Estimating clinical record rate of Peste Des Petits Ruminants disease among sheep and goats in Egypt

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Abstract: Reports of suspected outbreaks of Peste Des Petits Ruminants virus (PPRV) during 2013-2014 were investigated and biologic samples were collected for laboratory diagnostics. Swab samples were tested for specific PPRV antigen detection by Immune-capture ELISA Kits. Lung tissues from dead animals, blood buffy coat (Blood with anticoagulant) and conjunctival swabs samples were found to be the samples of the choice for PPRV outbreaks confirmations followed by the oral, nasal, and fecal swabs. The nucleotide sequence of the fragment analyzed (322bp) was compared to PPRV F gene sequences and confirmed the Egyptian 2013 outbreaks belonged to lineage 4. Few clinical PPR disease outbreaks were reported, during the 2 years periods of observation and only 52/100 (confirmed/suspected) clinical PPR virus outbreaks were confirmed: 33/58 outbreaks within the Nile delta region, 9/15 outbreaks within the Central region, 4/5 outbreaks within the Upper region, 4/16 outbreaks within the Western region and 2/6 outbreaks within the Eastern region. On the other hand the last country wide sero-survey September-November 2013 reiterated the high occurrence of recent PPR virus infection over a one year period in 6-18M old sheep and goats at both the animal and village epi-unite level. The clinical disease outbreaks were also common in sheep population which is different from some other workers, reported a PPR outbreak in a flock of sheep and goats with only the goats being affected. It was concluded that there is serious underreporting of PPR, active clinical and serological survey are the most unbiased method to determine the incidence of PPR and mirrors the pattern of results detected by passive reporting, and also PPR control strategy should be implemented.
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1. Introduction:

Peste des petits ruminants virus (PPRV; genus Morbillivirus, family Paramyxoviridae) causes severe infectious disease in sheep and goats in Africa and Asia. Pneumo-enteritis clinical signs are dominated by ocular and nasal discharge, and mortality rates are high [1]. PPRV epidemiologic systems: northern Africa, where all identified PPRVs belonged to lineage IV and were closely related to PPRV initially identified in Sudan [2], and western Africa, where all identified PPRVs belonged to lineages I and II [3, 4], lineage III in East Africa, Arabian and Southern India and lineage IV has been in the Middle East and Asia subcontinent, reaching east as far as Nepal and Bangladesh. All four lineages have been shown to be genetically distinct from the Rinderpest virus [5]. Historically, the disease was primarily associated with West Africa, but it extends in a belt across Africa immediately South of the Sahara, extending into Arabian Peninsula [4]. Morbidity and mortality can be as high as 100% and 90%, respectively. When associated with other diseases such as capripox, mortality can be100% [6]. It is hypothesized that PPRV spread into north and east Africa, moving up through trade routes into Egypt. PPRV has been detected among goats farm in Giza governorate during 1987 and 1990 [7]. More recent outbreaks were reported in Aswan province in 2006, highlighted the ability of infection occasionally be asymptomatic, while others develop a severe clinical disease [8]. Reemergence outbreaks also reported at Cairo, Giza, Menofia, Ismailia, Behera and south Sinai governorates between 2008-2009. [9].

Estimating the incidence and rates of disease is a key activity in the control pathway and serves diverse purposes as countries progress along. Methods available to measure PPR incidence in Egypt include counts of reported outbreaks, serological surveys, participatory approaches and active surveillance for clinical disease. All across Egyptian governorates September and November 2013 sero-survey reiterated the high occurrence of recent PPR virus infection at both villages Epi-unite (61.3%) and at the animal level (24.1%), over a one year period in sheep and goats between 6 and 18 months old [10].

In this paper, we had a trail to make a clear over view on the epidemiological situation of PPR disease among sheep and goats and we will compare results

from these methods and consider which are most appropriate for different purposes, in respond to the recent reemerge of PPRV outbreaks characterized in some occasions by sever clinical diseases high morbidity and mortality.

2. Materials and Methods

Outbreak investigation and Sampling:

All reports of suspected outbreaks of PPRV during 2013-2014. All outbreaks were investigated and biologic samples were collected for laboratory diagnostics Swab samples were tested for specific PPRV antigen detection by commercial ELISA Kits.

Clinical examination of the affected flock and necropsy of dead animal was carried out and ocular, nasal, fecal swabs moth mouth scraps from all suspected live animals organs and mesenteric lymph nodes from dead animal were collected for laboratory confirmation.

Ic-ELISA for detection of PPRV antigen:

Samples were tested by using ID Screan® antigen capture ELISA for detection of PPRV antigen, following the kit instruction manual.

Calculation of the mean OD value of the positive control (OD posC) >0.5 and the ratio of the Mean calculation of the (OD posC) and negative control (OD negC) >3 for the test plate validation and samples were considered positive if the (OD sample) is ≥20% of the mean (OD PosC) after subtracting the mean (OD negC) from both [(OD sample – OD negC)/ (OD posC – ODnegC)*100.

Tissue culture isolation:

Samples positive by Ic-ELISA were processed and cultured onto VERO cells (African Green Monkey Kidney cells) to obtain virus isolates following [11]. The PPRV was identified by cytopathic effects (CPE.

Sequencing:

The 15 samples from 6 outbreaks were sent to Nonvesicular reference laboratories, The Pirbright Institute, Ash Road, Pirbright, Woking, UK. Date sample sent to The Pirbright Institute: 23/04/14

STARS sample number: R1/14 samples 02, 09, 10, 12, 14 and 15, Sender Ref:RL/924B, RL/1013, RV/77A, SV/65, RL/975 and RL/810a, Date collected:25/10/13, 08/12/13, 27/01/14, 28/04/13, 25/11/13 and 09/07/13, Species: Ovine, Date of sequencing: 05/05/14, Material used: original sample, Gene sequenced: F gene, Method used: RT-PCR, Primers used: F1, F1b and F2d, Sequence length:322nt

3. Results

Ic- ELISA/virus isolation Results:

Out of 42 suspected PPRV 21 outbreaks were confirmed by Ic-ELISA. The confirmed Outbreaks were distributed within 11 governorates all over the country regions Bhera, Alexandria, Domiat, Sharqia, Gharbia Dkahlia within the Nile delta region, Giza, and Menia within the Central Egypt , and Aswan within the Upper Egypt, Matrrouh within the north Western of region, Port-said and Suez within the Eastern region (Table 1, Figure 1).

2014*:* Out of 58 suspected PPRV 31 outbreaks were confirmed by antigen capture ELISA. The confirmed Outbreaks were distributed within 14 governorates all over the country regions Bhera, Alexndria, Domiat, Sharqia, Gharbia Dkahlia, Kafr Elshaikh within the Nile delta region, Giza, Bani Swief and Menia within the Central Egypt , Qena and Aswan within the upper Egypt ,Ismailia and port said from the Eastern region, Matrrouh within the north Western of region (Table 1, Figure 1).

During the 2 years period 52/100 PPR outbreaks were confirmed. At the Governorate level the PPR clinical suspicion never reported in Mnofia, Asute, Sohag, Red Sea, South Sinai, North Sinai and El-wadi El-gadid governorates and never confirmed in the samples recived from Kafr El-Shakh, Fayoum and Seuz governorates.

By country regions 33/58 outbreaks within the Nile delta region, 9/15 outbreaks within the Central region, 4/5 outbreaks within the Upper region, 4/16 outbreaks within the Western regions and 2/6 outbreaks within the Eastern region (Figure 3).

By species during the year 2014, out of the 31 confirmed PPR clinical outbreaks 15/32 were sheep herd 7/13 were goat herds and 9/13 were mixed sheep and goat herds (Table 2).

PPRV antigen was successfully detected in different samples from 52 outbreaks 2013-2014. By using the Ic-ELISA the viral antigen was detected in: 28 (82.4%) out of 34 outbreaks Buffy coat received samples, 17 (65.4%) out of 29 outbreaks conjunctival swabs, 13 (54.2%) out of 24 outbreaks Nasal swabs, 22 (59.5%) out of 37 outbreaks oral swabs or scraps, 7 (53.3%) out of 14 outbreaks fecal swabs and in 10(83.3%) out of 12 outbreaks where lung tissues were sampled from dead animals.

Sequencing (Pribright Shipment sample results 2013):

The RNA from samples R1/14numbers 02, 09, 10, 12, 14 and 15 were tested in conventional RT-PCR assays, using a set of primers specific for the F gene of PPR. cDNA amplicons of the expected size were obtained from all samples.

Comparison of 322bp of the F gene of Egypt 2013.

Sequence analysis of the F gene cDNA amplicon derived from the samples confirmed PPRV. The nucleotide sequence of the fragment analysed (322bp) was compared to PPRV F gene sequences and

confirmed the Egyptian outbreak belonged to lineage 4.

Table 1: Confirmed PPR Outbreaks were distributed within governorates all over the country regions Egypt 2013-2014:

Region	Governorates	PPR outbreaks 2013		PPR outbreaks 2014		Total	
		Suspected	Confirmed	Suspected	Confirmed	Suspected	Confirmed
Nile delta	Alexandria	3	2	2	1	5	3
	Bhera	6	3	7	7	13	10
	Giza	3	2	7	4	10	6
	Domiat	1	1	1	1	2	2
	Gharbia	2	1	1	1	3	2
	Kalubia	1	0	2	1	3	1
	Dkahlia	1	1	10	3	11	4
	Kafr El-Shakh	-	-	3	0	3	0
	Mnofia	-	-	-	-	-	-
	Sharqia	5	4	3	1	8	5
Central	Asute	-	-	-	-	-	-
	Bani Swief	1	1	10	5	11	6
	Fayoum	1	0	-	-	1	0
	Menia	1	1	2	2	3	3
Upper	Aswan	3	2	1	1	4	3
	Qena	-	-	1	1	1	1
	Sohag	-	-	-	-	-	-
Eastern	Ismailia	-	-	1	1	1	1
	Port Said	2	0	1	1	3	1
	Red Sea	-	-	-	-	-	-
	Seuz	2	0	-	-	2	0
	South Sinai	-	-	-	-	-	-
	North Sinai	-	-	-	-	-	-
Western	El-wadi El-gadid	-	-	-	-	-	-
	Martouh	10	3	6	1	16	4
Total		42	21	58	31	100	52

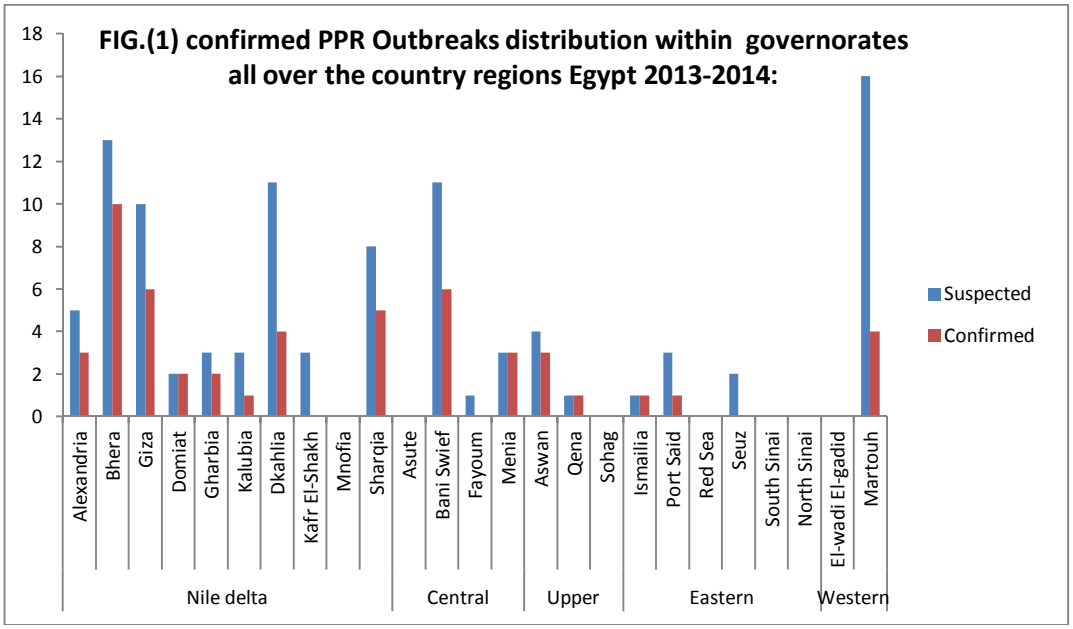


Fig 2 Map distribution of PPR confirmed outbreaks 2013-2014 (n=52)

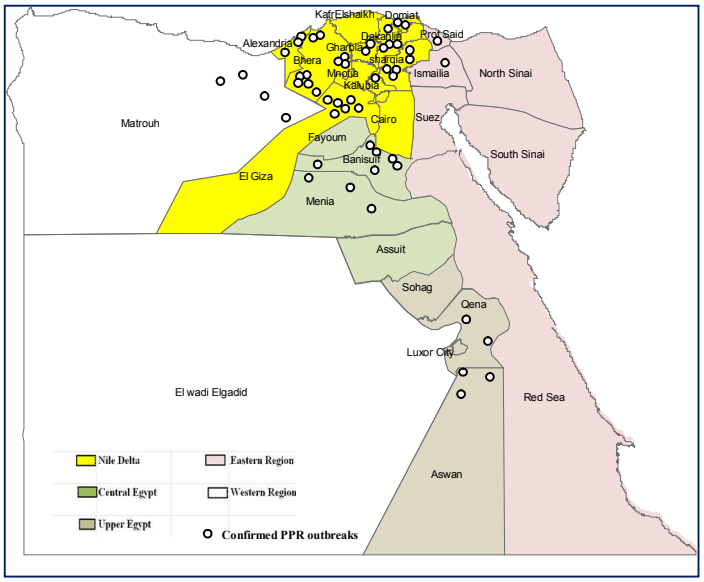


Table (2): PPR suspected and confirmed small ruminant outbreaks by Species Egypt 2014.

Sheep herds	Goats Herds	Mixed	Total
32	13	13	58
15	7	9	31

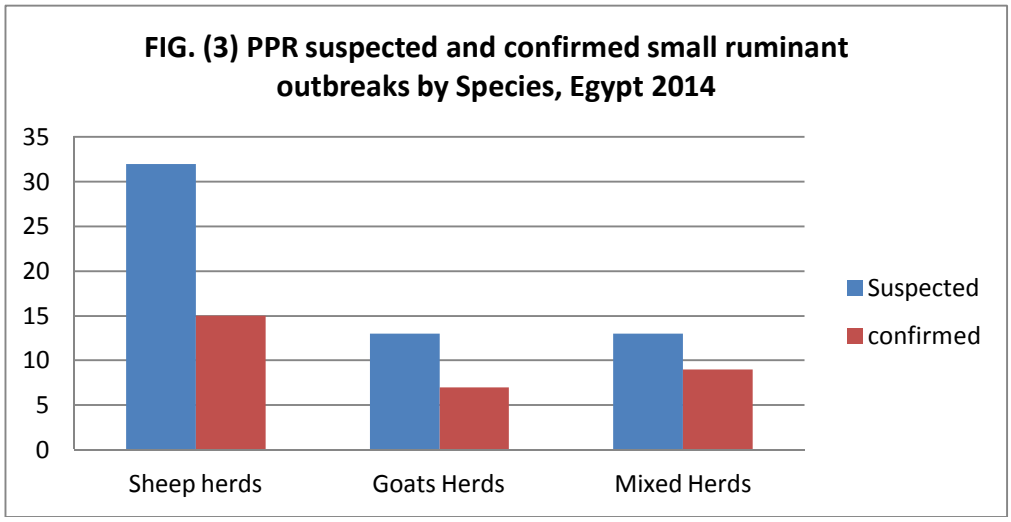


Table 3: Different PPRV field samples detection limits by using Ic-ELISA kit: (n=52 c**onfirmed outbreaks**)

Samples type	Pos.(outbreak)	Neg.(outbreak)	Total.(outbreak)	% Pos.
Blood	28	6	34	82.4
C.swab	17	9	26	65.4
Nasal Swab	13	11	24	54.2
Oral swab	22	15	37	59.5
Fecal	8	7	15	53.3
Lung Tissue	10	2	12	83.3

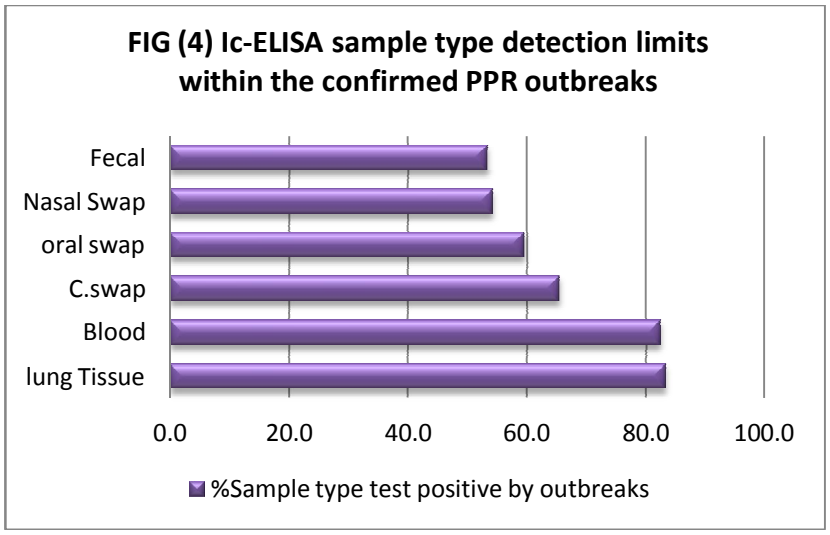
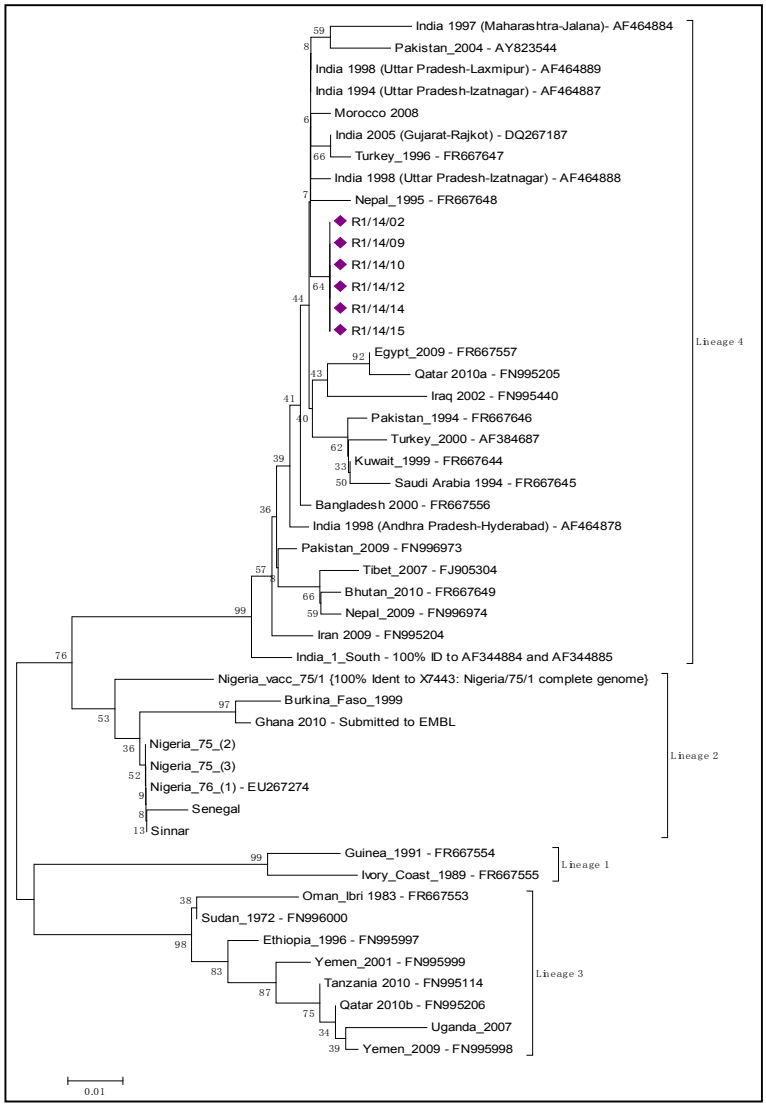


Fig. (5): Comparison Sequence analysis of 322bp of the F gene of samples confirmed PPRV Egypt 2013



4. Discussion

MAb-based Ic-ELISA [12] and sandwich ELISA (s-ELISA) [13] have been used extensively for detection of PPRV antigen in clinical specimens.

The immunocapture ELISA was developed in the world reference laboratory (CIRAD–EMVT, France) and is an internationally accepted assay for PPRV antigen detection [12]. This assay uses a biotinylated anti-'N' MAb against a cross-reactive epitope of RP/PPRV for capture or detection of PPRV antigen in clinical samples. Similarly, the s-ELISA kit developed at Division of Virology, IVRI, Mukteswar, India uses a MAb (4G6) directed against an epitope of N protein of PPRV [14], which is the routinely being used for clinical prevalence or detection of PPRV antigen in clinical specimens in India [13]. This assay was efficacious, with diagnostic sensitivities (89 %) and specficities (93 %), comparable to the immunocapture-ELISA [15].

Ic-ELISA kit sensitivity for PPR antigen detection can distinguish between different field samples from the same animals within the same outbreak as our results revealed that at the outbreak level, the Lung tissues from dead animals ,Blood buffy coat (Blood with anticoagulant) and conjunctival swabs are the samples of the priority for PPRV outbreaks confirmations followed by the oral, Nasal, and faecal swabs, although Parallel testing of different types of field samples from the same animal and or herd increasing the overall sensitivity and outbreak confirmation.

There were few clinical PPR disease reported, during a 2 years periods 2013-2014, local veterinary offices had reported only 52/100 (confirmed/suspected) clinical PPR virus outbreaks in acute and subacute forms, 33/58 outbreaks within the Nile delta region, 9/15 outbreaks within the Central region, 4/5 outbreaks within the Upper region, 4/16 outbreaks within the Western regions and 2/6 outbreaks within the Eastern region. On the other hand a country wide sero-survey reiterated the high occurrence of recent PPR virus infection, as in 61.3% CI 95% [53.1-69.5%] of the villages and at the animal level 24.1%, CI95% [21.18-27.2] of sheep and goats between 6 and 18 months [10].

At the Governorate level the PPR clinical disease never confirmed and or reported in Kafr El-Shakh, Mnofia, Fayoum, Asute, Sohag, Red Sea and Seuz. Governorates although the 6-12M small ruminant PPR specific antibodies prevalence during 2013 (Virus activity over a year period) were 23.3%, 19%, 26.7%, 21.4%, 29.2%, 33.3%, 66.7% respectively and no suspected clinical PPR disease were reported South Sinai and El-wadi El-gadid and also while no PPR specific antibodies could be detected in the serum samples collected which could be due to low sample size at prevalence estimates and need to be given the higher weightage [10].

The clinical disease outbreaks were common in sheep population (Table 2) although some workers have reported a PPR outbreak in a flock of sheep and goats with only the goats being affected [16, 17]. Goats were more susceptible and may have died from the disease, where as sheep may have survived [18].

Results also indicate that there is a high degree of subclinical infection and/or under-reporting of clinical outbreaks; approximately all the Egyptian governorates had serological evidence of recent PPRV infection in sheep and goats yet clinical signs had been observed and or confirmed in 10 governorates sheep population than goats. Other workers also reported the clinical signs range from subacute in sheep to fulminating fatal illness in goats although in apparent infections occur in both species especially in nomadic animals that are endemically exposed [19] In apparent infection with high PPR antibody provenances was also recognized in Egypt meanwhile 1993-2000 sero- surveillances through the (Pan African Rinder Pest Eradication) PARK project activities for Rinder Pest Virus eradication "data not shown". It was reported that morbidity and mortality rates due to PPR may vary from 0 to 90% depending on the animal husbandry, breed, age and other factors [20]. In Pakistan, goats react severely to the exposure of PPRV like other parts of the world where the disease is endemic. It has been also reported that during an outbreak of PPR in Pakistan, no clinical signs were observed in sheep kept with the sick goats in the same premises under one roof but they got sero-converted only [21]. Female sheep were 2.16 times CI 95% [1.24-3.75] more likely to be infected with PPR compared with male sheep ($P<0.05$)[10].The reasons for these outbreaks in different epidemiological condition, like those where flocks consist of only sheep or goats or both, are unknown and was further reported that partial N and H gene sequences of the virus isolate involved in sheep or goat outbreaks have not revealed a clue to explain this situation [22].

Sequence analysis of the F gene cDNA amplicon derived from the samples confirmed PPRV. The nucleotide sequence of the fragment analysed (322bp) was compared to PPRV F gene sequences and confirmed the Egyptian outbreak belonged to lineage 4 closely related to Morocco 2008. lineage IV is generally found in Asia [23, 24]. However, a recent appearance of lineage IV, which was associated with a large epizootic in Morocco 2008. Strains from Saudi Arabia (Saudi Arabia 99-7), Sudan (NSUD08), and Egypt (Egypt_2011). Genetic sequences are highly conserved in this group, particularly for

Erithria_Gulee_2005, which share 100% nucleotide identity with (Saudi Arabia 99-7), 99.6% identity with Egypt_2011 and 99.2% identity with (NSUD08). This group is closely related to the virus associated with a large epizootic in morocco during outbreaks 2008. The data suggest a colonal origin of the viruses belonging to this group, supporting hypothesis that Eretria should have been the gate way for Saudi Arabia 99-7 strain to spread through Africa [25], which confirms that there was a recent introduction related to the Re-emerge of PPR outbreaks associated with severe clinical disease characterized with high morbidity and motility.

There was only live attenuated homologous mono-valent vaccine available for the control of PPR. Vaccination against PPR was quite effective in protecting sheep and goats against the PPR disease, but that this efficacy only holds if the vaccination was done before exposure and when the animals were at a subclinical level of infection [26].

Emergency vaccination was started in response the outbreaks confirmed late 2013 using the locally produced live attenuated vaccine. Sheep and goats which have recovered from a PPR infection appear to be protected against a subsequent infection for the rest of their lives. Neutralising antibodies anti PPRV were found in sheep and goats up to three years after vaccination with the attenuated PPRV vaccine strain [27, 28].

This is in sharp contrast to the very limited number of official notifications reported to central GOVS: there is serious underreporting of PPR. Active clinical and serological surveys are the most unbiased method to determine the incidence of PPR. The number of outbreaks detected by active, clinical surveillance could be also closely mirrors the pattern of results detected by passive reporting. It can be also concluded also that the PPR control strategy should be implemented. Condition of animal movements, strengthen disease notification to limit spread of PPR circulation between areas, vaccination of small ruminants at risk and practice of farmers (small and large scale) regarding PPRV and PPRV disease control. The disease can be prevented by not introducing new stock from unknown sources, especially animals bought at livestock markets. In addition animals returned unsold from markets should be segregated unless the entire herd or flock has been vaccinated [29].

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