved competitive ELISA kit (CIRAD EMVT, Montpellier,

France). The test was performed according to the instruction of the manufacturer. Positive and negative controls were provided with the kit. Surveys for antibodies are very useful to determine the presence or absence of infection and its extent in a population. Competitive ELISA has now largely replaced the virus neutralization test (Murphy *et al*., 1999). PPRV antibody was detected by PPR C-ELISA kit obtained from (CIRAD EMVT, Montpellier, France) the competition percentage (S/N %) was calculated for each sample and then grouped as positive (S/N% ≤ 50%), negative (S/N% > 60%), or doubtful (50% <S/N% ≤ 60%). The lateral flow device (LFD) based test for PPRV was manufactured by the (Pirbright, Institute UK, Bach no.291113) were used monoclonal antibody C77 recognizing the H protein of PPRV (Anderson *et al*., 1990; Anderson and Mckay, 1994).

3.3 Data management and statistical analysis

All collected data was entered in to the Microsoft Excel Sheet Data Management and Analysis Window® 2007 and then it was analyzed using STATA version 11 soft –ware was used. Chi-square test would be used to compare the prevalence of the PPR disease in different variables & to determine the relationship between the categorical variables & the result. The prevalence of PPR infections calculated as the number of PPR positive animal would be examined by confirmatory test (c-ELISA **)** method to the total population at risk (Thrusfield, 2005).

4. Result

4.1. Prevalence of Peste des petits ruminants using c-ELISA

Table 1. Sero-prevalence of PPR disease in the three district of Metekel zone of Benshangule Gumuz Regional State (Oct-Nov 2017)

S.N	Study area	Number examined	Number of positive (%)	Number of Negative (%)	χ^2	(p-value)
1	Mandura	197	142(72.08%)	55(27.91%)		
2	Dangur	131	96(73.28%)	35(26.71%)	1.27	0.86
3	Debate	124	94(75.80%)	30(24.19%)		
Total		452	332(73.45%)	120(26.54%)		

Table 2. Sero -prevalence of PPR disease in shoat by age, species, sex, body condition and vaccination status analysis for the association between PPR and individual animal risk factor using chi- square test (Oct-Nov 2017)

S.N	Risk factors	Categories	Number of animal examined	Number of positive in%	χ^2	p-value)
1	Age	<1year	122	92 (75.40%)		
		1-2year	234	175(74.78%)	5.4	0.24
		>3year	96	65 (67.70%)		
2	Species	Ovine	153	100(65.35%)	9.17	0.01
		Caprine	299	232(77.59%)		
3	Sex	Male	70	47(67.14%)	4.09	0.13
		Female	382	285(74.60%)		
4	Body ondition	Good	36	8 (22.22%)		
		Medium	59	25 (42.37%)	103.73	0.000
		Poor	357	299 (83.75)		
5	Vaccination status	Yes	92	32 (35.16%)	86.74	0.000
		No	360	299(83.05%)		

Table 3. Sero –prevalenc of PPR disease in shoat at different selected kebele (PA) of study area (Oct-Nov 2017)

S.N	Site (PA)	Number of animal examined	Number of Positive in (%)	χ^2	(p-value)
1	Edida	46	43(93.47%)		
2	Mandur 02 kebele	40	25(62.5%)		
3	Duhanzabaguna	60	36(60%)		
4	Duhagubash	50	37(74%)		
5	Dangur 01 kebele	46	38(82.60%)		
6	Delsambi	48	36(75%)		
7	Azartkiteli	38	23(60.52%)		
8	Yamp	42	35(83.33)	31.66	0.24
9	Debate 01 kebele	43	31(70.09%)		
10	Parzite	39	28(71.79%)		
	Total	452	332(73.45%)		

The overall sero-prevalence of PPR in the study area was 73.45 %. The highest PPR sero-prevalence (75.80%) was observed in Debate district while the lowest sero-prevalence (72.08%) was recorded in Mandura district of Metekel Zone. There is no statistically significant variation ($\chi 2 = 1.27$, $p > 0.05$) in PPR sero-prevalence among the three districts.

The sero-prevalence of PPR in different age group, also show that it was higher in kids/lambs (75.40%) compared to adult (67.70%). In this study sero-prevalence of PPR in caprine show that it was higher (77.59%) as compared to ovine (65.35%). Similarly female (74.60%) were higher than males (67.14%) as indicated in (Table. 2).

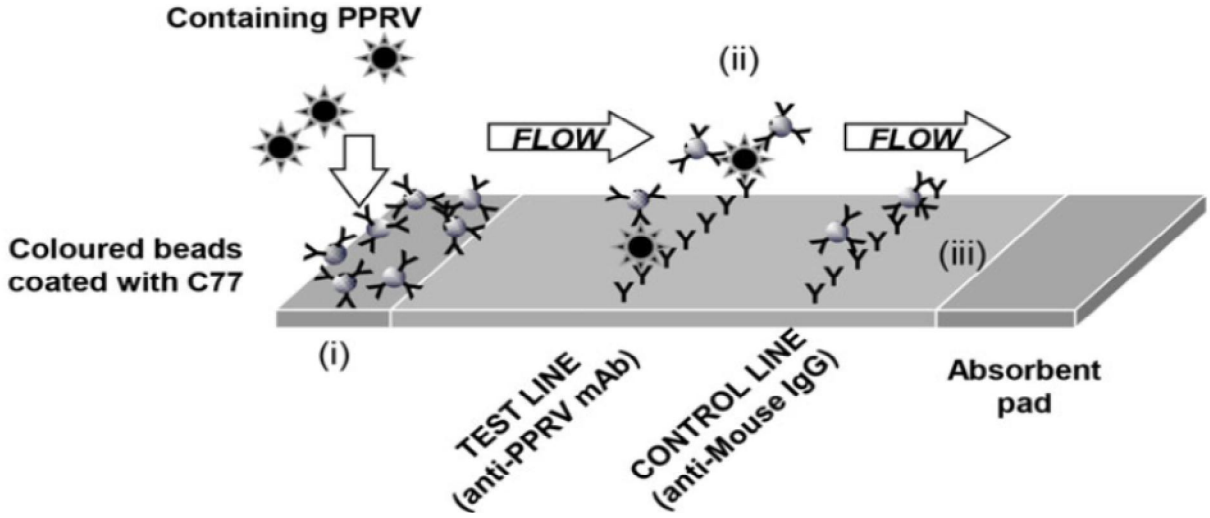
5.2. Apen-side test for the rapid diagnostic of PPRV at field

The pen-side test proved to be a useful technology for the rapid diagnosis of PPR. It helped reduce losses by employing early control measure to contain the infection. The lateral flow device (LFD) based test for PPRV was manufactured by the (Pirbright, Institute UK, Bach no.291113) were used monoclonal antibody C77 recognizing the H protein of PPRV (Anderson *et al*., 1990; Anderson and Mckay, 1994). By loading 4 drop of prepared sample on to a pad in sample window.

During contact with the sample diluents, the freeze-dried Mab - labeled microsphere, already present in the pad, were re-hydrated and moved by capillary action along the nitrocellulose strip, placed bellow the pad, towards the immobilized band of trapping antibody fig.2(A). Any PPR virus antigen in the sample was bound to the antibody on the microsphere and the whole complex was then captured by the immobilized band of antibody (specific against PPR virus) on the nitrocellulose membrane.

This result in the accumulation of the dyed microsphere which gave rise to a red line in the test window, indicating positive result. Excess Mab-labeled microspheres continued migrating along the membrane until reached the band of immobilized rabbit anti-mouse antibody and resulted in second red line in the control window fig. 2 (B). This internal control demonstrated that Mab-labeled microsphere had migrated along the length of the membrane.

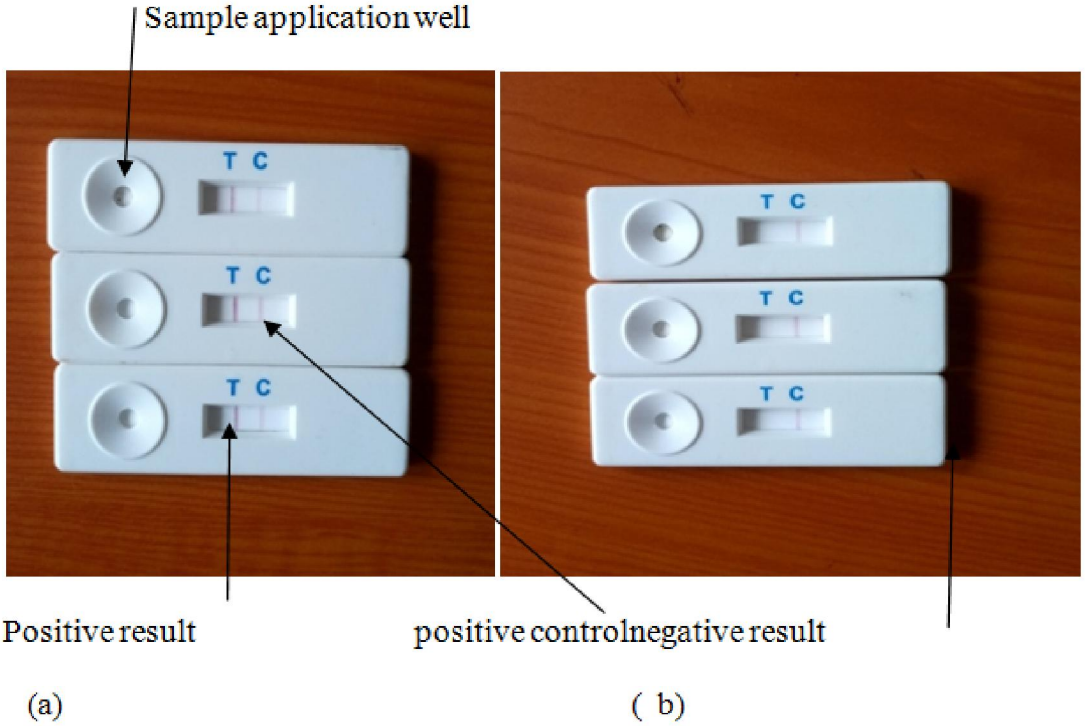
(A)



In the current study with suspected clinical sign of PPR observed in the field 8 swab sample were collected from goat and tested by LFD. Among the 8 animal tested five of them are positive to PPRV 62.5 % (5/8) this result indicated that the virus was circulating in the study area actively.

(B)

Fig2. Lateral Flow Device (LFD) based assay. (A) Basic operation of the pen-side test assay. Sample is added to the test port where it mixes with the beads in the reagent pad (i) virus antigen in the sample binds to bead there (ii) virus antigen bound to the bead will be immobilized on the test line, thereby immobilizing some of the beads and crating positive signal. Remaining beads are carried further along the strip until they come to the control line, where they are bound by anti-mouth IgG antibody.



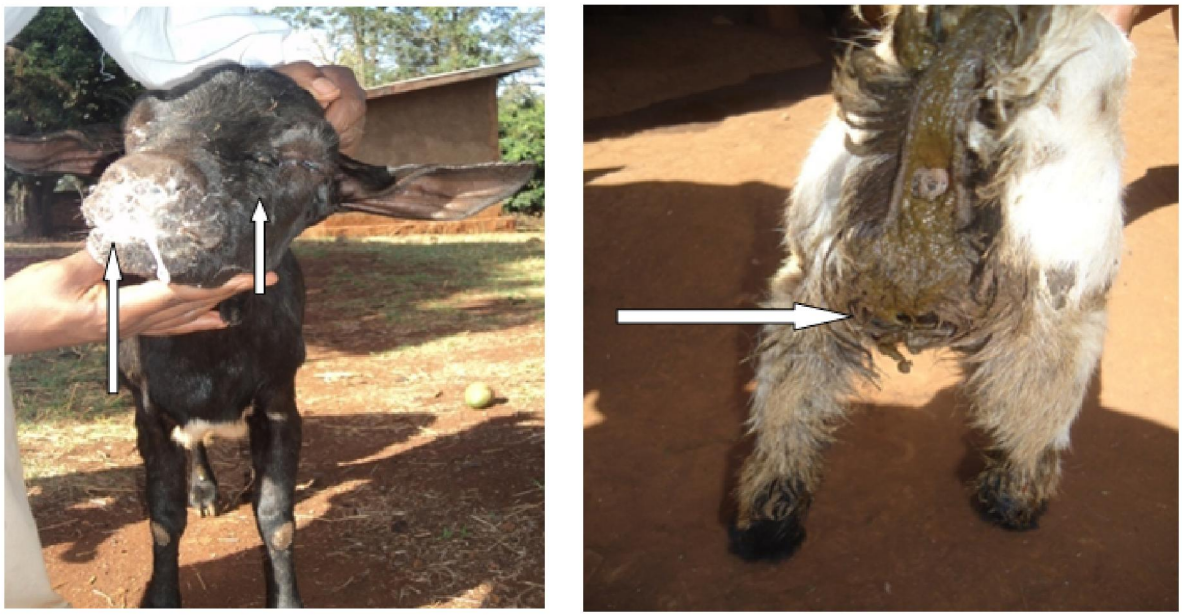


Fig 3. Typical Clinical sign in the field observation. (a) a clear nasal discharge that eventually became grey and sticky exudates with severe inflammation of the mucous membrane of the nose, causing respiratory distress, congestion of conjunctiva with the matted eyelids is the characteristic sign of PPR (b) profuse diarrhea, watery, fetid and/ or blood-stained.

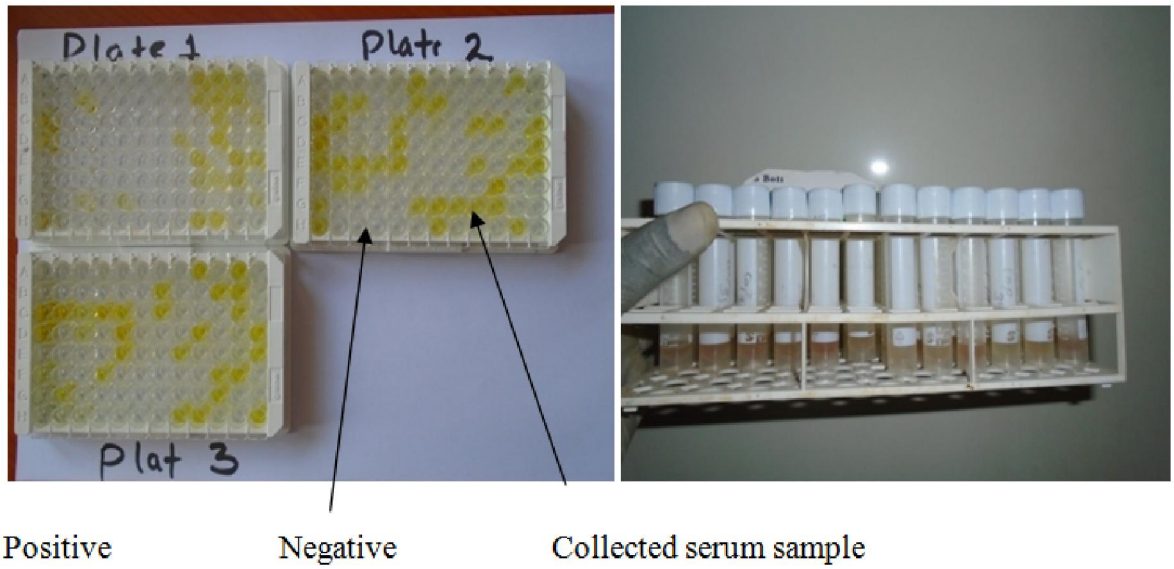


Fig 4. Competitive-ELISA microplate showing positive and negative reaction after stop solution

5. Discussion

The current study revealed that the overall sero-prevalence of antibodies against PPRV in shoats 452 serum samples collected from the three district of Metekel zone was found to be (73.45%). This finding indicated that the disease was more important which needs a particular attention in the region as it was the most economically important disease affecting both productivity and production.

The overall sero-prevalence in this study was found to be lower than the sero-prevalence (93.8%) reported from different country (Banyard *et al.,* 2010). The current study showed that the overall prevalence (73.45%) of PPR in the three district of Benishangul Gumuz Regional State of Metekel zone was somewhat similar with previous studies with over all prevalence (70.2%) reported by (Shuaib, 2011). Whereas, compared to other previous studies which is higher the prevalence of reported of (52.5%) from Somalia region Ethiopia (Waret-Szkuta *et al*., 2008).

Moreover, the result of current study is also higher than the finding of (57.6%) in Uganda (Mulindwa *et al.,* 2011), (55%) in Nigeria (EL-Yoguda *et al.,*2013), (63.40%) in Egypt (Abd EI-Rahim *et al.,*2010), (55.95%) in Saudi Arabia (Elshemey *et al.,* 2011), (61.8%) in Sudan (Abdella *et al.,* 2012). From the above finding it can be speculated that the prevalence of PPR was significantly varied among the country due to geographical variation, different animal production and husbandry system practices in each area. Difference in the size of sample tested in each study could also result in the noticed variation.

This finding is agreed with the result reported by (Shuaib, 2011, and Muse *et al*., 2012). The plausible explanation for the higher sero-prevalence found in this study could be well organized vaccination campaign are not practiced to control the disease. This finding is agreed with the report of (Intisar *et al.,* 2009). On the other hand some owners and herders do not have the desire to vaccinate their shoat because they think that vaccination causes the disease itself, rather than protecting their shoats against it. Lack of quarantine for infected animal and free movement of shoat, grazing and sharing of water sources are all factors that can play a significant great role in spreading of PPRV, facilitating its transmission among population of small ruminants in to new uninfected areas.

A statistically significant difference between the sero-prevalence estimated from the three investigated district in this study. The sero- prevalence (75.80%), (73.28%) and (72.08%) was in Debate, Dangur and Mandura district respectively. This finding was agreed with (Shuaib, 2011), reported sero- prevalence of (74.5%) in North Kordofan.

In the present study also revealed the higher prevalence of (74.60%) in female shoat than male (67.14%) agreed with previous report of (EL-Yoguda *et al.,* 2013, Afera *et al*., 2014, Nizamani *et al.,*2015, Bello *et al.,* 2016). In agreed to this finding, sex with sero prevalence of PPR also reported higher in male than female (Thakor *et al.,* 2016). The higher prevalence in female than male in current study may be due to physiological difference where female reveal some degree of predominance infection as a result of production and reproduction stress which female more prone to infection (Megersa *et al.,* 2011).

The age wise sero prevalence was (75.40%), (74.78%) and (67.70%) in less than 1 year, 1-2 year

and greater than 3 year of age group, respectively. This finding was agreed with the report of (Mahajam *et al*., 2013 and Afera *et al*., 2014). However (EL-Yogudaet *al.*,2013 and Rahman *et al*., 2017) have been reported higher sero prevalence in in adult than young shoats. The higher prevalence less than 1year may due to the poor immunity and poor nutrition as responsible factor for the disease prevalence and sub-clinical load of parasitic infection which causes immune-suppressive effect of *E.coli* infection causes fimbrial adhesion with intestinal mucosa which enhance effect of PPR virus (Kumar *et al.,* 2001).

In this study sero prevalence of PPR in caprine show that it was higher (77.59%) as compared to ovine (65.35%) which is agreement with another study done by (Gelagay,1996, Ozkul *et al*.,2002, Al-Majali *et al*., 2008, Waret-Szkuta *et al*., 2008) reported a higher sero-prevalence in goats than in sheep linked to higher fecundity in goats compared to sheep. It was suggested that new born kids accounts for a large proportion of the goat flock each year, which increases the size of susceptible population.

The current study showed the number of vaccinated sheep and goat (shoat) is very small with number of positive 32/92(34.78%) and non-vaccinated animal with the number positive 299/360 (83.05%). It is obvious this low number of vaccinated shoat against PPR in the study area will not lead to effective containment and control of PPR due to the fact that the region has a number of susceptibility host shoat. The owner and the harder, their un awareness of the benefit of vaccination would be probable explanation why only very small number of animals vaccinated.

6. Conclusion And Recommendations

The findings of this study confirmed that the circulation of PPR virus among populations of sheep and goats (Shoats) in the study areas and prevalence in actual outbreaks situation, which should be kept in mind while deciding the vaccination strategy for the control of the disease. The overall sero-prevalence of PPR in shoats in the selected districts of Dangur, Debate and Mandura was 73.45% while the flock level prevalence was 100%. A flock with at least one positive animal was considered a positive flock for PPR. This shows the transmissibility of the virus within herds is very fast when compared between herds. The fact that antibodies of PPR virus were detected in the whole peasant associations and districts suggests the endemicity of the disease in the studied districts. Because of the economic impact, morbidity and mortality increament, attention was given towards the disease regionally as well as nationally through time. Disease outbreak reporting needs awareness, harmonization, and network of all partners (region, district and field professionals) to mitigate the potential risk factors.

Therefore, based on the above conclusion the following recommendations are forwarded:

- It is necessary to plan out strategic vaccination not only in the studied district but also in the regions with a history of recurrent disease outbreaks in order to prevent the circulation of the virus.
- It needs harmonization in the control and eradication of the disease between the study districts and the neighboring countries specially Sudan where there is active movement of livestock across the border.
- In addition, strict sero surveillance and monitoring of PPR is recommended, together with uninterrupted vaccination of migratory flocks at the borders between districts or provinces or regions, for effective control of the disease.
- Further research should be undertaken on the development of differentiating infection from vaccinated animal's vaccine which is the most important measure for prevention and also identify the gene sequences and lineage of the PPR virus isolated in this study area.

Acknowledgement

The author is very much indebted to the Benishagul Gumuz regional state, Regional Veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory for funding the study and also grateful to the staffs for their unlimited support during the study**.**

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9/25/2018