Short Communication

Peste des Petits Ruminants Virus Lineage II and IV From Goats in Southern Tanzania During an Outbreak in 2011

Gerald Misinzo[1,*], Tebogo Kgotlele[1], Epaphras A. Muse[1], Jan Van Doorsselaere[2], Mikael Berg[3], and Muhammad Munir[4]

[1]Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania; [2]Higher Institute for Nursing and Biotechnology, VIVES University College, Wilgenstraat 32, 8800 Roeselare, Belgium; [3]Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, P.O. Box 7028, SE-750 07 Uppsala, Sweden; [4]The Pirbright Institute, The Pirbright Institute, Ash Road, Pirbright, Surrey, GU24 0NF, UK.

Abstract | Peste des petits ruminants virus (PPRV) causes a highly contagious disease in both domestic and wild small ruminants, and in camels. Based on sequence analysis of the nucleoprotein (N) gene, the PPRV has been classified into four lineages. Serological investigations in Tanzania indicate that peste des petits ruminants (PPR) was introduced in 2004 in Ngorongoro district bordering Kenya before official confirmation of the disease in most districts of Northern Tanzania in 2008. In 2011, the presence of PPRV in goats of southern Tanzania district of Tandahimba bordering Mozambique was reported. The aim of this study was to perform molecular typing of PPRV strains that caused outbreak in Tandahimba district in 2011. A total of 17 (sheep=0, goats=17) out of 27 (sheep=3, goats=24) were positive for PPRV N gene. The nucleotide sequence and phylogenetic analysis clustered the PPRV from Tandahimba into lineages II and IV. The results give evidence of at least two separate introductions of PPRV into Southern Tanzania, underlining the transboundary nature of the disease, particularly in regions with uncontrolled livestock movements.

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***Correspondence** | Gerald Misinzo, Sokoine University of Agriculture, Morogoro, Tanzania; **E-mail** | gmisinzo@gmail.com
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Peste des petits ruminants virus (PPRV) causes a highly contagious disease in both domestic and wild small ruminants, and in camels. Owing to its high morbidity (80-100%) and mortality (20-80%), the disease is considered a major constraint to productivity in small ruminants industry particularly in Africa, Asia and Middle East (Munir et al., 2013). Based on sequence analysis of the fusion (F) and nucleoprotein (N) genes, the PPRV has been classified into four lineages distinct to different geographical areas. The PPRV isolates of lineage I and II are found in Western and Central Africa, lineage III is most prevalent in Eastern Africa and the southern part of the Middle East, whereas, lineage IV is most prevalent in Asian countries (Munir et al., 2012b; Munir et al., 2013).

Serological investigations in Tanzania indicate that peste des petits ruminants (PPR) was introduced in 2004 in Ngorongoro district bordering Kenya (Karimuribo et al., 2011) before official confirmation of the disease in most districts of Northern Tanzania in 2008 (Swai et al., 2009). In 2011, the presence of PPRV in goats of Southern Tanzania district of Tandahimba bordering Mozambique was reported (Muse et al., 2012). However, the genetic nature of the circulating

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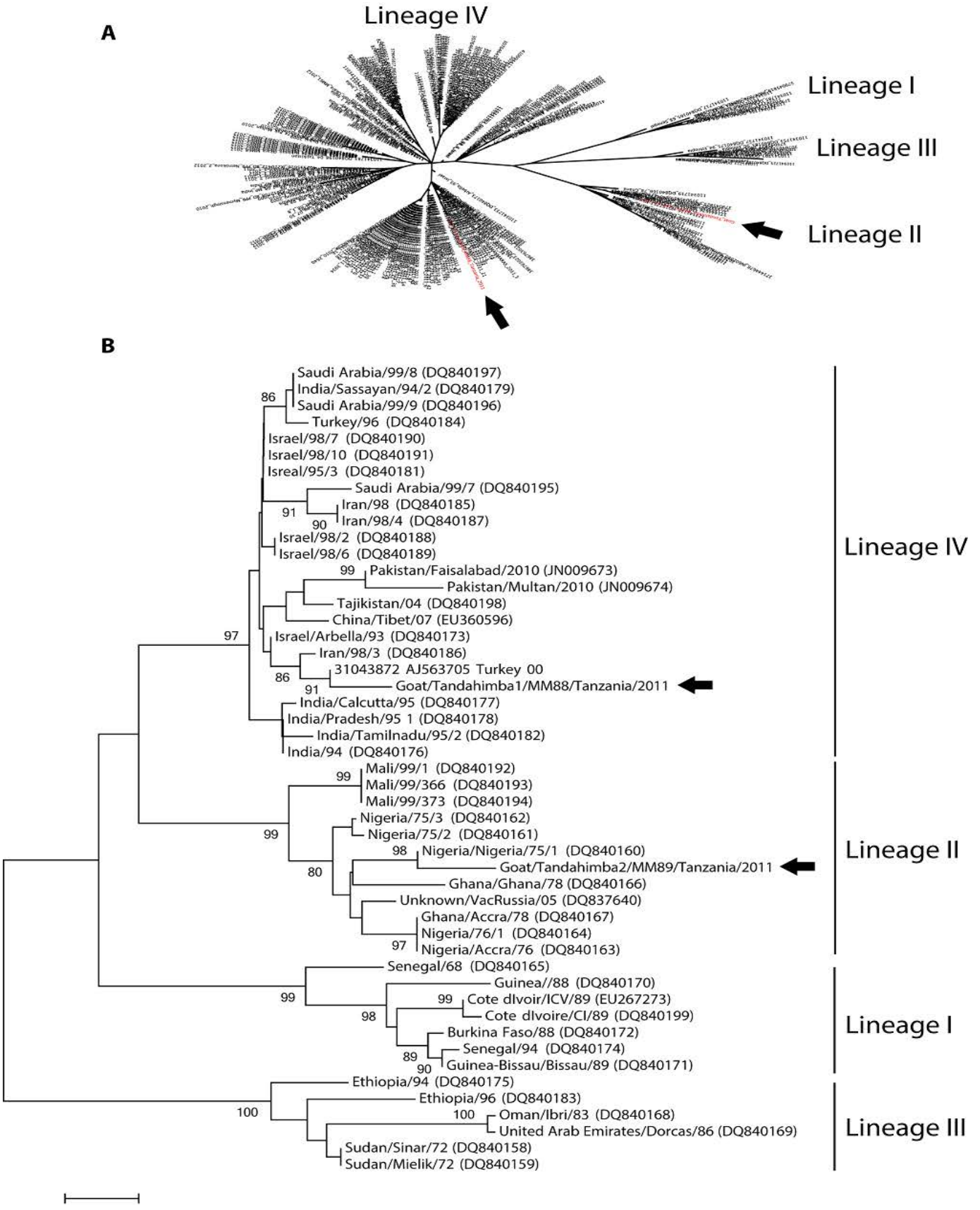

Figure 1: *Majority rule consensus tree of Peste des petits ruminants viruses based on the variable region of the N gene (255 bp) constructed by Bayesian MCMC analysis implemented in MrBayes programme. A) Branching pattern of the Tanzanian PPRV strain against all available sequences in the GenBank. B) Tree topology of Tanzanian PPRV strain with selected sequences representing all the lineages. The samples used in this study are marked with black arrows.*

PPRV, which is an essential step to establish epidemiological links for this novel disease in Tanzania remains to be determined. The aim of this study was to provide information on the phylogenetic relationship of PPRV strains that caused outbreak in Tandahimba district as reported by Muse and colleagues (Muse et al., 2012).

Nasopharyngeal and ocular swabs obtained from three sheep and 27 goats by Muse and colleagues (Muse et al., 2012) were resuspended in universal transport medium (BD, Maryland, USA). Each swab suspensions (200µl) was spotted onto FTA cards (Whatman, Florham Park, NJ, USA) , which preserve genomic material and lyse the cells and viruses. The elution of the total RNA from FTA cards was performed as previously described (Munir et al., 2012a). A real-time reverse transcription polymerase chain reaction (qRT-PCR), targeting the PPRV N gene (Kwiatek et al., 2012), was performed to screen the presence of PPRV genome in a Rotor-Gene 6000 real-time analyzer (Qiagen, Valencia, USA) using AgPath ID one-step RT-PCR kit (Applied Biosystem, Foster City, CA, USA). A total of 17 samples (sheep=0, goats=17) appeared positive and were used in conventional RT-PCR for the amplification of the hypervariable 3´-end of the N gene of PPRV using NP3 (5´-TCT CGG AAA TCG CCT CAC AGA CTG-3´) and NP4 (5´-CCT CCT CCT GGT CCT CCA GAA TCT-3´) primers (Couacy-Hymann et al., 2002) for sequencing and subsequent phylogenetic analysis.

The sequence of the N gene obtained from Tandahimba strains of PPRV showed 83-98% nucleotide identity level compared with sequences available in GenBank using the BLASTn tool (www.ncbi.nlm.nih.gov/blast). Phylogenetic analysis was performed with two representative sequences (GenBank accession numbers: KF672745 and KF672746) from this study and N gene sequences representing all 4 lineages using GTR substitution model in MrBayes software and was confirmed by neighbor-joining methods in MEGA5 software.

Interestingly, PPRV strains from Tanzania were clustered in two distinct lineages. One group of Tanzanian strains clustered within lineage IV with previously characterized strains from Middle East (Figure 1). This lineage was previously shown to be prevalent only in Asian and Middle East countries, however; recently it is reported from several countries of Africa (Luka et al., 2012; Maganga et al., 2013), including Uganda that border Tanzania (Luka et al., 2012). Another strain clustered within lineage II, a lineage described in Uganda (Luka et al., 2012) and showed 98% identity with a virus from Nigeria (Nig/75/1), which is used as vaccine virus strain in many countries. There was no history of PPR vaccination in Tandahimba at the time of sampling (Muse et al., 2012), suggesting that obtained sequences originated from circulating field viruses related to Nig/75/1 rather than being vaccine derived. This suggestion was supported by the clinical presentation typical of PPR (Muse et al., 2012). Moreover, it also gives evidence of at least 2 separate introductions of PPRV into Southern Tanzania, underlining the transboundary nature of the disease, particularly in regions with uncontrolled livestock movements. Epidemiological investigation into the introduction and factors for the spread of PPR in Southern Tanzania show that the purchase of goats from Dar es Salaam may be a source of PPRV in Tandahimba (Muse et al., 2012).

In conclusion, we confirm the presence of PPRV in Tanzania, and provide genetic characterization of detected viruses, knowledge that is fundamental for control, prevention, and in the long run, eradication of the disease. Since our previous studies, an emergency vaccination programme has been launched and implemented in Tanzania. Additionally, it was recommended by the FAO that vaccination should be considered in the area bordering Malawi, Mozambique and Zambia.

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