

Research Article

Peste Des Petits Ruminants - A Permanent Burden on the Development of Small Ruminant in Ivory-Coast

Couacy-Hymann E^{1*}, Koffi YM¹, Godji P¹, Kouassi LA¹, Kouakou KC¹, Krou AH¹ and Nana P¹

¹LANADA - Central Laboratory for Animal Diseases - Virology Laboratory, Ivory-Coast

*Corresponding author: Couacy-Hymann E, LANADA - Central Laboratory for Animal Diseases, Virology Laboratory, BP 206 Bingerville, Ivory-Coast

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Abstract

PPR is a major constraint of the development of small ruminants in areas where it is endemic such as Ivory-Coast.

On the period 2009-2012, we conducted a PPR survey in four selected regions (Agboville, Bongouanou, Dabou and Yamoussokro). Based on reports from the field veterinary technicians we investigated fourteen outbreaks of PPR causing estimated mortality cases of 1300 goat's and 450 sheep. Appropriate tissue and swabs samples were collected along with 746 randomly collected serum samples. Using the N-cELISA technique, an overall seroprevalence of 35.6% (266/746) was found ranging from 12.9% in Yamoussokro region to 56.7% in Bongouanou region with a yearly seroprevalence varying from 25.2% in 2012 to 40.7% in 2010. All the tissue and swabs samples were positive by RT-PCR and the PPR virus was isolated from eight tissue samples on the monkey CV1 cell line expressing sheep-goat SLAM protein. These strains fell into PPRV lineage II.

Keywords: PPR; RT-PCR; ELISA; Disease surveillance; Small ruminant; Ivory-Coast

Introduction

Peste Des Petits Ruminants (PPR) is a serious and contagious plague of small ruminants, mostly sheep and goats, in many developing countries in Africa, near and Middle-East and southern Asia [1]. Within Africa, PPR has now extended to southwards in Tanzania, Democratic Republic of Congo and Angola [2,3]. Outbreaks of PPR have been also reported across North Africa including Algeria, Morocco, Tunisia [4-6] along with the European part of Turkey [7]. In southwest Asia, China has reported PPR spread all over the country starting during year 2007 in Tibet region [8]. The current spread of PPR over large geographically areas can be due to the eradication of RPV from the world. Animal of all ages are susceptible and the transmission route remains oral and respiratory secretions following close contact between infected and naive population.

The disease is highly contagious and case fatality rates in some outbreaks can approach 90% in susceptible populations and, as a consequence of the effects of epidemics, the local and rural economies of the affected countries can be devastating [9,10]. Since small ruminants are a major resource for the rural populations then they are more seriously affected by epidemics of PPR. Nowadays there are efficient attenuated vaccines to be used to prevent this disease and to control its extension [11-13]. In addition, there are new diagnostic tools available after a quick report of any outbreak of PPR.

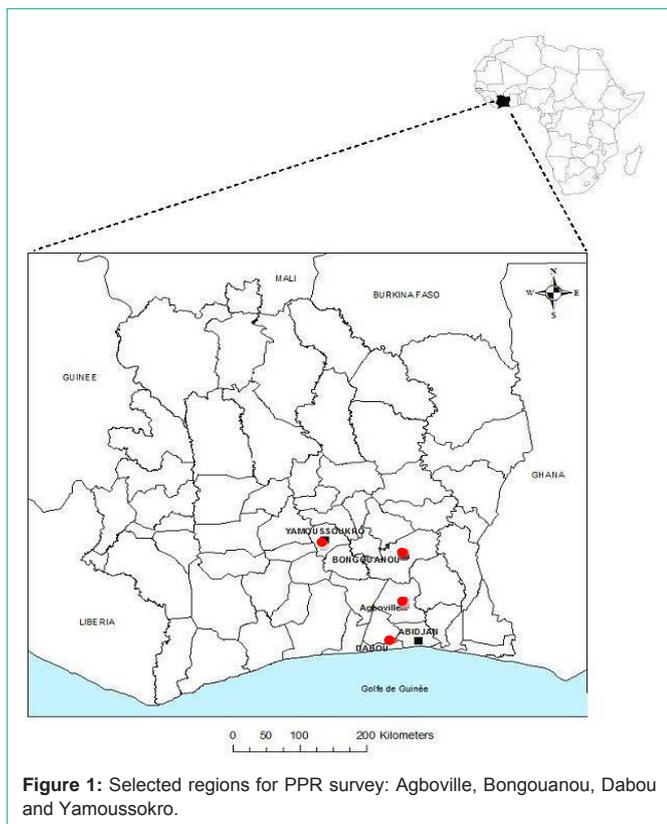
Depending on any predisposing factors and the virulence of the infecting virus, clinical manifestation for PPR can be seen in per-acute, acute, sub-acute and sub-clinical forms. However, PPR in sheep and goats is generally observed as an acute disease. The per-acute form of disease is often seen in kids infected at the age of 4 months and older during the time frame whereupon any pre-existing maternal antibody levels wane. This per-acute form of disease has a

short incubation period (2 days) with a rapid development of pyrexia with body temperature rising to 40-42°C. Depression, congestion of mucous membranes, oculo-nasal discharge, dyspnoea and profuse watery diarrhea lead to the death of infected animals within 4-5 days.

In the acute form of disease a 3-4 day incubation period precedes development of pyrexia and the onset of other clinical disease signs, including watery oculo-nasal discharge, congestion of the mucous membranes of the buccal cavity, conjunctiva of the eye and the vulva. A diarrhoeic phase follows, often resulting in the generation of bloody faecal matter leading to dehydration and ultimately death of the animal. As the disease progress, the watery oculo-nasal discharge may become mucopurulent and can occlude the nostrils, predisposing to dyspnoea.

In the sub-acute form of disease, the animals do not develop severe clinical disease and low mortality rates are seen. With this form of infection, the animals may develop temperatures ranging from 39-40°C, but do not develop the characteristic clinical signs normally associated with PPRV infection. Animals usually recover from the disease within 10-14 days. A sub-clinical form of disease is also seen in large ruminants (buffalo and cattle), where the infected animals are able to clear virus in the complete absence of clinical disease, but seroconvert to PPRV, often generating strong neutralizing antibody responses. Sheep and goat are the main susceptible host of PPRV.

The causative agent, Peste Des Petits Ruminants Virus (PPRV) is a negative-strand RNA virus with a monosegmented genome of length 15,948 and containing 6 genes encoding 6 structural proteins which are the Nucleocapsid protein (N), the Phosphoprotein (P), the Matrix protein (M), the Fusion protein (F), the Haemagglutinin protein (H) and the Polymerase protein (L). In addition, the P gene also encodes the three non-structural proteins V, W, C. It belongs



to family *Paramyxoviridae* and the genus *Morbillivirus* together with Rinderpest Virus (RPV), Measles Virus (MV), Canine Distemper Virus (CDV) and marine mammalian Morbilliviruses [14]. There are four lineages of PPRV based on the differentiation determined by the sequence comparison of a small region of the F gene [15] or the N gene [16,17]. However, it has been demonstrated recently that the N gene is more divergent therefore more suitable for phylogenetic distinction between closely related PPRV viruses [18]. Historically, African PPRV isolates were lineages I, II, III (with mainly West African countries harbouring lineages I and II and East African countries with lineage III) and the Middle-East and Asian isolates were lineage IV. Within a short time this distribution has deeply changed : in West Africa : lineage II is spreading with the trend to push out lineage I confined to Ivory-Coast, Conakry Guinea and Burkina-Faso with a co-circulation of lineage I. Moreover in 2009, lineage II was identified in Ivory-Coast [19] where probably the two lineages are co-circulating. Lineage IV has been found in Africa with an extension from East Africa (mainly Sudan, lineages III and IV) up to Nigeria where lineage II is still circulating [4,20] and to Southern part : Democratic Republic of Congo (DRC) and Angola (Diallo, personal communication). Northern African countries have Lineage IV too [20].

The present study aimed to investigate PPR outbreaks on time based on the early reports from field staff and early detection and confirmation from the laboratory that will help to trace the lineage strain in circulation within the country. It is also to make a differential diagnosis with other syndromic diseases such as pasteurellosis

Table 1: Tissue and Swabs samples collected from outbreak investigations on the period 2009 - 2012 from Agboville, Bongouanou and Dabou's regions.

Date of Sample Collection	Identification	Tissues	Species	Epi Unit	Regions
July 2009	CIV/09-OVP	Lung	sheep	Fronobo	Bongouanou
July 2009	CIV/09-OVG	Lymph node	sheep	Fronobo	Bongouanou
July 2009	CIV/09-OVF	Liver	Sheep	Fronobo	Bongouanou
July 2009	CIV/09-OVR	Spleen	Sheep	Fronobo	Bongouanou
July 2009	CIV/09-01P	Lung	Goat	Amonkro	Bongouanou
July 2009	CIV/09-01G	Lymph node	Goat	Amonkro	Bongouanou
July 2009	CIV/09-01F	Liver	Goat	Amonkro	Bongouanou
July 2009	CIV/09-01R	Spleen	Goat	Amonkro	Bongouanou
July 2009	CIV/09-02P	Lung	Goat	Amonkro	Bongouanou
July 2009	CIV/09-02F	Liver	Goat	Amonkro	Bongouanou
July 2009	CIV/09-02R	Spleen	Goat	Amonkro	Bongouanou
July 2009	CIV/09-02N	Kidney	Goat	Amonkro	Bongouanou
October2009	CIV/09S1	Swabs	Goat	IRA	Dabou
November2009	CIV/09S2	Swabs	Goat	Bongouanou	Bongouanou
Jananuary2010	CIV/10S1	Swabs	Goat	Dabou	Dabou
February2010	CIV/10S2	Swabs	Goat/sheep	Loviguié I –II	Agboville
July2011	CIV/11-07	Lung	Goat	Offoumpo	Agboville
July2012	CIV/12-04	Spleen	Sheep	Offoumpo	Agboville
July2012	CIV/12-05	Lung	Goat	Offoumpo	Agboville
October2012	CIV/12-06	Lymph node	Goat	Offoumpo	Agboville
October2012	CIV/12-07	Liver	Goat	Offoumpo	Agboville
October2012	CIV/12-08	Spleen	Goat	Offoumpo	Agboville
October2012	CIV/12-09	Lung	Goat	Offoumpo	Agboville
October2012	CIV/12-10	Liver	Goat	Offoumpo	Agboville
October2012	CIV/12-11	Spleen	Goat	Offoumpo	Agboville
October2012	CIV/12-12	Kidney	Goat	Offoumpo	Agboville

Epi Unit : Epidemiological unit

Table 2: Collected serum samples and Seroprevalence determination on the period 2009-2012.

Species/Year	2009		2010		2011		2012		Total	
Goat	101		105		35		78		319	
Sheep	137		143		28		119		427	
Total collected sera	238		248		63		197		746	
Total positive	90	Goat: 34	101	Goat: 41	25	Goat: 15	50	Goat: 19	266	Goat:109
		Sheep: 56		Sheep: 60		Sheep: 10		Sheep:31		Sheep:157
Seroprevalence X100	37,8		40,73		39,7		25,2		35,6	

Table 3: RT-PCR and Virus isolation results on tissue and swabs samples collected from PPR investigated outbreaks on the period 2009 – 2012.

Samples ID	Tissues/Swabs	RT-PCR	Isolation on CV1-Slam cell
CIV/09-OVP	Lung	+	Not isolated
CIV/09-OVG	Lymph node	+	Not isolated
CIV/09-OVF	Liver	+	Not isolated
CIV/09-OVR	Spleen	+	Not isolated
CIV/09-01P	Lung	+	<i>Isolated-1 day pi</i>
CIV/09-01G	Lymph node	+	<i>Isolated-2 days pi</i>
CIV/09-01F	Liver	+	Not isolated
CIV/09-01R	Spleen	+	Not isolated
CIV/09-02P	Lung	+	<i>Isolated-2 days pi</i>
CIV/09-02F	Liver	+	<i>Isolated-4 days pi</i>
CIV/09-02R	Spleen	+	Not isolated
CIV/09-02N	Kidney	+	Not isolated
CIV/09S1	Swabs	+	Not tested
CIV/09S2	Swabs	+	Not tested
CIV/10S1	Swabs	+	Not tested
CIV/10S2	Swabs	+	Not tested
CIV/12-04	Spleen	+	Not isolated
CIV/12-05	Lung	+	<i>Isolated-1 day pi</i>
CIV/12-06	Lymph node	+	<i>Isolated -2 days pi</i>
CIV/12-07	Liver	+	Not isolated
CIV/12-08	Spleen	+	Not isolated
CIV/12-09	Lung	+	<i>Isolated-2 days pi</i>
CIV/12-10	Liver	+	<i>Isolated- 4 days pi</i>
CIV/12-11	Spleen	+	Not isolated
CIV/12-12	Kidney	+	Not isolated

pi: post infection

or internal parasite infestation. This is based on the collection of appropriate specimen on the field.

Materials and Methods

Sampling sites and samples collection

Four regions were selected to perform this survey on PPR: Agboville, Bongouanou, Dabou, in the southern part of the country which is the forest region and Yamoussokro in the centre being the Presavannah region (Figure 1). They were visited by the Virology-Laboratory's team in close collaboration with local veterinary field personnel. A total of 28 villages were visited. Each village, being an

epidemiology unit, with animals flocks living and grazing together on the same pasture. Both active and passive disease surveillance were used during this study and appropriate outbreak investigation was conducted along with the collection of good quality samples. From the outbreak investigation, tissue samples and oral, ocular and nasal swabs were collected on the period 2009-2012 from the three southern regions (Agboville, Bongouanou and Dabou) and from all sick animals of the concerned flocks (Table 1). In addition, a total of 746 serum samples (319 and 427 samples from goats and sheep respectively) were randomly collected from each epidemiology unit including Yamoussokro region with a minimum of 25 serum samples per unit (Table 2).

These samples were kept in an ice box containing ice packs for a quick transportation to the laboratory within 24 hours where they were stored at -80°C for tissue and swabs samples and at -20°C for serum samples until treatment.

Serology analysis

Serum samples were analyzed using the PPR competitive ELISA (cELISA) kit, with Monoclonal Antibody (Mab) specific to N protein, from CIRAD – Montpellier (France) [21], according to the manufactures' instructions. Briefly, this kit uses the recombinant N-protein of PPR virus as the capture antigen to coat the plate and a mouse Mab against the N-protein of the PPR virus as the competitive antibody and so called N-cELISA. Briefly, 50µL of 1/3000 diluted PPR N-protein antigen in coating buffer (PBS 0.01M, pH 7.4-7.6) was dispensed in every well and incubated at +37°C for 1 h on an orbital shaker. After washing 3 times with the washing solution (PBS 0.002M at pH 7.2-7.6 with 0.05% Tween20), 45µL of the blocking buffer (PBS 0.01M at pH 7.4-7.6 with 0.05% tween 20 and 0.5% negative sheep serum supplied with the kit) were dispensed in each well. The two conjugate control wells receive an additional 55µL of blocking buffer while the monoclonal antibody wells control wells receive only 5µL. Then 5µL of testing sera were dispensed in the test wells giving a dilution of 1/10 of test sera. Strong positive, weak positive and negative controls were included in duplicate. Next 50µL of Mab 1/100 in blocking buffer were dispensed in each well except the conjugate control wells. The plates were incubated for 1 hour on a shaker followed by 3 wash. 50µL of conjugate (anti-mouse Ig G coupled with radish peroxidase, Dako A/S, Denmark) diluted 1/1000 in blocking buffer was added to each well and incubate for a further 1 hour followed by 3 washes. Finally, 50µL of substrate/chromogen, OPD/H₂O₂ solution was added to each well and incubate in a dark room for 10 minutes. The color development was stopped with 50µL of sulfuric acid 0.1M. The optical density was measured with a Multiskan MKII plate reader at 492nm. Optical Density (OD) readings were converted

to Percentage Inhibition (PI%) values using the following formula : PI (%) = 100 - [mean of OD of tested serum / median of Mab control OD] * 100

Pi% values greater than or equal to 50% were considered as positive.

RNA extraction, cDNA synthesis and amplification

Tissue samples were minced and ground with sterile sand using a mortar and pestle to give a 10% suspension in a serum-free DMEM media. The suspension was clarified by low speed centrifugation and 100µL were used for RNA extraction. The cotton swabs (oral, ocular and nasal) were individually placed in a 2.5mL syringe and 250 µL of PBS (0.1M, pH 7.4-7.6) was added. The cotton swab was compressed three times and the solution collected in a 1.5mL Eppendorf tube. One hundred µL of the suspension were used for RNA extraction. The extraction of RNA was performed using the Qiagen RNeasy kit (Qiagen, Germany) following the manufacturer's instructions. Fifty µL of RNase-free water were used for the elution of bound RNA from the matrix and 10µL of the extracted RNA applied to the cDNA synthesis and amplification of the targeted region of Np gene that were carried out using the set of primers NP3/NP4 (1232-1255: TCTCGGAAATCGCCTCACAGACTG/1583-1560: TCAGCCGATCTTTGAGCCTCACGAG) according to the protocol described by Couacy-Hymann [17]. The target fragment size is 351bp.

Virus isolation

An aliquot of tissue samples was sent to Vienna at IAEA's Laboratory for virus isolation using the new cell line, monkey CV1 cell line expressing sheep-goat SLAM protein [19] with the developed protocol accordingly. Briefly, 0.5mL of the sample homogenate (obtained as describe above) allowed to adsorb for 1 hour on at 37°C onto the cell monolayer at 80% of confluence in 25 cm² tissue culture flasks with shaking every 15 min for one hour. After this time, 5 mL of cell growth medium supplemented with 2% antibiotics was added to the flask and it was returned to the incubator. One day after infection, the cell supernatant was removed and replaced by 5mL of fresh growth medium containing 2% antibiotics. The cell was examined daily by microscopic observation for the detection of the virus Cytopathic Effect (CPE). The cell culture medium was changed every 2 days, the concentration of the antibiotics being reduced to 1% from day 4 onwards. At each medium change, an aliquot of 200µL was kept for RNA extraction and detection of PPRV specific nucleic acid by conventional RT-PCR. Cells in flasks where no CPE was detected after 7 days of incubation were trypsinised for a blind passage.

Results

Outbreak investigations

A network was established involving the veterinary field personnel in the selected regions. Thus, on reports of any cases of PPR based on the clinical symptoms (pyrexia, nasal discharge, lachrymation, oral erosions, pneumonia, and diarrhea) to the Virology Laboratory, a team was quick mobilized to investigate this outbreak. From 2009 up to 2012, several outbreaks of PPR were investigated where the mortality was observed in sheep and goat but mostly goat specie was concerned. Indeed, estimated 1300 goats and 450 sheep died in the seven listed epidemiological units of the three regions with the most mortality cases in Bongouanou having 45% followed by

Agboville with 40% and Dabou with 15%. During this period of study, 14 outbreaks of PPR were investigated (Agboville: 5 outbreaks, Bongouanou: 7 outbreaks and Dabou: 2 outbreaks) and confirmed by the Virology Laboratory.

Serology of PPR

The total serum samples were analyzed with the N-cELISA technique. Any serum samples with PI% greater than or equal to 50% is positive. Based on the test threshold, 266 out of 746 serums samples were found positive giving an overall positive rate of 35.6% ranging from 12.9% (Yamoussokro) to 56.7% (Bongouanou) seroprevalence. The details breakdowns are as follows:

- Agboville: 46.4% (109/235)
- Bongouanou : 56.7% (63/111)
- Dabou : 34.3% (68/198)
- Yamoussokro: 12.9% (26/202).

The yearly seroprevalence was ranging from 25.2% in 2012 to 40.7% in 2010. Out of the positive serum samples, 14.6% sera were collected from goat (109/746) and 21% (157/746) from sheep species (Table 1). The difference is not significant ($\chi^2 = 0.07$; $p < 0.001$).

PPRV Np gene detection

RT-PCR technique was carried out on tissue and swabs samples collected during the outbreak investigations. All the collected samples, after amplification with the set of primers NP3/NP4, showed fragment of 351bp by gel electrophoresis and therefore, were found as PPR positive samples (Table 3).

Virus isolation

Isolates of PPRV were obtained from 8 out of 24 tissue samples using monkey CV1 cell line expressing sheep-goat SLAM protein showing CPE and confirmed by RT-PCR (Table 2). There were 4 isolates from Amonkro (region of Bongouanou with 13 samples) and 4 isolates also from Offoumpo (region of Agboville with 11 samples) (Table 3). The virus prevalence between the mentioned regions is not significantly different ($\chi^2 = 0.46$; $p < 0.001$). Swabs samples were not tested for virus isolation.

Discussion

On reports from the field staff, the Virology Laboratory's team reacted on time for the investigation of PPR outbreaks and adequate samples were collected for confirmation. A total of fourteen outbreaks reported during 2009-2012 were involved into the present study. Of note, current reported system was running a passive surveillance activity that relied on active reports of animal owners. Enhance, this epidemiological situation may be largely under-estimated. The field staff does not have any means to move into their region therefore even the passive surveillance of any diseases is totally weak or absent. However the network which was established in the selected regions allowed to investigate those outbreaks.

It was estimated that 1300 goats and 450 sheep died during these reported outbreaks causing an estimated losses of 57millions FCFA (117,283.95USD or 87,000 Euros) reducing deeply owners' livelihood. The spread of the disease is mainly due to a trade of sick or in incubation phase animals (introduction of new animal into the

flock) and also due to a lack of animal movement control [22].

An overall seroprevalence rate of 35.6% was found ranging between 12.9% (Yamoussokro) and 56.7% (Bongouanou). However, the analysis of the results from the three southern regions (Agboville, Bongouanou, Dabou) showed the prevalence rate was 44.1% (240/544) which confirmed previous study with 40% in the forest region twenty years ago [16]. Yamoussokro is located in the centre of the country. The central region registered the lowest seroprevalence rate, 2%, due to an implemented annual vaccination against PPR [16]. The yearly seroprevalence showed that the disease is endemic in the country on the studied period with a seroprevalence above 25%. With regard to species, 14.6% and 21% out of the total serum samples collected from goat and sheep respectively, were positive. However the difference was not significant ($p < 0.001$).

It was observed that goat specie is more susceptible than sheep in Ivory-Coast as it is in most endemic countries [9].

These PPR outbreaks were confirmed using RT-PCR technique on samples collected from sick animals and height isolates of PPRV were obtained by cell culture with CV1 cell line expressing sheep-goat SLAM protein [19]. After sequencing of the Np gene, it was found that these isolates were lineage II [19] probably co-circulating with lineage I which is known to circulate in Ivory-Coast [4]. Further studies should be undertaken to clarify this epidemiology situation.

There is a possibility of inadequate disease reporting as is the case in most other developing countries. So an effective disease surveillance system shall be established as a core system within the Veterinary services with an adequate and pragmatic programme. This is a key for any disease surveillance and control.

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