



International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 2 Number 6 (June-2014) pp. 197-205

www.ijcrar.com



Molecular detection and differentiation of Peste des Petits Ruminant virus and Rinderpest virus in sheep and goats with PPR-like symptoms in Dangme West District of Ghana

M.Ayim-Akonor^{1*}, F.Y.Obese², C.Arthur¹, D.D.Owusu-Ntumy¹ and H.R.Otsyina³

¹Council for Scientific and Industrial Research-Animal Research Institute, Accra, Ghana

²Department of Animal Science, University of Ghana, Legon, Accra, Ghana

³School of Veterinary Medicine, University of Ghana, Legon, Accra, Ghana

*Corresponding author

KEYWORDS

Ghana,
Goats,
Peste des Petits
Ruminant Virus,
Rinderpest Virus,
Sheep.

A B S T R A C T

Peste des Petits Ruminant Virus (PPRV) and Rinderpest Virus (RPV) cause diseases clinically indistinguishable in small ruminants. Laboratory diagnosis is needed to identify the specific agent(s) during a disease state particularly in areas where both viruses have co-existed in recent past. Here we explored the use of the PCR technique in the rapid diagnosis and differentiation of PPRV and RPV in small ruminants in Ghana. Mucosae swabs were collected from sheep and goats exhibiting symptoms typical of PPR in the Dangme West District of Ghana. RNA was extracted from all samples and tested for PPRV and RPV in a single tube one step-RT-PCR using gene specific primer NP3/NP4 for PPRV and B2/B12 for RPV. Amplicons were resolved on a 1% ethidium bromide stained agarose gel and visualized with a UV transilluminator. One hundred and eighteen (118) samples were collected and analyzed during a 12 month period. Goats were the most affected making 83.1% (98) of the samples and sheep 16.9% (20). All the samples (100%) tested negative to RPV. 112 (94.9%) of samples tested positive to PPRV and 6 (5.1%) tested negative to both viruses. None of the samples tested positive to both viruses. The prevalence of PPR in sheep and goat was found to be 15.3% and 79.7% respectively. The overall prevalence of PPR and Rinderpest was 95% and 0% respectively. Small ruminants in the Dangme West District showing symptoms typical of PPR are only infected with PPRV and not RPV or a mixture of both viruses. There is the need to develop a suitable control strategy to protect small ruminants against PPR in the District and thereby increase the productivity of these ruminants.

Introduction

Peste des Petits Ruminants (PPR) and Rinderpest (RP) have been important viral diseases of ruminants in developing

countries for many decades. The Rinderpest virus (RPV) has been reported to occur in the Middle East, Africa, and Asia causing

huge economic losses to large ruminants especially cattle and buffaloes (Couacy-Hymann et al., 2002; Banayard et al., 2006). The transfer and migration of unvaccinated large ruminants across borders facilitates spread to neighbouring countries (Forsyth and Barrett, 1995). In cattle and buffaloes, clinical manifestation of Rinderpest varies from per-acute, through acute to mild forms with varying symptoms including poor appetite, depression, high fever, keratitis, cataract, mucosal congestion and hemorrhagic diarrhoea. Mortality rates are very high, approaching 100% in naive populations (African Union - Interafrican Bureau for Animal Resources, 2011).

First described in the 1940s in La Cote d'Ivoire, PPR predominantly affects sheep and goats (Gargadennee and Lalanne, 1942). Geographic distribution of PPR appears to overlap with that of Rinderpest (Abraham et al., 2005; Bailey et al., 2005). Affected animals show symptoms of severe pyrexia, nasal and ocular discharges, pneumonia, ulceration of mucous membrane and inflammation of the gastrointestinal tract leading to severe diarrhoea (Gibbs et al., 1979; Lefevre and Diallo, 1990). Morbidity and mortality rates vary but can reach 100% and 90% respectively (Farooq et al., 2008).

PPR continues to remain a major challenge to the development of sheep and goats in sub-saharan Africa. In most of these areas, major control programmes have not yet been put in place to control the disease (Couacy-Hymann et al., 2009). Although Peste des Petits Ruminant Virus (PPRV) predominantly affect small ruminants, infections of large ruminants have been reported in many parts of the world. In India, Banayard and his co-workers (2006) reported of buffaloes dying of PPRV

infection. In West Africa, Anderson and McKay (1994) reported 25% and 96% prevalence of PPRV antibodies in cattle sera collected from Nigeria and Ghana respectively and 10% and 16% in camels and cattle respectively in Ethiopia (Abraham et al., 2005). Balamurugan et al., (2012) recently reported a 4.58% prevalence of PPRV antibody in cattle and buffaloes in Southern peninsular of India.

Although Rinderpest virus (RPV) predominantly affect large ruminants, infections of small ruminants with RPV have also been reported in Africa and India (Taylor 1986; Anderson et al., 1990). Large ruminants infected with PPRV remain asymptomatic, but small ruminants infected with RPV may be asymptomatic or symptomatic and can transmit the infection to cattle causing a more serious disease (Anderson et al., 1991; Couacy-Hymann et al., 1995). In cases of symptomatic infection of small ruminants with RPV, the symptoms exhibited are clinically indistinguishable from those of PPRV (Couacy-Hymann et al., 1995). Laboratory diagnosis is therefore needed to identify the etiological agent involved during a disease state particularly in areas where both viruses have co-existed in the recent past.

Small ruminants in Ghana are very important to the livelihood of farmers especially the rural poor. Goats and sheep serve as a ready source of income to the farmer. They are also a source of meat and milk and play significant roles in socio-cultural activities such as funerals, dowries (Tuah, 1990). However, PPR remains a major constraint to the productivity of these ruminants resulting in high morbidity and mortality.

Global eradication of Rinderpest has recently been achieved after years of rigorous eradication programmes (African

Union □ Interafrican Bureau for Animal Resources, 2011). Like in many other developing countries in sub-Saharan Africa small ruminants in Ghana were not enrolled in a similar rigorous PPR eradication programme during the global Rinderpest eradication. Diagnosis of PPR in the country is mainly based on clinical symptoms of host species, which is not definitive in many cases as clinical symptoms of PPRV and RPV are indistinguishable in small ruminants. Also the use of laboratory confirmation and differentiation methods such as the Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) technique, a robust, sensitive and specific diagnostic tool developed for the rapid diagnosis and differentiation of PPRV and RPV is very much limited in Ghana. In this study we explored the use of RT-PCR technique in the direct detection and differentiation of PPRV and RPV in sheep and goats showing PPR-like symptoms. This should enable the development of suitable control strategies to increase small ruminant productivity.

Materials and method

Animals

The study was conducted in the Dangme West District of the Greater Accra Region in Ghana from December 2009 to December, 2010. Small holder ruminant farmers in eight (8) communities within the district were used in the study. The communities were frequently visited by the team during the study period and the animals inspected for PPR-like symptoms. Farmers were also encouraged to voluntarily report suspected cases of PPR to the team based on the clinical symptoms they are familiar with. Sheep and/or goats showing PPR-like symptoms were selected

for sampling. Not more than five (5) animals were considered for sampling in a particular herd.

Sample collection

A sterile cotton swab was used to firmly swab the nasal mucosae of the animals using one cotton swab per animal. Swab was immediately put in a labeled 2ml microfuge tube containing 700µl of 0.1M Phosphate Buffer Saline (PBS) (pH 7.4-7.6). The swab stick was cut just at the level of the tube's edge and the tube firmly closed. Samples were immediately put in a cool box with ice packs and transported to the Molecular Biology Laboratory of the Council for Scientific and Industrial Research (CSIR) - Animal Research Institute (ARI) for processing.

RNA Extraction

The tube containing the swab was vigorously vortexed for about 20s 60 µl of the sample was collected with a 200µl barrier pipette tip into a pre-labeled 2 ml sterile microfuge tube. The RNeasy Mini Kit (Qiagen, Germany) was used to extract RNA from all the samples. The protocol adopted was that of tissue extraction specified by the. The manufacturer's instructions were followed. PBS (0.1M) was used as an extraction negative control and tissue obtained from goat confirmed previously to be infected with was used as positive control. All extracted RNA was stored at -20°C until needed.

Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR)

The Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) was performed in a 200 µl thin walled PCR tube in a final reaction volume of 50 µl. Each RNA

extract was tested for both PPRV and RPV in separate tubes. The protocol used was according to Couacy-Hymann et al., (2002) with some slight modifications. The Qiagen OneStep RT-PCR kit was used. Briefly into each tube, the following reagents were added: 2 µl of dNTP mix (10 mM of each dNTP), 10µl of 5X OneStep RT-PCR buffer, 2.5 µl (10 pmoles/µl) of each forward and reverse primer: NP3/NP4 for PPRV detection or B2/B12 for RPV detection (Invitrogen), 2 µl of 1-step RT-PCR enzyme mix and 26 µl of RNase-free water. 5 µl of extracted RNA was added. Pipetting of all reagents was done on ice. The GeneAmp PCR system 9700 from Applied Biosystems was used for the amplification reaction. The following cycling conditions were used: reverse transcription at 50°C for 30 mins and initial PCR activation step at 95°C for 15 mins. This was followed by 35 cycles of 94°C for 30s (Denaturation), 55°C for 30s (Annealing), 72°C for 1 min (Extension) and a final extension at 72°C for 10 mins. A PCR positive control and negative control (Nuclease free water) were added in each reaction run.

Agarose Gel Electrophoresis and Visualization of DNA amplicons

A 1% agarose gel was prepared in a conical flask by weighing 0.6g of agarose powder with a sensitive scale (Mettler Toledo, USA) and a disposable weighing boat and measuring 60 mls of 0.5X TBE buffer with a glass measuring cylinder. Agarose was melted in a microwave oven for a total of 4 mins, pulsing to swirl the flask intermittently. 5 µl of ethidium bromide (5µg/ml) was added to the melted agarose and swirled to mix well. The mixture was poured into the gel tray already fitted with gel combs. Agarose was allowed to solidify for about 30-40 mins after which combs

were removed. 0.5X TBE buffer was added to the gel tank to submerge the solidified agarose gel. 10 µl of 6X gel loading dye were added to the PCR product in each tube to make a final concentration of 1X. Pipette tips were changed in between samples. 5 µl of 100 bp ladder (1 µg/ml) was loaded into the first and last wells while the remaining wells were loaded (1 sample per well) with 15 µl of the sample containing the dye. Electrophoresis was carried out at 60V for 90 min.. DNA amplicons were visualized on a High Performance UV transilluminator (UVP, USA) and photographed with a Kodak digital camera.

Data Analysis

Data was analysed using simple descriptive statistical analysis of Microsoft Excel (2007). The prevalence of PPR and RP among flock was calculated using the formula:

$$\text{Prevalence} = \frac{\text{Number of positive samples detected}}{\text{Total number of samples analysed}} \times 100$$

Result and Discussion

A total of one hundred and eighteen (118) samples were collected from sheep and goats showing PPR-like symptoms and analyzed for both PPRV and RPV. Samples were obtained from animals in all the eight (8) communities visited. Ninety five percent (95%) of suspected animals were less than 24 months old. Out of 118 samples collected, 98 goats (83%) and 20 sheep (16.9%) were found to be infected (Figure 1).

The primer pairs NP3/NP4 and B2/B12 both targets the Nucleoprotein gene of PPRV and RPV respectively and have previously been published (Table 1). Of the 118 samples analyzed, 112, representing 94.9% tested positive for Peste des Petits

Ruminant Virus (PPRV) and six (5.1%) tested negative. All the 118 samples analyzed (100%), tested negative to RPV (Figure 3).

None of the samples (0%) tested positive to both PPRV and RPV. 112 samples were found to be positive for PPRV but negative for RPV. Six (5.1%) of the samples were found to be negative for both PPRV and

RPV (Figure 4). Of the 112 samples that tested positive to PPRV, 83.9% (94) were from goat and 16.1% (18) from sheep. The prevalence of PPR in goats in the district was approximately 80%. The prevalence of PPR in sheep in the district was determined to be 15.3%. The overall prevalence of PPR in small Ruminants in the Dangme West district was 95% (Table 2).

Table.1 Primer sequences used for identification and differentiation of PPRV and RPV by RT-PCR

Primer name	Primer Sequence	Reference
NP3	5' TCTCGGAAATCGCCTCACAGACTG 3'	Couacy-Hymann et al., 2002
NP4	5' CCTCCTCCTGGTCCTCCAGAATCT 3'	
B2	5'ATCCTTGTCGTTGTATGTTCTCGG 3'	Couacy-Hymann et al., 2006
B12	5'CAA GGG GGTGAGATCCAGCACAA 3'	

Table2: Prevalence of PPRV in small ruminants in Dangme West dsitric

Animal	Number tested	Number of negatives detected	Number of positives detected	Prevalence (%)
Goat	98 (83.1)	4 (66.7)	94 (83.9)	79.7
Sheep	20 (16.9)	2 (33.3)	18 (16.1)	15.3
Total	118 (100)	6 (100)	112 (100)	94.9

* Figures in parenthesis represent percentages.

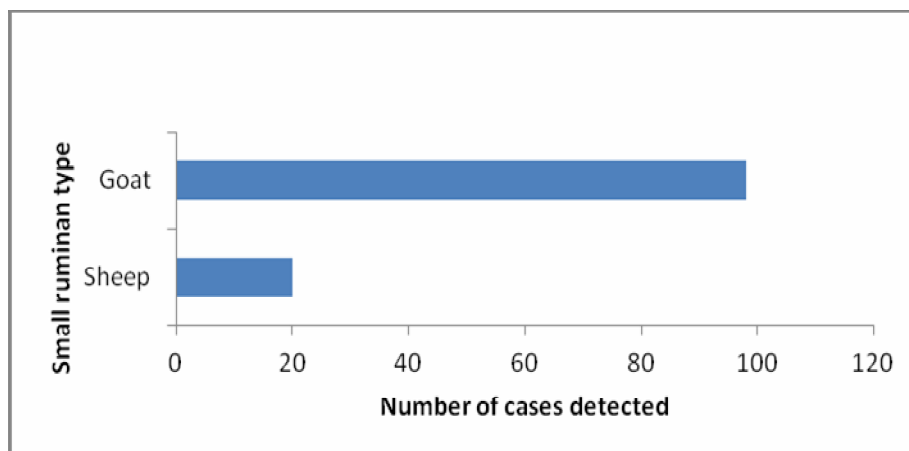


Figure.1 Number of small Ruminants showing PPR-like symptoms

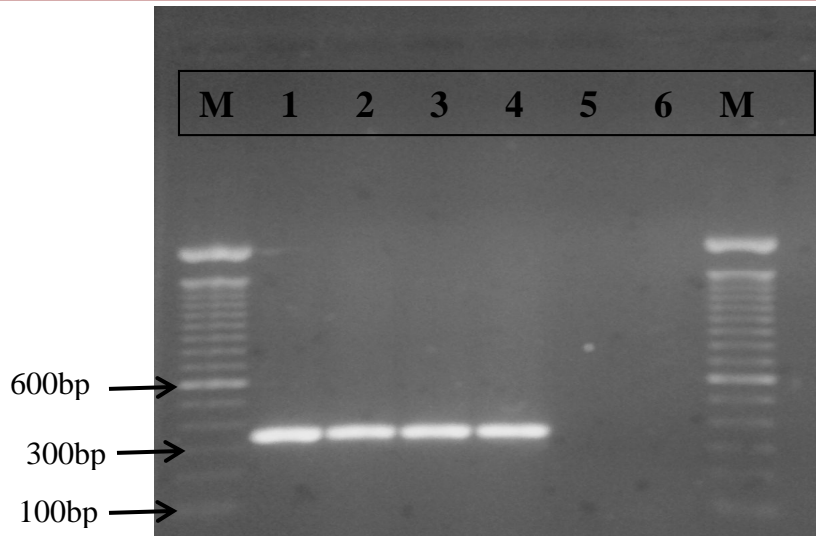


Figure.2 Resolved RT-PCR products of PPRV on 1% agarose gel stained with ethidium bromide. **Lanes:** M = 100bp Marker, 1 Extraction positive control, 2= PCR positive control, 5=Extraction negative control (PBS), 6= PCR negative control (nuclease free H₂O), 3&4=Positive samples.

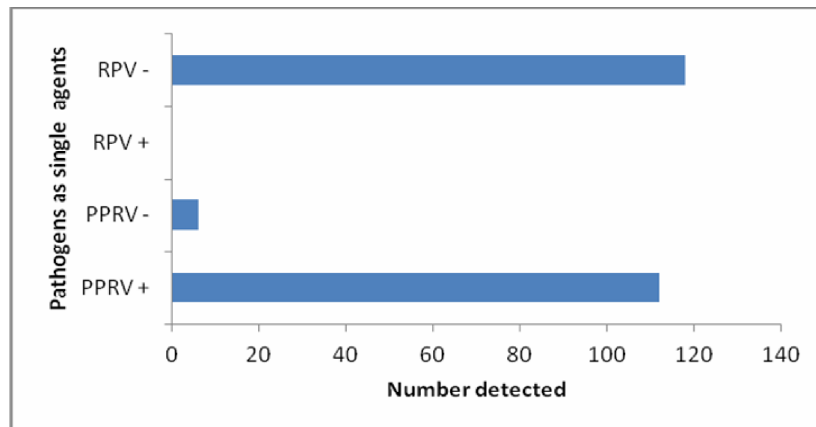


Figure.3 Viral distribution as single agent detected in the mucosae samples analyzed

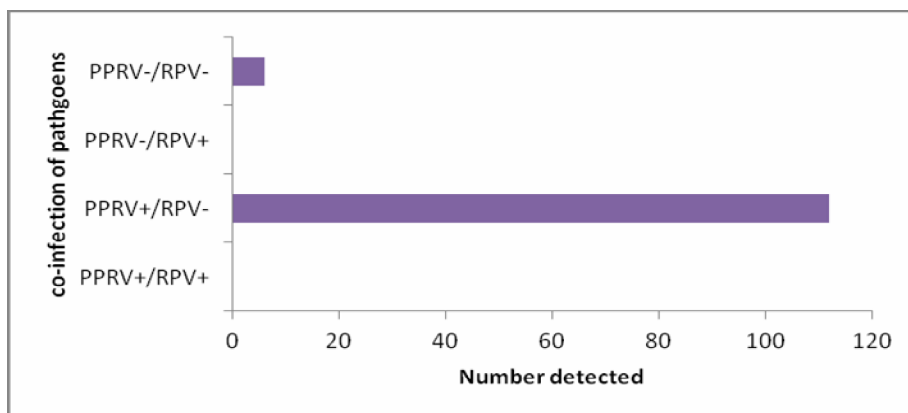


Figure.4 Viral distribution as co- agents detected in the mucosae samples analyzed

References

- Abraham, G., A. Sintayechu, G. Libeau, E. Albina, F. Roger, Y. Laekemariam, D. Abayneh and Awoke, K. M. 2005. Antibody seroprevalence against peste des pestits. ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. *Prev. Med.* 70:51-57.
- African Union □ Interafrican Bureau for Animal Resources (2011). The Eradication of Rinderpest from Africa A Great Milestone. Nairobi Kenya.P 1-103.
- Anderson, J and McKay, J. A. 1994. The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epi. and Infect.* 112: 225-231.
- Anderson, E. C., E. Hassan, T. Barrett and Anderson, J. 1990. Observations of the pathogenicity and transmissibility for sheep and goat of the strain of the virus isolated during the rinderpest outbreak in Sri Lanka in 1987. *Vet. Microb.* 21: 309-18.
- Anderson, J., M. Corteyn and G. Libeau. 2006. Diagnosis of Rinderpest virus and Peste des petits ruminants virus. In: T. Barrett, P. Pastord and W. P. Taylor (Eds.). pp. 15-6. Rinderpest and Peste des Petits Ruminants Virus Plague of Large and Small Ruminants (Biology of Animal Infection). Academic Press. U.K.
- Anderson, J., J. A. McKay and Butcher, R. N. 1991. The use of monoclonal antibodies in competitive ELISA for the detection of antibodies to rinderpest and peste des petits ruminants viruses. In: proceedings of the Final Research Coordination Meeting of the FAO/IAEA/SIDA/OAU/ IVAR/PARC ‘ The Sero-monitoring of Rinderpest throughout Africa; phase one’ Publisher IAEA-TECDOC 623. p. 43-53.
- Baah, J., A. K. Tuah, W. Addah and Tait, R.M. 2012. Small ruminant production characteristics in urban households in Ghana. *Lives. Res. Rural Dev.* 24(5).
- Bailey, D., A. Banyard, P. Dash, A. Ozkul and Barrett, T. 2005. Full genome sequence of Peste des petits ruminants virus, a member of the Morbillivirus genus. *Virus Res.* 110:119-124.
- Balamurugan, V., P. Krishnamoorthy, B. M. Veeregowda, A. Sen. K .K. Rajak, V. Bhanuprakash, M. R. Gajendragad and Prabhudas, K. 2012. Seroprevalence of Peste des petits ruminants in cattle and buffaloes from Southern Peninsular India. *Trop. Ani. Hth. Prod.* 44(2):301-6.
- Banayard, A. C., B. K. Rima and T. Barrett. 2006. The morbilliviruses. In: T. Barrett, P. Pastord and W.P. Taylor (Eds.). pp. 15-6. Rinderpest and Peste des Petits Ruminants Virus Plague of Large and Small Ruminants (Biology of Animal Infection). Academic Press. U. K.
- Barrett, T., C. Amarel-Doel, R. P. Kitching and Gusev, A.1993. Use of the polymerase chain reaction in differentiating rinderpest field virus and vaccine virus in the same animals. *Rev. Scienti. Tech. Off. Inter. Epi.* 12: 865-872.
- Brindha K., R. Dhinakar, G. P. I. Ganesan, V. Thiagarajan, A. M. Nainar and Nachimuthu, K. 2001. Comparison of virus isolation and PCR for diagnosis of PPR. *Act Virol.* 45:169-172.
- Couacy-Hymann, E., S. C. Bodjo, T. Danho, M. Y. Koffi and Akoua-Koffi, C. 2006. Diagnosis and surveillance of rinderpest using reverse transcription – PCR. *Afr. J. Biotech.* 5 (19):1717-172.
- Couacy-Hymann, E., F. Roger, C. Hurard, J. P. Guillou, G. Libeau and Diallo, A.

2002. Rapid and sensitive detection of peste des petits ruminants virus by a polymerase chain reaction assay, *J. Virol. Meths.* 100: 17–25.
- Couacy-Hymann, E., S. C. Bodjo, M. Y. Koffi, C. Kouakou and Danho, T. 2009. The early detection of Peste-des-Petits-Ruminants (PPR) virus antigens and nucleic acid from experimentally infected goats using RT-PCR and immunocapture ELISA techniques. *Res. Vet. Sci.* 87: 332–335.
- Couacy-Hymann, E., K. Bidjeh, A. Angba, J. Domenech and Diallo, A. 1995. Protection of goats against rinderpest by vaccination with attenuated peste des petits ruminants virus. *Res. Vet. Sci.*, 59(2):106-109.
- Diallo, A., 1990. Morbillivirus group: genome organization and proteins. *Vet. Microb.* 23: 156–163.
- Diallo, A., T. Barrett, M. Barbron, M. S. Shaila and Taylor, W. P. 1989. Differentiation of rinderpest and peste des petits ruminants viruses using specific cDNA clones. *J. Virol. Meths.* 23, 127–136.
- Farooq, U., Q. M. Khan and Barrett, T. 2008. Molecular based diagnosis of rinderpest and peste des petits ruminants virus in Pakistan. *Inter. J. Agric. Biol.* 10:93-96.
- Forsyth, M. A., and Barrett, T. 1995. Evaluation of polymerase chain reaction for the detection and characterization of rinderpest and peste des petits ruminants viruses for epidemiological studies. *Virus Res.* 39: 151–63.
- Gargadennée, L., and Lalanne, A. 1942. La peste des petits ruminants. *Bull. Serv. Zoot. Epiz. Afriq. Occid. Fran.* 5: 16-21.
- Gibbs, E. P. J., W. P. Taylor, M. P. J. Lawman and Bryant, J. 1979. Classification of peste des petits ruminants virus as a fourth member of the genus Morbillivirus. *Inter. Virol.* 11: 268–274.
- Lefevre, P. C., and Diallo, A. 1990. Peste des petits ruminants virus. *Rev. Sci. Tech. Offi. Int. Epiz.* 9:951-965.
- Libeau, G and P. Bonnet. 2012. Effective control methods against against peste des petits ruminants. CIRAD. www.cirad.fr/en/content/download/6458/.../FS9-LibEAU-ppr-ENG.pdf. 23/01/14
- Libeau, G., A. Diallo, F. Colas and Guerre, L. 1994. Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. *Vet. Rec.* 19:300-304.
- Libeau, G., A. Diallo, R. Lancelot, F. Colas and Guerre, L. 1995. Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant N protein. *Res. Vet. Sci.* 58:50–55.
- Murphy, F. A., E. P. J. Gibbs, M. C. Horzinek and Studdert M. J.. 1999. Classification and nomenclature of viruses. In: EDITORS pp. 411-428. *Veterinary Virology*. Academic Press. San Diego, Calif.
- Pandey, K. D., M. D. Baron and Barrett, T. 1992. Differential diagnosis of rinderpest and PPR using biotinylated cDNA probes. *Vet. Rec.* 131: 199-200.
- Saliki, J. T., C. C. Brown, J. A. House and Dubovi, E.J. 1994. Differential immuno-histological staining of peste des petits ruminants and rinderpest antigens in formalin-fixed, paraffin embedded tissues using monoclonal and polyclonal antibodies. *J. Vet. Diag. Invest.* 6: 96–8.
- Saliki, J. T., G. Libeau, J. A. House, C. A. Mebus and Dubovi, E.J. 1993. Monoclonal antibody-based blocking enzyme-linked immunosorbent assay for specific detection and titration of

- peste des petits ruminants virus antibody in caprine and ovine sera. *J. Clin. Microb.* 31:1075–1082.
- Shaila, M. S., D. Shamaki, M. Forysth, A. Diallo, L. Goatley, P. Kitching and Barrett T. 1996. Geographic distribution and epidemiology of peste des petits ruminants viruses. *Virus Res.*, 43: 149–53.
- Subir, S., and Hemayeatul I. Md. 2011. Prevalence and Risk Factor Assessment of Peste des petits ruminants in Goats in Rajshahi, Bangladesh. *Vet. Wld.* 4(12):546-549.
- Taylor, W.P., 1986. Epidemiology and control of rinderpest. *Rev. Sci. Tech.*, Off. Int. Epiz. 5: 407- 410.
- Taylor, W. P., S. Abusaidy and Barret, T. 1990. The epidemiology of PPR in the sultanate of Oman. *Vet. Micro.* 22: 341-352.
- Taylor, W. P., and Abegunde, A. 1979. The isolation of peste des petits ruminants virus from Nigerian sheep and goats. *Res. Vet. Sci.* 26: 94-96.
- Tuah, A. K. 1990. Utilization of agricultural by product from village and commercial production in sheep rations in Ghana. In: proceedings of the first joint PANESA/ARNAB workshop ‘utilization of research results on forage and agricultural by-products materials as animal feed resources in Africa’. Publisher ILCA, Lillongwe, Malawi. Pg. 57-69.
- Wilson, T. R .1991. Small ruminant production and the small ruminant genetic resource in Tropical Africa. *FAO Animal Production and Health Paper*, No.88.