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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

The confirmation of *Peste des petit ruminants* (PPR) in Kenya and perception of the disease in West Pokot

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Abstract

Marakwet district is an arid district in Kenya where livestock keeping is a major activity. There have been severe outbreaks of PPR in Kenya since when it was first suspected in 1992 and confirmed in Turkana District in 2007. There has been no description of the disease in other geographical areas. PPR is a relatively new disease and is easily confused with other infectious conditions especially contagious caprine pleuro-pneumonia, dermatophilosis contagious ecthyma which occurs in the same areas where PPR occurs and sometimes occur simultaneously. It is therefore important to describe the outbreaks that occur. In this study, the main clinical signs as observed by pastoralists were examined and PPRV confirmed through RT-PCR and ELISA tests. The main signs were nasal and ocular discharges, depression, loss of appetite, fever and diarrhoea. The prevalence of PPR was found to be 21.2%.

Keywords: PPR; Kenya; ELISA; RT-PCR; West Pokot

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Introduction

Peste des Petits Ruminants (PPR) is an acute, highly contagious and infectious disease specific to small ruminants and small wild stocks (Nussieba et al., 2009a). The disease is caused by Peste des Petits Ruminants virus (PPRV) classified under Paramyxo-virinae, and genus Morbillivirus (Chauhan et el., 2009). Transmission of PPR is achieved by direct contact from infected to susceptible animals by close contact or through respiratory and oral routes (Chauhan et al., 2009). The disease is endemic and common in goats and sheep in Asia, China, Middle East, Eastern parts of Europe, West, Central and East Africa (Banyard et al., 2010). Goats are more affected than sheep (Nussieba et al., 2009b) and PPR occurs in an epizootic form with a morbidity of 80-90% and mortality between 50 and 80% (Chauhan et al., 2009). PPR reduces the pastoralist's income generation impacting negatively their livelihoods and food security (Banyard et al., 2010). Women and children depending on small ruminants are even more affected (Kumar et al., 2003). In Kenya, it was first suspected in 1992 (FAO, 2008) with further serological reports by Wamwayi et al. (1995) and confirmed in Turkana district in 2007 (Pro Med-Mail, 2007). Other participatory epidemiological studies indicated that Turkana herders noticed the disease in 2005 (Bett et al., 2009). The disease has since spread to all the arid pastoral districts in Kenya.

The aim of the study was to describe the presence of PPR virus in small ruminants and describe the perception of the disease by farmers in West Pokot, one of the pastoral areas. It is a relatively new disease in Kenya and it is important to establish the perception of farmers towards the disease and how it ranks in their priorities.

Materials and Methods

Study area

The study was undertaken in Marakwet East district where cases of PPR were reported during the 2008 outbreaks. Marakwet East district is divided into three topographical zones that run parallel to each

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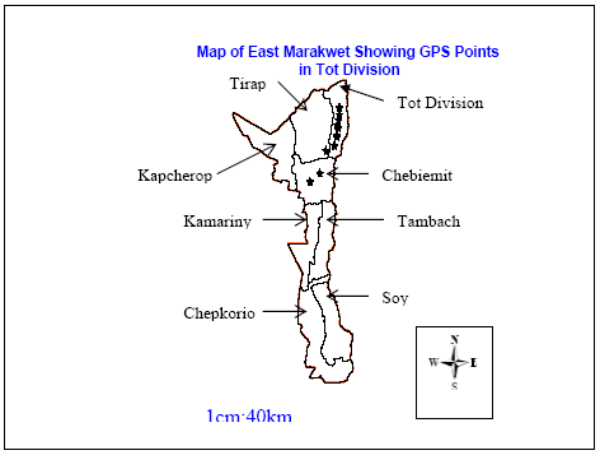

Fig. 1: Map of Marakwet East district showing GPS points in Tot division

other; the highlands, the escarpment and the Kerio valley. Altitude ranges from the highest point at 800 2000m above sea level with Kerio valley as its main drainage. Rainfall is highly influenced by altitude and ranges from high (1100mm) in the highlands to low (750mm) in the valley (Fig 1).

A cross-sectional disease search was carried out in in Marakwet East district where nasal and eye swabs were collected aseptically in cryovials containing 0.5ml of Trizol from 10 PPR suspect goats in Kabetwa location, Tot division of Marakwet East District. The sampling was done purposively from goats that showed clinical sign (s) that characterize the clinical case definition. Temperature readings were done early in the morning before environmental temperature rose. The samples were processed and tested by the conventional reverse transcriptase Polymerase Chain Reaction (RT-PCR) as described by Durrani et al. (2010). For the serological analysis, blood samples were randomly taken from 33 goats that were five to nine months old in Marakwet district in November, 2009.

RNA extraction from eye and nasal swabs

An RNA extraction method using the TRIzol® (Lifetechnologies, Carlsbad, CA) (RT-PCR protocol by Institute of Animal Health, Pirbright; 1994) was used. Each sample of the processed eye and nasal swabs was handled separately to avoid cross-contamination. 500µl of each sample was transferred onto a 2ml micro-centrifuge tube. One ml of the TRIzol® reagent was added into each tube and vortex mixed for 20 seconds. The samples were then left to stand at room temperature for 5 minutes. To each sample, 200µl of chloroform was added and vortex mixed for 20 seconds. The samples were left to stand at room temperature for 3 minutes. They were then centrifuged at 10,000 rpm for 15 min. The aqueous phase was collected and transferred into a fresh tube. 500µl of absolute ethanol was added and then mixed by vortexing for 20 seconds. The RNA was precipitated by storing at -20°C for 2 hours then pelleted by centrifuging at 10,000 rpm for 10 minutes and supernatant slowly discarded. The RNA pellet was washed by re-suspending in 1ml of 75% ethanol and centrifuged at 10,000 rpm for 10 minutes. This later step was repeated one more time. The supernatant was discarded and the RNA pellet air dried for 10-20 min at room temperature. The RNA pellet was re-dissolved in 50µl of TE buffer and kept at -20°C ready for the next step.

A total of 33 serum samples were obtained from goats that were five to nine months old in Marakwet district in November, 2009.

RT-PCR

For the RT-PCR, RNA, organ suspensions were used as a source of RNA for RT-PCR detection of viral genomes. Virus RNA was extracted using acid guanidium-phenol-chloroform-isoamyl alcohol mixture as described by Chomczyn and Sacchi (1987). The RNA pellet was dissolved in 20 ml of sterile distilled water and used for cDNA synthesis. Complementary DNA synthesis was initiated by incubation of tubes at 70°C for 5 minutes to denature probable secondary structures in the RNA. The synthesis of cDNA was carried out in a mixture of 25 mM Tris-HCl, 25 mMKCl, 4 mM MgCl2, 10 mM DTT, 50 ng random hexamere primers, 200 U Moloney murine leukemia virus reverse transcriptase (MMLV-RT) (MBI, Fermentas, Lithuania), and 10 U of RNase inhibitor. The reaction mixture was incubated first at 25^0C for 10 minutes, which was followed by a second step incubation at 37°C for 1 h. Moloney murine leukemia virus reverse transcriptase was then inactivated by holding at 70°C for 10 minutes. Polymerase chain reaction amplification was carried out by adding 3 ml of cDNA in the master mix containing 75 mM Tris-HCl (pH8.8), 20 mM $NH_4(SO_4)2$, 1.5 mM $MgCl_2$, 15 pmole of each primer,0.2 mMdNTP, and 0.5 U of Taq DNA polymerase. The amplification was completed in 30 ml of total reaction mixture in a thermal cycler (Techne, Oxford, UK). The steps of amplification on thermal cycler were set up as follows: initial denaturation for 6 minutes at 94°C followed consecutively by 1 minute at 65°C, 1 minute at 72°C,and 45 seconds at 94°C, which were repeated 40 times. Amplification was terminated by final extension at 70°C for 10 minutes. A PPR-specific primer set used in the study was selected from the F protein-coding gene sequence. Designed upper (sense) and lower (antisense) primers were as follows; PPRF1b-59-ATCACAGTGTTAAAGCCTGTAGAG-39 (positions 760-784) and PPRF2d - 59-GAGACTGAGT TTGTGACCTACAAG-39 (positions 1183–1207). Using this pair of primers, it was expected to amplify a

448-bp DNA product. The resulting DNA products (amplicon) were analyzed on agarose gel (1.5%) after electrophoresis at 80 V for 30 minutes. The DNA bands were observed under ultraviolet light and photographed.

A Cross-sectional study

In the field a questionnaire was administered to livestock keepers of Tot division in Marakwet East district of Kenya in November, 2009. Eleven months after vaccination against PPR was carried out in the district. The number of questionnaires was determined using the formula by Goodchild et al. (1994). The questionnaire was pretested and then administered to 78 livestock keepers in the division in order to describe what was perceived as the presentation of PPR clinically and determine the risk factors that were associated with the disease. The unit of questionnaire administration was the division (Tot).

Competitive ELISA: PPR c-ELISA kit for PPRV antibody detection was obtained from Institute for Animal Health (Pirbright Laboratory, Surrey, UK). c-ELISA was performed strictly as per the protocol outlined in the user manual supplied with the kit Libeau et al. (1995). Optical density (OD) values were read at 492nm with ELISA plate reader (Immunoskan BDSL, Thermo Lab. Systems,Finland). The absorbance was converted to percentage inhibition (PI) using the formula with help of ELISA Data Interchange (EDI 2.3) software (FAO/ IAEA).

PI = (100-[OD in test well/OD in 0% competition well]) × 100.

The test serum samples showing PI value of 50 or above were taken as positive for PPR antibodies.

Results

The prevalence of PPR in goats was found to be 21.2% (Table 1).

Table 1: Sero-prevalence of PPR in goats in Marakwet East District

Sex	Number of samples	Number positive	Percentage	Overall Percentage
Male	12	2	16.7%	21.2%
Female	21	5	23.8%	

PCR Confirmation

The key clinical signs observed in 10 goats were depression, loss of appetite, a rectal temperature of between 39.9^{0}C and 41.3°C, diarrhoea in all the goats that were sampled and ocular and nasal discharges. There were no mouth lesions in all the goats examined. The goats from which samples were taken aged between six and eleven months.

In this study, ocular and nasal swab samples collected from a female animal aged nine months from the 10 goats were observed and tested positive for PPR RNA on RT-PCR.

The respondents reared goats, cattle, sheep poultry and donkeys in order of diminishing numbers (Fig. 2). About 95% of the respondents use the extensive pastoral mode of production while the rest practice complete or partial zero grazing. The constraints reported included disease outbreaks, lack of pasture, poor or lack of veterinary services, lack of water, animal rustling or insecurity, ticks, lack of dips, predation, lack of markets or poor marketing and unavailability of veterinary drugs (Fig. 3). The diseases considered important ranged from contagious caprine pleuro-pneumonia as the most important to foot rot as the least important (Fig. 4).

Those livestock keepers that were reporting to have an experience of PPR in their sheep and goats reported that it started occurring from the December of 2007. Livestock keepers referred to PPR as a mysterious disease. Fifty one farmers (72%) reported an outbreak while the rest (28%) of the farmers had no outbreaks in their area.

Discussion

Peste des Petits Ruminants (PPR) is a severe and highly infectious viral disease of small ruminants. The PPR virus (PPRV) belongs to the genus *Morbillivirus* in the family Paramyxoviridae (Gibbs et al., 1979) Currently, the disease is recognized as responsible for mortality and morbidity across most of the sub-Saharan African countries situated north of the equator, in the Arabian Peninsula, in India and in numerous other countries in Asia (Dhar et al., 2002).

The clinical signs observed in this study were depression, loss of appetite, fever, and diarrhoea as well as ocular and nasal discharges. Pastoralists ranked PPR third after CCPP and heartwater in relation to health issues that are significant in West Pokot. The presence of respiratory signs in both PPR and Contagious caprine pleuro-pneumonia led to problems in disease recognition.

The PPR virus was confirmed by PCR test to be present from nasal swabs obtained from infected goats. Conventionally, detection of PPPRV in field samples is carried out serologically or by virus isolation; which besides being less sensitive and cumbersome are more difficult to interpret. The nucleic acid based detection assay like RT-PCR, overcome these limitations and have been used successfully for detecting the virus as described by Tiwari (2004). Among the various techniques developed for the detection of PPR virus, RT-PCR test developed by using F-gene primers (F1-F2) which is known to amplify a 372bp fragment of F-gene

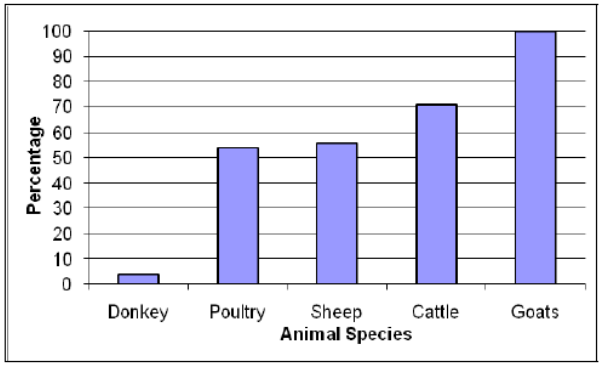

Fig. 2: Livestock species reared and percentages of farmers rearing each species

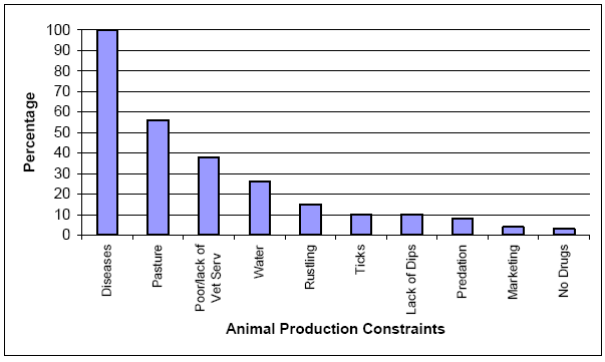

Fig. 3: Animal production constraints in Marakwet East District

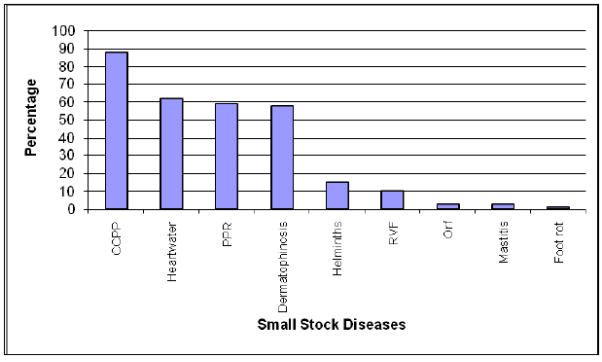

Fig. 4: Disease constraints in Marakwet East District

(Forsyth and Barrett, 1995; Shaila et.al., 1996). However, Dhar et al. (2002) described another F-gene primer pair (F1b-F2d), which amplifies a 448 bp fragment of the F gene encompassing the sequence amplified by primer pair FI-F2.

The respiratory signs in both caprine pleuro-pneumonia and PPR led to problems in disease recognition in diagnosis but this can be resolved through laboratory confirmation. The sero-prevalence of PPR in goats in Karamajong in Uganda which neighbours Kenya was found to be 57.6 % (Mulindwa et al., 2011) which is close to 52.5% reported earlier in Ethiopia (Waret-Szkuta et al., 2008). PPR is endemic in Ethiopia (Abraham and Berhan, 2001) and the main animal husbandry practiced here is nomadism which is marked by a vibrant cross border trade associated with widespread illegal movements of animals and human beings which easily spreads PPR. The 21.2% sero-prevalence in the goats reported here compares well to the locality specific PPR sero-prevalence that was recorded 22% in Ethiopia (Abraham, 2005). The overall sero-prevalence of PPR in goats and sheep was found to be 45.4% in Tanzania (Swai et al., 2009) which is higher than the sero-prevalence of 21.2% reported here. The borders between Kenya and Tanzania are porous and pastoralists mover livestock across them. Although the sero-prevalence was low in this study than it has been shown elsewhere being as high as 45.5, 78 and 92.5%, in Cameroon, Nigeria and Sudan respectively (Ekue et al., 1992; Obidike et al., 2006; Osman et al., 2008).

The medium prevalence of PPR in this study may be related to the medium level of contact of small stock during movement in search of water or pasture and in addition, for trade purposes. In the present study, lambs and kids were affected among the susceptible population, while those which were vaccinated earlier remained unaffected. In conclusion, timely vaccination targeted at the susceptible ruminants is important if the disease is to be controlled. Awareness on the new disease can be enhanced by building on the already known health status by pastoralists.

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