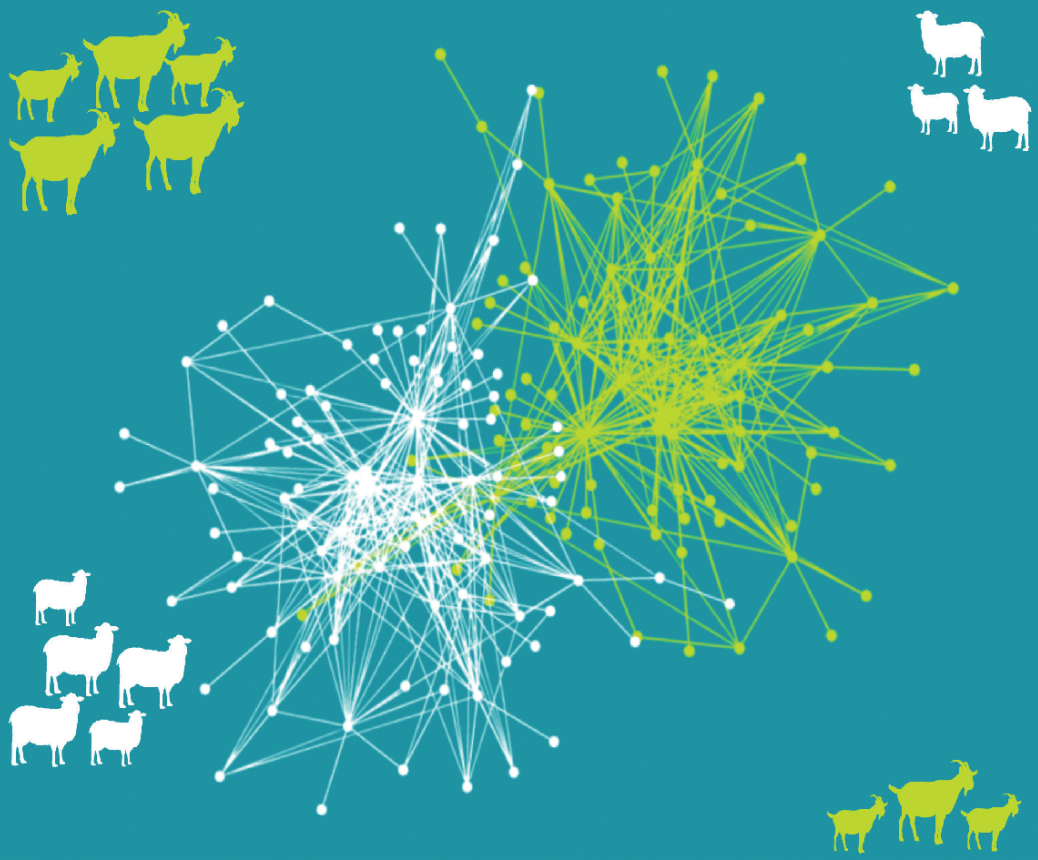
From the Institute of Virology
of the Department of Veterinary Medicine
the Freie Universität Berlin



Epidemiology of Peste des petits ruminants in Uganda

Inaugural-Dissertation
for the award of the degree of
Doctor of Philosophy (PhD)
in Biomedical Sciences
at the Freie Universität Berlin

submitted by
Joseph Nkamwesiga
Molecular Biologist from Kampala, Uganda

Berlin 2024
Journal-Nr.: 4483

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“Give me a lever long enough, and a place to stand,
and I will move the world”
(**Archimedes)**

Dedication

This is for you, Tyra Amanya Marilyn.

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List of abbreviations

Abbreviation	Definition
CIRAD	The French Agricultural Research Centre for International Development
DVO	District Veterinary Officer
ELISA	Enzyme linked immunosorbent assay
FAO	The Food and Agriculture Organization of the United Nations
IAEA	International Atomic Energy Agency
IgG	Immunoglobulin G
MAAIF	The Ministry of Agriculture, Animal Industry and Fisheries
NADDEC	National Animal Disease Diagnostics and Epidemiology Centre
PCR	Polymerase chain reaction
PPR	Peste des petits ruminants
PPRV	Peste des petits ruminants virus
UBOS	Uganda Bureau of statistics
USD	United states dollar
VI	Virus isolation
VNT	Virus neutralization test
WOAH	World Organisation for Animal Health, formerly Office International des Epizooties (OIE)

Chapter 1: Introduction

Peste des petits ruminants (PPR) is a devastating transboundary disease in domestic small ruminants and to some extent, a threat to wild small ruminant populations. The disease was first reported in 1942 in Côte d'Ivoire and by 2020, it had spread to more than 70 countries in Africa and Asia (FAO & WOAH, 2022). The disease was once reported on the European continent, in Bulgaria in 2018, thanks to the swift disease control efforts from the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA), the threat was quickly neutralized through animal movement restriction, banning of livestock trade from affected areas coupled with an efficient surveillance system (IAEA Bulletin, 2020). However, considering that PPR still circulates in western Turkey, a part of continental Europe (Altan et al., 2019), and in North African countries that are closer to Europe such as Algeria and Morocco (Baazizi et al., 2017; Parida et al., 2016), an increased threat of PPR incursion into Europe still exists. In countries where PPR is endemic, it is associated with huge global economic losses estimated at between USD 1.5 billion to 2.1 billion per year (Jones et al., 2016). These losses result majorly from direct animal losses, productivity reduction (milk and meat), lost international trade opportunities and disease management related expenses such as vaccination and management of secondary opportunistic infections following the viral infection (Jones et al., 2016).

The huge economic burden posed by PPR indicates that the disease has significantly contributed to increased hunger, malnutrition and poverty, especially among vulnerable poor smallholder farmers including women and the youth who tend to dominate the small ruminant production enterprise (FAO & WOAH, 2022). Small ruminant production is attractive to smallholder farmers due to the short generation interval, quick conversion into cash in case of need and generally the relatively low capital input required to start the enterprise (Sargison, 2020). Small ruminant production sustains lives of more than 300 million people across Africa and Asia where more than 80% of global small ruminant populations are kept (Devendra, 1994; FAO, 2019).This small ruminant population is now threatened by PPR which affects almost 100% of the animals in flocks and kills close to 90% of them, especially when it occurs in naïve flocks (Dundon et al., 2017).

In Uganda, the first recorded outbreak of PPR was officially confirmed in 2007, and was associated with death of over 500,000 small ruminants valued at about USD 30 million in the Karamoja sub-region, northeastern Uganda, alone. However, the disease has since spread to several districts in central and western regions of Uganda, home to the next highest number of the small ruminant population after Karamoja (Nkamwesiga et al., 2022; UBOS & MAAIF, 2024).

In 2012, the devastating effects of PPR prompted FAO and the World Organisation for Animal Health (WOAH) to initiate a multi-national, multi-stage PPR global control and eradication program (PPR-GEP) by 2030 (FAO & WOAH, 2016). The PPR-GEP was designed to be implemented in four stages: (i) Assessment, (ii) control, (iii) eradication and (iv) post eradication. The PPR-GEP stage 1 is meant to allow individual countries to conduct self-assessment of epidemiological and socio-economic risk factors and demonstrate or develop efficient surveillance and diagnostic capacities. Stages 2 and 3 were designed to ensure that disease control efforts such as risk-based vaccination are initiated, in addition to significantly revamping surveillance and diagnostic capacities. The final stage was designed to ensure the disease is sustainably eradicated and disease-free status maintained through ensuring proper stakeholder engagement, enabling legal framework and sustainable surveillance and diagnostic systems (FAO & WOAH, 2022).

Uganda is currently (2024) at stage 2 of the PPR-GEP as per the recent self-assessment exercise conducted (Ayebazibwe et al., 2022). However, as much as control activities such as vaccination are being implemented, the country is yet to exhaust pertinent activities in the assessment stage. For example, there is still need to update the epidemiological situation in different small ruminant production systems. Additionally, the absence of innovative approaches to guide optimal utilization of available control options such as rational distribution of the already scarce vaccines, identification of suitable districts to institute quarantine and surveillance among others, make it difficult to effectively control PPR especially without requisite epidemiological evidence. These and many other challenges have contributed to Uganda's slow progression from one PPR-GEP stage to the next. To contribute to the global efforts of control and eradication of PPR in Uganda, this PhD study was designed to directly address some of these gaps to contribute to the national as well as the global effort of control and eradication of PPR by the year 2030.

This thesis was designed with four aims organized in four chapters as follows:

i. characterize locations (districts) in Uganda where PPR outbreaks tend be more frequently reported and identify the high-level spatial conditions associated with such locations using purely spatial modelling and statistical regression approaches.

ii. determine the prevalence of PPRV specific antibodies across the main small ruminant production systems in Uganda (pastoral, agropastoral and mixed crop-livestock production systems), and identify production system- and animal-level risk factors for PPRV transmission.

iii. estimate PPR incidence and determine PPRV lineages and other relevant coinfections circulating in all suspected PPR outbreaks in Uganda (2020—2022).

iv. identify suitable locations (districts) for active surveillance, targeted vaccination, quarantine and other relevant PPR control measures through small ruminant movement network analysis of a 9-year dataset using social network analysis and graph theory approaches.

Chapter 2: Literature Review

2.1 Peste des petits ruminants virus

Peste des petits ruminants virus (PPRV) is a mono serotype, negative-sense, enveloped RNA virus between 450-500 nm in size when viewed under a negative-stain electron microscopy (Gibbs et al., 1979). Similar to other viruses that cause important veterinary and public health diseases such as measles, rinderpest, canine distemper, and phocine distemper, PPRV is a member of the genus *Morbillivirus*, subfamily *Paramyxovirinae*, family *Paramyxoviridae*, order Mononegavirales and currently the only member of the species *Morbillivirus caprinae* (Gibbs et al., 1979; Parida et al., 2015; Postler et al., 2016).

It is believed that PPRV genetically departed from Measles virus and Rinderpest virus between the 19th and 20th century (Gibbs et al., 1979). The PPRV envelope is composed of two main virulence factors; Hemagglutinin (H) and Fusion (F) proteins which help the virus to attach and fuse to the host cells respectively during infection of the host cell. The viral genome is wound by nucleocapsid protein (N) which protects the viral RNA from host cell degradative mechanisms such as nucleases (De Nardi et al., 2012). These proteins (H & N) are highly antigenic and are consequently incorporated in PPRV immunodetection assays (Libeau et al., 1995; Parida et al., 2015). The PPRV genome is made up of ~15,948 nucleotides and carries six functional genes (structural protein transcription units); Nucleocapsid (1,689 nucleotides), Phosphoprotein (1,655 nucleotides), Matrix protein (1,484 nucleotides), Fusion protein (2,410 nucleotides) and Hemagglutinin protein (1,957 nucleotides). Additionally, the genome contains two non-structural proteins C (177 amino acids) and V (298 amino acids) (Banyard et al., 2010).

2.2 Epidemiology and economic impact of PPR

About 60% of the world small ruminant population (n ~2.1 billion heads) are kept in Asia, 34% in Africa and only about 6% are kept in other parts of the world (FAO, 2019). By the year 2020, PPR had been reported in almost the whole of Africa, most parts Asia, western Turkey and Bulgaria (Banyard et al., 2010; FAO & WOAH, 2022). PPR was first recognized in Côte d'Ivoire in 1942, however by the year 2020, the disease had spread to the rest of Africa including north African countries, such as Morocco and Algeria, which had been PPR free for a long time until 2008 (Albina et al., 2013; Soltan & Abd-Eldaim, 2014).

The losses caused by PPR in Africa were most felt after 2007; the time when most countries experienced their first PPR outbreaks, including the Republic of Congo, Uganda and Kenya. Economic losses associated with PPR losses result from lost trade opportunities, direct animal losses and losses in production which negatively impact the livelihoods of the affected farming

households and their entire small ruminant value chain. For example, the economic loss due to PPR outbreaks in Turkana County in Kenya was estimated to be ~ USD 19.1 million (Kihu et al., 2015) . PPR significantly affects the economy of Africa's small holder farmers who are the majority small ruminant keepers. About one third of the global financial burden posed by PPR is suffered by countries in Africa (FAO & WOAH, 2016).

There are four PPRV lineages that can be effectively identified and characterized by partial sequencing of the F and N genes in the PPRV genome (Banyard et al., 2010; Shaila et al., 1996). Lineages I – III were found to be stable in Africa where PPRV lineage I was first confirmed in Côte d'Ivoire, West Africa around the 1970s. Lineage II was confirmed in West Africa in the 1980s while PPRV lineage III was later confirmed in Eastern Africa (Sudan) and Oman in the 1990s (Kwiatek et al., 2011) whereas PPRV lineage IV was originally thought to be exclusive to Asian countries (Libeau et al., 2014). However, PPRV Llineage IV has in the recent past been confirmed to circulate in many African countries (Libeau et al., 2014). Similarly, PPRV lineage III which is ideally the East African lineage has also been confirmed in some Asian countries including Oman (Kwiatek et al., 2011). This cross-territorial circulation of PPRV lineages suggests cross-border movement of unscreened animals for trade and other movement purposes such as social functions, paying dowry and gifting.

Many different African regions and individual countries have more than one circulating PPRV lineage (OIE, 2013). Three PPRV lineages have previously been reported to circulate in small ruminant populations in Uganda (Luka et al., 2012; Muniraju, Munir, Parthiban, et al., 2014). However, recent re-analysis of all the F and N gene nucleotide sequences in GenBank ruled out lineage II and confirmed that only lineages III and IV as the only virus lineages detected in Uganda following the first confirmed outbreak in 2007 (Muniraju et al., 2016; Nkamwesiga et al., 2019).

2.2.1 Epidemiology and economic impact of PPR in Uganda

A febrile small ruminant disease characterized by diarrhea, matting and clogging of eye and nasal mucosal surfaces with tears and mucus respectively was first reported in Karamoja subregion, north-eastern Uganda around April 2007 (Luka et al., 2012). Studies were initiated to investigate the "strange disease" in July 2007. For instance, PPRV molecular detection using F gene sequencing confirmed PPRV for the first in Uganda (Luka et al., 2012). Additionally, samples were shipped to the PPR reference laboratory at Pirbright Institute in the UK for confirmation. Two years later (2009), the majority (57.6 %;95% CI: 48.8 – 66.4) of small ruminants in Karamoja had been exposed to PPRV (Mulindwa et al., 2011) whereas a relatively small proportion of goats and sheep from surrounding districts tested positive for PPRV specific antibodies (Ruhweza et al., 2010). PPRV specific antibodies have since been

detected in goats and sheep in urban and peri-urban areas including the capital Kampala, which indicates that the virus has rapidly spread to new areas in Uganda (Lernfelt, 2013). PPR remains one of the most important constraints to over 6 million small ruminants kept in Karamoja, and to the national small ruminant flock in Uganda that counts over 21 million (UBOS & MAAIF, 2024). There is no study in Uganda today that demonstrates the exact magnitude (or projection) of the compound economic loss due to PPR in Karamoja sub-region or in Uganda as a whole. By 2020, the PPRV seroprevalence in the different production systems was still not known.

2.3 Peste des petits ruminants virus transmission

Peste des petits ruminants virus infects its hosts via the respiratory route. The virus uses its envelope proteins (i.e., Hemagglutinin and Fusion) to attach and fuse with the host epithelial cell membrane respectively (De Nardi et al., 2012). The virus organ predilection sites include the lymph nodes, spleen and lungs. Infected animal secretions such as saliva, tears, nasal mucus and feces potentially contain sufficient concentrations of PPRV that could be transmissible to susceptible hosts (OIE Terrestrial Manual, 2013). Fomites (water troughs, beddings, ropes) potentially play a role in transmission of PPRV; however, these inanimate objects do not remain infective for more than 2 hours in tropical conditions where the temperature and humidity are relatively high (Des et al., 2009). Furthermore, laboratory evidence suggests that PPRV is reportedly inactivated when incubated at 37°C for about 2 hours (OIE Terrestrial Manual, 2013). Successful transmission therefore requires close contact between sick and naïve susceptible hosts (Des et al., 2009).

To sustain PPR infection, there must be a population of naïve PPR susceptible hosts large enough to maintain the domestic cycle (OIE Terrestrial Manual, 2013). This condition is often provided by the high turnover rates for small ruminants where over 15% of young animals are born into a typical flock per year. Moreover, small ruminants tend to be sold more often for slaughter purposes, a scenario that further dilutes herd immunity if any. PPRV does not present with an asymptomatic carrier status; and PPRV infection becomes self-limiting if the affected PPRV naïve small ruminant population is not large enough to warrant maintenance (OIE Terrestrial Manual, 2013).

2.3.1 Epidemiological drivers of PPRV transmission

Infectious diseases that require close contact for their transmission such as PPR are best spread in areas where wild and domestic small ruminants usually congregate, for example water points, communal grazing areas, or holding grounds for vaccination among others (VanderWaal et al., 2017). The congregation points are potential risk determinants for PPR because they facilitate the likelihood of direct contact between herds (VanderWaal et al.,

2017). Animal movements for various reasons such as trade, search for water and pasture has also proved to be a potential risk determinant for PPRV transmission. Distance from arid areas, where nomadic pastoralism with mass movement of small ruminants prevails, can also be a key risk factor for PPR exposure. National parks and other wildlife protection areas could also play a role in the transmission and spread of PPR (Ruget et al., 2019).

2.4 Diagnosis of PPR

A range of diagnostic methods with differing advantages, disadvantages and applicability are available for use in diagnosis of PPR. They include clinical detection methods (based on clinical signs), direct (detects the virus or its part) and indirect (detect past exposure to the virus through specific antibodies) methods. Field veterinarians often diagnose PPR clinically, using typical PPR characteristic symptoms such as inappetence, lacrimation, nasal discharge, diarrhea and sudden death of kids and lambs (OIE Terrestrial Manual, 2013). Traditional laboratory diagnostic methods include virus isolation (VI), virus neutralization, and immunostaining. The WOAH gold standard diagnostic tests for PPR such as virus neuralization test (VNT) and VI require advanced capability to handle live viruses and biosecurity-compliant infrastructure that is expensive to establish and maintain. The advent of modern serological and molecular biology techniques has greatly improved the turnaround time and accuracy of PPR diagnosis. Modern diagnostic techniques include monoclonal antibody-based Enzyme-linked Immunosorbent assays (ELISA) (direct, indirect, competitive, sandwich), reverse transcriptase polymerase chain reaction (RT-PCR), real time reverse transcriptase PCR (rRT-PCR), loop mediated isothermal amplification (LAMP), lateral flow assay (LFA), among others (Balamurugan et al., 2014a; Banyard et al., 2010).

2.4.1 Clinical/tentative diagnosis

The PPRV incubation period is typically 2-6 days after natural infection (Roeder & Obi, 2018). Depending on the virus lineage involved, animal breed / animal species (both domestic and wild) and immune status of the host, the average incubation period is 21 days. PPRV infected small ruminants suddenly become pyretic, restless, and present with a dull coat and dry muzzle. Within the first few days post-exposure, their gums become hyperemic, and erosive abrasions develop in the oral cavity causing extreme salivation. Excessive lacrimation with sequential congestion of conjunctiva which often results in matting of the eyelids follows. In the later stages, PPRV infected animals develop intense blood-stained watery diarrhea and mucopurulent nasal discharge resulting occasionally into fatal respiratory distress. Animals that survive this wave of acute viremia develop life-long immunity (≥ 3 years) against PPRV (OIE Terrestrial Manual, 2013).

2.4.2 Confirmatory / definitive diagnosis

The definitive diagnosis of PPR involves laboratory tests that detect the PPRV genome or its parts in the infected animal body fluids (blood, secretions and excretions). The WOAH-recommended laboratory tests include antigen detection, virus isolation and nucleic acid-based techniques. Antibody-based techniques are recommended to confirm previous exposure to PPRV or vaccination as there is currently (June 2024) no test that differentiates vaccinated animals from those naturally infected.

2.4.2.1 Antibody detection

Monoclonal antibody-based ELISA specific to PPRV are commercially available for confirmation of previous exposure to PPRV. These kits are supplied with microtiter plates precoated with purified recombinant antigens of either PPRV Hemagglutinin or Nucleocapsid proteins to enable detect IgG antibodies against PPRV with high sensitivity (99.4%) and specificity (94.9%). One of the WOAH recommended serological test is the ID Screen® PPR Competition ELISA that was developed by Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) in collaboration with a commercial partner (IDvet, Montpellier) based on the Nucleocapsid protein for detection of the most abundant PPRV proteins in serum upon infection (Libeau et al., 1995). Indirect ELISAs on the other hand have not received as much attention but have been used by previous studies to detect PPRV-specific antibodies in serum with reliable specificity and sensitivity (Balamurugan et al., 2007).

Serodiagnosis of PPRV has proven very useful, especially in providing a means of assisting in surveillance efforts through tracing of exposure to PPRV. Serodiagnosis has been applied albeit to a less extent in evaluating vaccination campaigns because the currently available serological tests do not discriminate between naturally infected from vaccinated animals (Balamurugan et al., 2014a; Mulindwa et al., 2011). The antibody-based techniques benefit from advantages of being relatively cheap (cost per sample), short turnaround time (average 1.5 hours to test at least 90 samples), among others as compared to VI and VNT (OIE Terrestrial Manual, 2013). However, Antibody detection methods, face a challenge of requiring basic equipment such as incubators, colorimetric equipment for reading the plates such as ELISA readers, which are not easily available in resource-limited settings.

2.4.2.2 Virus isolation and culture

To isolate PPRV, sample material (nasal swab, ocular swab, whole blood or tissue) from suspected animals is inoculated onto a monolayer of cell lines (e.g. Vero-DogSLAM, CHS20 etc) grown on either culture plates or flasks. Minimum essential medium (MEM) or basal medium Eagle (BME) supplemented with 2mML-Glutamine and 10% fetal bovine serum are often used for PPRV culturing (Eloiflin et al., 2019). The cultures are monitored for development of characteristic PPRV cytopathic effects days after inoculation (OIE Terrestrial Manual, 2013). The Spearman–Karber method is one of the reliable methods used to titrate virus solutions on cell lines (Freeman et al., 1986). The titrated virus solutions can then be used to infect other cell lines for further studies or stored at ultra-low temperature conditions (in liquid nitrogen) for future use. This test is the gold standard for most viral diagnostic tests as recommended by the WOAH. The assay allows for further studies to be carried out on the isolated organisms and it provides for direct measure of infectivity. Nonetheless, this technique suffers a number of drawbacks such as long time periods (4 - 14 days) required for virus detection and may require multiple reagents and consumables which makes it quite costly (Rodríguez et al., 2009). Samples from animals with high PPRV-specific antibody titers may not be culturable regardless of how much many genome-copies present due to viral neutralization (Logan et al., 2016).

2.4.2.3 Nucleic acid-based techniques

Molecular detection of PPRV genetic material is done using conventional or real time reverse transcriptase polymerase chain reaction (RT-PCR /rRT-PCR) primer pairs targeting genes encoding the most abundant viral proteins. For example, RT-PCR protocols that amplify the Nucleocapsid, Fusion and Hemagglutinin genes on the complementary DNA (cDNA) template are widely available. These PCR techniques give very specific amplification with no cross-reactivity with other related morbilliviruses. These assays can now be performed in one step using the One step RT-PCR commercial kits that contain enzymes for both reverse transcription and polymerase activity. Available also, are real time PCR platforms for real time detection of viral RNA even in the earliest infections. This allows quantification of the viral RNA copies in the sample thus giving the lowest limit of detection with little or no chance for PPR misdiagnosis due to low viremia (Abera et al., 2014).

2.5 Peste des petits ruminants virus genotyping

PPRV has been studied in most of the countries where the disease is endemic posing a threat to small ruminant health and production. Genotyping is typically done by nucleotide sequencing using either capillary or next generation sequencing platforms. The viral Fusion (F) and/or Nucleoprotein (N) genes have been recommended as adequate targets to efficiently delineate the different PPRV lineages. The rate of mutations in the N gene is higher than that of the F gene making the N gene a better candidate for delineation of the four PPRV lineages (Padhi & Ma, 2014). Some studies have reported the *Hemagglutinin* (H) gene to have a higher PPRV lineage discrimination power than that of the *Nucleoprotein* (*N*) gene (Liang et al., 2016; Rahman et al., 2018), however there are relatively fewer studies that have sequenced the H gene than F and N. Whole genome sequences of the four PPRV lineages from different countries including Uganda, exist in the GenBank (Sahu et al., 2017). Much as PPRV has one serotype and all antibodies raised against any of the four lineages are protective against all forms of PPR disease, continuous genotyping helps with tracking of virus changes overtime in addition to tracking the dissemination and spread of the disease from one area to another.

2.5.1 Capillary and Next generation sequencing

Sanger sequencing uses the principle of dideoxy ribonucleotide triphosphate (ddNTPs) as elongation chain terminators. Typically, the DNA template is divided into four different aliquots each containing all the four standard nucleotides (dATP, dCTP, dGTP and dTTP), DNA polymerase, short primer (dye or radioactive labelled) and only one of the chain terminators (ddNTP). In the presence of these necessary reaction components, the DNA polymerase extends the primer by adding complementary nucleotides until the chain terminator is incorporated. At the end of the reaction, a mixture of extension products of varying lengths is then resolved on a denaturing polyacrylamide gel in four parallel lanes. The exact sequence of nucleotides is then manually read-off the gels following the migration patterns in the four lanes (Karger & Guttman, 2009).

In capillary sequencing, the primer and the chain terminator nucleotides are labelled with fluorescent dyes that enable optical detection of the bases in a single tube. The equipment set up comprises of a capillary tube studded with a thick gel polymer that allows separation of migrating DNA fragments at a resolution of a single base once the electric current is applied. The sample is automatically injected into the capillary tube with the aid of an electric current. The negatively charged DNA fragments migrate towards the anode and at the fluorophore on each labeled base is excited by the lesser beam that fires through the tube. Each base is labeled with a fluorophore that emits light of a particular wavelength unique to each base (e.g. A = Green, C = Blue, G = Black and T = Red) (Figure 1). These four different colors are

detected by the inbuilt camera and captured as chromatograms containing peaks of each base and the sequence can then be determined (Karger & Guttman, 2009). When the sample (purified PCR amplicon) is very good (strong visible bands on the gel) and the sequencing primer is good, single-colour evenly spaced chromatogram peaks are often observed. It is normal for peak heights to vary sometimes up to 3-fold. There may be “noise” at the base of the peaks, but this is always minimal when the template is good, and this minimal noise does not interfere with automated base-calling and human eye doublechecking (Figure 1).

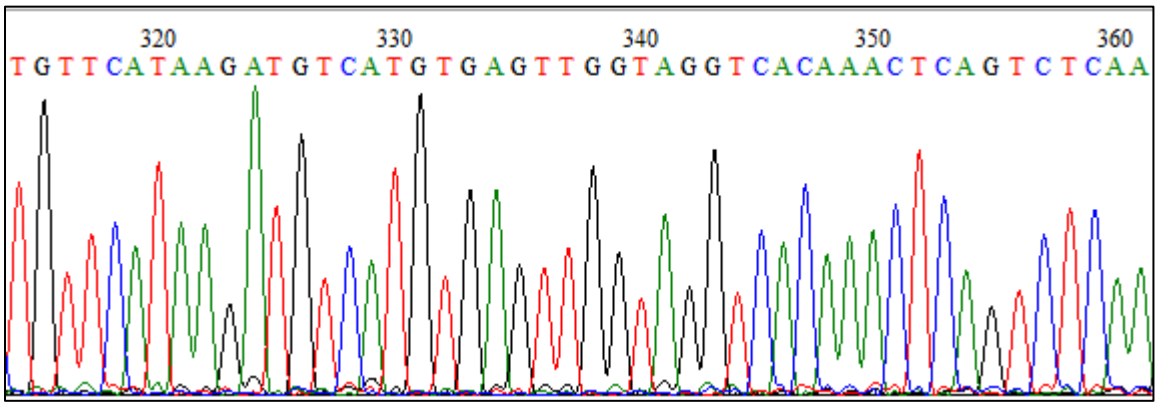

Figure 1: Sequence Chromatogram

Next generation sequencing (NGS) platforms on the other hand, are a series of technologies designed to continuously optimise and improve the Sanger sequencing method in terms throughput and cost effectiveness. Larger genomes can now be sequenced in a couple of hours as compared to multi-billion multi-year genome projects in the past, except, the number of bases sequenced at a time are much lower than in Sanger sequencing (Rajesh & Jaya, 2017). The accuracy of base calls is also not as good as the Sanger /capillary sequencing method. The NGS DNA sequencing methods (Massively Parallel Signature Sequencing, Polony Sequencing, 454 Pyrosequencing, Reversible, Terminator Sequencing by Synthesis, Sequencing by Oligonucleotide Ligation Detection, Single Molecule Real-Time Sequencing by Synthesis, Ion Torrent—Sequencing by Synthesis etc.) are completed in three common steps: The first step is usually library preparation by breaking down DNA molecules into multiple short fragments using either enzymatic or mechanical methods such as sonication. In the next step, the prepared DNA libraries are ligated to specially developed DNA adapters that aid specific binding of DNA fragments onto a solid surface for amplification using PCR and clonal amplification. The last step is to sequence the libraries using an array of techniques depending on the platform in question (Rajesh & Jaya, 2017).

2.6 PPR control methods

The hope of successful PPR control relies on vaccination, animal movement control and eventual elimination/eradication by test-and-slaughter technique. There is an effective live attenuated vaccine that has been proven safe and efficacious against all the four known PPRV lineages for close to three decades (Diallo et al., 1989). Recent research advances are trying to improve this vaccine formulation in respect to thermal stability, targeting more than one small ruminant disease pathogen and making it a marker vaccine (Mariner et al., 2012, 2017; Sen et al., 2010).

2.6.1 Global strategy for control and eradication of PPR (PPR GCES)

WOAH and FAO have earmarked PPR eradication by the year 2030. This eradication campaign banks on the success of the Rinderpest campaign, where effective diagnosis, efficacious vaccine that provides lifelong immunity and PPRV just like RPV, having no carrier status which led to RPV eradication in 2011 (FAO & WOAH, 2016). In addition, other control measures relative to specific PPR epidemiology also need to be addressed (Mariner et al., 2016). The 15-year PPR eradication strategy was designed with four progressive stages to ensure sustainable control of PPR and eventually other important small ruminant diseases through improved veterinary service delivery. The strategy comes with a PPR monitoring and assessment tool (PMAT) to enable categorize individual countries into four different PPR control-stage categories (FAO & WOAH, 2016). The PMAT results for each country will be presented and reviewed during annual PPR regional roadmap stakeholder meetings to establish each country's progress. Individual countries and consequently regions will benefit from the incentives that come with WOAH recognition of PPR freedom including improved profitability of the small ruminant venture for instance through access to lucrative international markets (FAO & WOAH, 2016).

2.6.2 Uganda national PPR eradication strategy

Following national key stakeholders' (Research, academic institutions, private sector and relevant government ministries) consultation meetings, the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), developed a 15-year national PPR eradication plan. This plan was designed to align with that of the regional Intergovernmental Authority on Development (IGAD) and the continental Pan African and Global strategies for PPR control and eradication (AU-IBAR, 2019). The conversations were shaped by the global PPR eradication strategy 2030 guidelines. As it is the case for the global strategy, the Uganda national PPR strategy follows the four progressive stages of PPR control guided by the three integrated components of the global eradication strategy (FAO & WOAH, 2016). By 2017, Uganda was on stage one,

carrying out assessment of the national PPR situation, with much more resources required to ramp up the efforts to document the PPR status to inform rational deployment of control measures (stage 2) across the country (personal communication). It is against this background, that research institutions, external funding bodies have come on board to help propel Uganda to the next steps of this eradication campaign.

The commercially available live attenuated vaccine designed in the 1980s from a PPRV lineage II strain (Nigeria 75/1) has assumed world-wide application (Diallo et al., 1989). One dose of this vaccine provides lifelong protection (≤ 3years) against all known lineages (Albina et al., 2013; Diallo, 2015). Despite the availability of an efficacious vaccine and a national PPR control strategy, there is no evidence-based national PPR control strategy. The lack of an evidence-based vaccination strategy is believed to have led to endemicity of PPR in the original Karamoja focus and its uncontrolled spread to new central and south-western Uganda foci.

Chapter 3: Spatio-temporal cluster analysis and transmission drivers for Peste des Petits Ruminants in Uganda

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ORIGINAL ARTICLE

Spatio-temporal cluster analysis and transmission drivers for Peste des Petits Ruminants in Uganda

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Abstract

Peste des Petits Ruminants (PPR) is a transboundary, highly contagious, and fatal disease of small ruminants. PPR causes global annual economic losses of between USD 1.5 and 2.0 billion across more than 70 affected countries. Despite the commercial availability of effective PPR vaccines, lack of financial and technical commitment to PPR control coupled with a dearth of refined PPR risk profiling data in different endemic countries has perpetuated PPR virus transmission. In Uganda, over the past 5 years, PPR has extended from northeastern Uganda (Karamoja) with sporadic incursions in other districts /regions. To identify disease cluster hotspot trends that would facilitate the design and implementation of PPR risk-based control methods (including vaccination), we employed the space–time cube approach to identify trends in the clustering of outbreaks in neighbouring space–time cells using confirmed PPR outbreak report data (2007–2020). We also used negative binomial and logistic regression models and identified high small ruminant density, extended road length, low annual precipitation and high soil water index as the most important drivers of PPR in Uganda. The study identified (with 90–99% confidence) five PPR disease hotspot trend categories across subregions of Uganda. Diminishing hotspots were identified in the Karamoja region whereas consecutive, sporadic, new and emerging hotspots were identified in central

and southwestern districts of Uganda. Inter-district and cross-border small ruminant movement facilitated by longer road stretches and animal comingling precipitate PPR outbreaks as well as PPR virus spread from its initial Karamoja focus to the central and southwestern Uganda. There is therefore urgent need to prioritize considerable vaccination coverage to obtain the required herd immunity among small ruminants in the new hotspot areas to block transmission to further emerging hotspots. Findings of this study provide a basis for more robust timing and prioritization of control measures including vaccination.

KEYWORDS
GIS, hotspots, Peste des Petits Ruminants, regression models, transmission drivers, Uganda

1 | INTRODUCTION

Peste des Petits Ruminants (PPR) is a distressing viral disease of domestic small ruminants (goats and sheep) in Africa, Asia and the middle East caused by Peste des Petits Ruminants virus (PPRV) (family *Paramyxoviridae*) (Amarasinghe et al., 2019; Banyard et al., 2010; Parida et al., 2015).

PPRV has one serotype with four distinct phylogenetic lineages. The PPRV lineages I, II and III are stable in Africa whereas lineage IV is predominant in Asia and the middle East. However, there has been recent geographical expansion of lineage coverage with lineage IV reported in many parts of Africa including Tanzania, Ethiopia and South Sudan, countries neighbouring or close to Uganda. PPRV lineage IV territorial expansion has been attributed to uncontrolled cross border animal movements (Alemu et al., 2019; Padhi & Ma, 2014; Tounkara et al., 2018). Three (I, II and III) of the four virus lineages have been confirmed to circulate in Uganda. During the past 7 years, most of the PPR occurrences in Uganda have been as a result of PPRV Lineage III (Dundon et al., 2020; Muniraju et al., 2014; Nkamwesiga et al., 2019).

PPR is endemic in most parts of Africa and Asia. Coincidentally, Africa and Asia are home to more than 80% of the global small ruminant population. The estimated PPR seroprevalence in Africa and Asia is about 40% (Ahaduzzaman, 2020). The disease presents with sudden increase in temperature (40—41.3°C). In the early days post infection, the animals look visibly weak, dull, restless with reduced appetite. This is usually followed by serous discharge from the eyes and nose that later becomes mucoid sometimes leading to matting of the eyelids and blockage of the nasal passage. Diarrhea usually follows leading into dehydration and emaciation. After 10–12 days, affected animals either die or recover to obtain immunity from subsequent PPR infections (Balamurugan et al., 2014; Diallo et al., 2007). The morbidity rate in naïve small ruminant populations can reach up to 100% whereas the mortality rate ranges between 23% and 100%, depending on the breed of the animals and the virulence of the PPRV lineage involved (Chowdhury et al., 2014). Even though PPR-induced small ruminant mortality and morbidity is much lower in PPR endemic areas, PPR still causes significant production losses through reduced milk yield, poor animal body condition and cost of treating secondary bacterial infections in unvaccinated flocks. The global annual losses as a result of PPR are estimated to be between USD 1.45–2.10 billion (Jones et al., 2016; OIE-FAO, 2015). This indicates that PPR significantly affects the livelihoods and wellbeing of smallholder livestock farmers in Africa and Asia. In recognition of its socioeconomic importance, the World Organisation for Animal Health (OIE) and the Food and Agriculture Organisation (FAO) launched the PPR Global Control and Eradication Strategy (PPR GCES) in 2015. It is anticipated that affected countries (or regions) will develop and implement the progressive control pathway for PPR (PCP-PPR) and eradicate the disease by the year 2030 (OIE-FAO, 2015). The strategy is built around four stages which are (i) assessment stage, (ii) control stage, (iii) eradication stage and (iv) post-eradication stage (OIE-FAO, 2015). Uganda is currently at stage 2 of this PPR-GCES and has drafted a PPR-GCES aligned PPR national control strategy that is pending approval and publication.

Effective PPR control requires deep understanding of the disease epidemiology in the affected countries (Mariner et al., 2016). The disease majorly spreads from infected to susceptible animals through human activities such as animal movements for purposes of breeding, social functions, livestock trade, returning unsold livestock to the flocks without observing quarantine measures and communal animal husbandry practices such as sharing water sources (FAO, 1999; Fournié et al., 2018). PPRV natural and experimental infection studies have indicated possible source of PPRV infection from a range of atypical domestic livestock hosts such as pigs, cattle, camels and dogs, which therefore, need to be included in surveillance plans either as sources of infection or at least as surveillance indicators of PPR transmission (Gortázar et al., 2021; Rahman et al., 2020). Livestock species such as pigs have been experimentally proved to be sources and amplifiers of PPRV (Schulz et al., 2018). A significant number of wild artiodactyls have also been previously reported as susceptible although with low levels of infection believed insufficient for sustained transmission among wild ruminants (Jones et al., 2021).

The available PPR control measures include vaccination, animal movement restrictions (quarantine), good biosecurity measures such as proper carcass disposal, and proper management practices that

restrict chances of direct contact between flocks, among others. To be able to achieve the 2030 PPR GCES, PPR endemic regions (or eco-zones) and individual countries first need to fully understand PPR eco-epidemiology (OIE-FAO, 2015). However, most disease endemic countries including Uganda have not documented the full eco-epidemiology of PPR. Isolated studies in Uganda indicate that PPR has been endemic in northeastern Uganda (Karamoja region) for the past decade. PPR recently extended to isolated districts in central and southwestern Uganda (Fernandez Aguilar et al., 2020; Lernfelt, 2013; Luka et al., 2012; Mulindwa et al., 2011; Ruhweza et al., 2010). Grey literature, namely PPR passive reports, from the Ugandan Ministry of Agriculture Animal Industry and Fisheries (MAAIF) further indicates that PPRV is rapidly spreading to previously non-endemic districts in Uganda.

Although spatiotemporal and broader epidemiological studies are necessary primers for designing and implementing PPR surveillance and risk-targeted control programs, for example vaccination (A. K. M. A. Rahman et al., 2021; Abdrakhmanov et al., 2022; M. H. Rahman et al., 2021; Ma et al., 2019; Ruget et al., 2019), such studies have not been undertaken for Uganda. As such, there is a dearth of information about PPR hotspot patterns, and epidemiological drivers of PPR transmission. Consequently, PPR has not been prevented from spreading from its initial northeastern Uganda (Karamoja) focus to other regions, even though effective attenuated PPRV vaccines are commercially available. This has put the population of 16 million small ruminants in Uganda at risk of PPRV infection. To bridge this information gap, we used spatiotemporal cluster analysis and statistical regression approaches to fit a purely spatial model to identify the high-level spatial conditions associated with places in which PPR tends to be present and characterize those places in which the disease is frequent using epidemiological factors, such as past laboratory-confirmed outbreak reports (2007–2020), animal movements and environmental data sets. This information will support the design and implementation of PPR GCES for Uganda.

2 | MATERIALS AND METHODS

2.1 | Study area

The study included all districts of Uganda that reported at least one PPR confirmed outbreak during the study period (2007–2020). Uganda is a landlocked country located in East Africa. It borders South Sudan to the north, Kenya to the east, Democratic republic of Congo to the west, Tanzania to the south and Rwanda to the south-west (Figure 1). Uganda is divided into nine subregions (Karamoja, Acholi, Lango, Western, South Western, Central, East Central, West Nile, Elgon and Teso) and five administrative divisions (districts, counties/municipalities, sub-counties/town councils, parishes/wards and villages).

In 2019, Uganda had 135 districts. Disease reporting is usually aggregated at district level where there is a functional veterinary services department. Due to the temperate climate in all but the north-eastern parts of the country, the major economic activity in Uganda is agriculture, with crop growing and livestock keeping as the backbone of the economy. As such, Ugandans keep about 16 million small ruminants (12,344,407 goats and 3,410,371 sheep), 11,434,795 cattle, 3,184,297 pigs, 37,443,881 chickens, 1,458,253 ducks and 348,314 turkeys (MAAIF & UBOS, 2008).

2.2 | Data source and curation of dependent variables

We obtained PPR outbreak reports (passive and active surveillance) data from 2007 to 2020 from MAAIF. Reports with accompanying laboratory reports in which at least one of the samples tested positive by either OIE recommended polymerase chain reaction (PCR) or Enzyme-linked immunosorbent assay (ELISA) PPR protocols, were considered as confirmed PPR outbreaks. All reports with no corresponding laboratory report were excluded from the analysis. Two potential response variables, (i) discrete total number of confirmed outbreak reports per district and (ii) binary report data (whether 'yes' or 'no'; a district reported at least one confirmed outbreak), during the study period were generated. This was done in Microsoft Excel (Microsoft Office suite 365, version 2106, Build 14131.20320).

2.3 | Preparation of potential explanatory variables

Different sets of variables hypothesized to directly or indirectly facilitate/support the PPR mode of transmission were considered in this study as supported by recent scholarly literature and specific epidemiological aspects of PPR virus transmission. PPR transmission and spread is usually facilitated by human socioeconomic activities, bioclimatic conditions, topographic and environmental factors that tend to favour suitability of PPR disease occurrence (Gao et al., 2019). These factors, acting singly or in combination may contribute significantly to the transmission and spread of the PPR virus resulting into re-introduction or introduction of such infectious diseases into new areas. It is therefore paramount to evaluate the interplay between anthropogenic and bioclimatic factors for better control of infectious diseases (Niu et al., 2021). We obtained human population data from the Uganda Bureau of Statistics (UBOS) (UBOS, 2009). Livestock density data sets (goat, sheep, cattle, pig, horse and small ruminant) were obtained from the Gridded Livestock of the World v2.0 high resolution raster files at cell size 30s < https://livestock.geo-wiki.org/home-2 / > (Robinson et al., 2014). Environmental variables such as landcover type, soil water index and digital elevation were obtained from Copernicus global website < https://land.copernicus.eu/global / > (Buchhorn et al., 2020). Topographic slope was calculated from the elevation data using the geodesic method with GIS extension 'Slope' (Ligas & Banasik, 2012) in ArcMap v.10.7 (Esri, Redlands, CA, USA). We also obtained variables for wildlife protected areas, distance from major towns (as defined by the Uganda Bureau of statistics according to population size and infrastructure) as proxies for wildlife population density and livestock

FIGURE 1 Uganda's location in Africa (in set) and national administrative sub-region boundaries

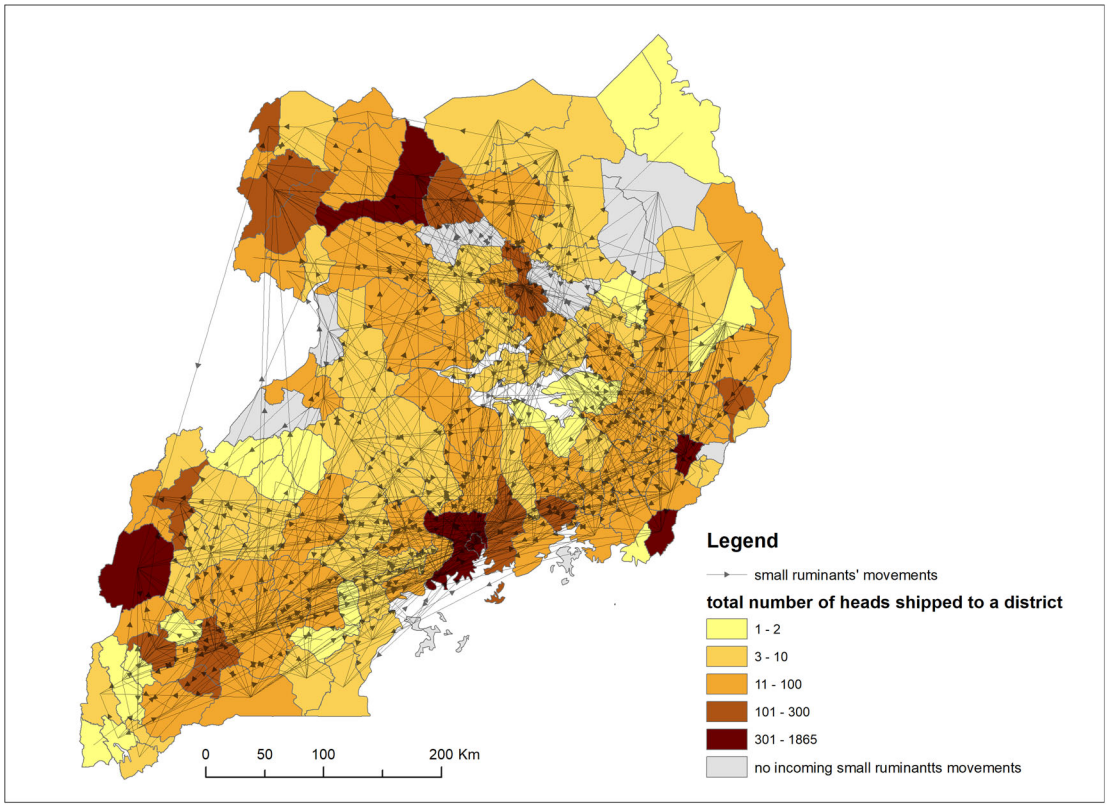

FIGURE 2 Animal movement transactions across Uganda districts (2010–2019). Each transaction line contains details pertaining to small ruminant origin, means of movement and purpose of movement as summarised using the SQL queries in ArcMap 10.7 software

markets/slaughterhouses respectively. The 19 bioclimatic variables along with solar radiation, wind speed, water vapour pressure were obtained from < https://www.worldclim.org/data/worldclim21.html > (Fick & Hijmans, 2017). Solar radiation has previously been reported to rapidly inactivate PPR virus within a couple minutes in an in vitro experiment (Latif et al., 2016), thus including such a variable might be useful in characterizing areas with likely high or low PPR transmission rates. The variables on road density and road length were computed from the roads dataset obtained from the Uganda Road network < https://geonode.wfp.org/layers/geonode:uga_trs_roads_osm >. A series of different variables were created from livestock movement data which was obtained from animal movement permits issued by officials at MAAIF (2013-2019). Movement permits were digitized in Microsoft Excel to generate a table containing all the attributes of the animal movement permit (animal species, mode of transportation, purpose of movement, number of heads moved, origin and destination among others). To this table, centroid GPS coordinates for animal origin and destination were calculated and added (since the movement permits did not include actual GPS coordinates) to create an animal movements geo-database. The frequency and the total number of heads of individual (and /combined) livestock species (goats, sheep, pigs and cattle) translocated to each destination district were computed from the created geo database (Figure 2).

All predictor variables, except for animal movement variables, were extracted from high resolution raster files available in open-source repositories. They were summarized by district followed by calculating the median values per district. Spatial Analyst, an ArcMap Desktop 10.7 extension (https://www.esri.com/en-us/arcgis/products/arcgis-desktop/resources), was used to perform raster calculations. The entire geo-database containing all 44 variables was then exported in a comma-separated (csv) file for further analysis (Supplementary Table S1).

2.4 | Building the regression models

Variable testing and regression analysis were performed with R software, version 4.05 (R Core Team, 2021). We tested all the 44 variables for multicollinearity by calculating the Variance Inflation Factor (VIF) for each independent variable. Before computing VIF, the 'alias' function in R was used to check for and later remove any perfectly correlated independent variables. Using R software packages 'car' and 'plyr' (Fox & Weisberg, 2019; Wickham, 2011), we fitted a linear regression model to the data and set out to sequentially drop all predictor variables with VIF threshold greater than 2.5 (Table 1) (Robinson et al., 2014).

Using R software package MASS (Venables & Ripley, 2002), we used a Generalised Linear Negative Binomial Regression (GLMNB) method for the count data. This was the preferred method of choice because our dependent variable [discrete total number of outbreaks

TABLE 1 A list of potential explanatory variables that were deemed eligible for use in the regression models selected based on variance inflation factor (VIF) threshold of 2.5

Variable (unit of measure)	VIF	Range	Source	Reference
Annual Precipitation (mm)	1.74	721–1935	https://www.worldclim.org/data/worldclim21.html	(Fick & Hijmans, 2017)
Precipitation Seasonality (mm)	1.83	31–60		
Digital elevation (m)	1.53	646–2219		
Median annual wind speed (ms^{-1})	1.94	1.7–2.5		
Soil Water Index for June 2019	1.46	0–250	https://land.copernicus.eu/global/products/swi	(Yao et al., 2021)
Land cover type (km^2)	2.20	2–21		
Road density (length per km^2)	2.07	0.3–15	https://geonode.wfp.org/layers/geonode:uga_trs_roads_osm	(UBOS, 2009)
Road length (km)	1.77	0–458		
Cattle density (head per km^2)	1.85	0–336	https://livestock.geo-wiki.org/home-2/	(Robinson et al., 2014)
Pig density (head per km^2)	1.50	0–122		
Sheep density (head per km^2)	2.37	0–79		
Neighbouring country reporting PPR cases	1.61	0 or 1	MAAIF	This study
Number of cattle movement transactions	1.55	0–2233		
Number of shipped heads by hoof, 2016–2020	1.22	0–3773		
Distance to the nearest 'major' city (km)	1.28	1977–79048	https://geonode.wfp.org/layers/geonode:uga_trs_roads_osm	(UBOS, 2009)
Protected area within a district (km^2)	1.49	0–2302		
Percentage of wetland areas (km^2)	1.51	0–42		

per district for the entire study period (2007–2020)] was over dispersed (i.e. the ratio between the conditional variance to conditional mean was 3.2, three times greater than the recommended 1). The GLMNB was applied using the stepAIC function that uses the Akaike information criterion (AIC) to sequentially remove all variables that are not statistically significant ($p > .05$) and generate the best-fitting model with the lowest AIC. To further assess the accuracy of our findings, we also similarly attempted fitting a logistic regression model on the cases data (yes/no; for a district that had reported a confirmed outbreak for the entire study period).

2.5 | Testing whether animal movements could explain the observed outbreaks

We performed a logistic regression test to determine whether the animal movements by year for each district were associated with the presence of PPR outbreaks so as to justify applicability of either pure spatial or spatiotemporal models. Three movement types passed the multicollinearity test [with variance inflation factor (VIF) less than 2.5]: (i) movements of small ruminants, (ii) movements by hoof (trekking) and (iii) the total numbers of movement transactions to each district were considered in the analysis.

Using SQL queries on the animal movement data (Figure 2), corresponding movement types were segregated by year and by destination district. Logistic regression models for each of the three movement types were then fit to test whether each of the movement type was significantly associated with the presence of PPR outbreaks (Outbr_bin).

2.6 | Testing for spatial autocorrelation of the model and residuals

The final model residuals and fitted values were annexed to the attributes table containing all Ugandan districts in ArcGIS. Global Moran's I method was used to test the observed and fitted values for spatial autocorrelation (Mitchell, 2005) in order to detect potential clustering and to decide whether or not the obtained set of explanatory variables allows adjusting for spatial autocorrelation. Spatial autocorrelation was tested using the Row standardization of features' spatial weights that allows for mitigation of bias due to features having different number of neighbours. Results of the analysis include *Z*-scores and *p* values, which together indicate a statistical significance of the observed pattern (standard deviations and corresponding probabilities). Moran's I index represents a measure of statistically significant *Z*

and *p* values. Positive values of I indicate a tendency towards clustering while negative values indicate a tendency towards dispersion.

2.7 | Space–time analysis and visualization

To analyse and visualize the change in PPR infection status at the district level throughout the study period (2007–2020), we applied a space–time analysis using the space–time cube data aggregation approach (Abdrakhmanov et al., 2017; Kraak & Koussoulakou, 2005). This technique generates space–time hotspots and their trends across the entire study area. The total number of confirmed PPR outbreaks was aggregated by Ugandan districts as space units, while 1 year was used as a time step for the analysis. The Getis-Ord Gi* statistics was used to generate hotspots in each location (Ord & Getis, 1995). An *Emerging Hot Spot* analysis was applied to identify trends in the clustering of outbreaks in neighbouring space–time cells. This was followed by the Mann–Kendall statistics approach that detects trends in hotspot emergence (Hamed, 2009). Depending on the trend category revealed, this analysis assigns each district a particular pattern of a hotspot emergence through time (new, consecutive, oscillating, diminishing etc.), thus allowing conclusion making about the tendency of outbreaks to emerge or to fade within each district over the considered period (2007–2020 in our case).

3 | RESULTS

3.1 | Descriptive statistics

During the study period (2007–2020), a total of 221 PPR passive surveillance reports were recorded at the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) of MAAIF. Of these, 172 reports were confirmed as PPR outbreaks based on ELISA and/or PCR test results and covered about 40% (55/134) of districts in Uganda. Confirmed outbreaks per district in the entire 14-year period ranged between 0 and 12. The average number of confirmed PPR outbreaks per year and per district were 13 and 2, respectively (Figure 3). The spatial distribution of each of the 17 potential explanatory variables in Uganda was also generated (Figures S1–S3)

3.2 | Logistic regression model analysis of the animal movement variables

All the three logistic regression models revealed that animal movements were not significantly associated with the likelihood of outbreaks in any district (Outbr_bin). In our case therefore, animal movements were not significant predictors of the observed outbreaks ($p > .1$ and Null deviance nearly equals Residual deviance) (Table 2).

These results demonstrate that animal movement does not contribute to the explanation of the observed outbreaks, so the final regression models did not lose their goodness of fit with the exclusion of this variable. This further validates the fact that this set of variables was not statistically significant and was thus eliminated during the stepwise best model selection based on AIC criteria.

3.3 | Negative binomial regression (NBR) analysis

A total of 17 independent variables were fit into the negative binomial regression (NBR) model. The final model contained a set of seven variables with six of them being significantly associated with number of outbreaks in each district ($p < .05$). Increase in the road length, cattle density and soil water index were significantly associated with increase in PPR outbreaks. The model further revealed that as road density, annual precipitation and wildlife protected areas decrease in a district, the number of outbreaks tends to significantly increase (Table 3). The goodness-of-fit chi-squared test was not statistically significant ($p = .2875$); AIC: 384.48; thus, this model fits our data reasonably well.

3.4 | Predicted number of outbreaks by NBR model results

The negative binomial regression model predicted a range of 5–11 outbreaks in the Karamoja subregion and about 1–2 in the Lake Victoria crescent area (east central region). The model also predicted between 2 and 3 outbreaks to occur in the central and southwestern regions of Uganda except for Rakai and Isingiro districts with a similar range of predicted outbreaks like the Karamoja region (Figure 4a). The model residuals exhibited a nearly random pattern (Figure 4b), indicating a fairly good fit.

3.5 | Logistic regression model analysis of the likelihood of occurrence of confirmed outbreaks

A total of 17 independent variables were fit into the logistic regression model. The final model revealed a combination of five variables, with four of them being significantly associated ($p < .05$) with the likelihood of occurrence of confirmed PPR outbreaks. All the variables in this model were similar to those predicted by the NBR model except for the median annual windspeed that was negatively associated with PPR outbreak in a district (Table 4). The goodness-of-fit chi-squared test was not statistically significant ($p = .072$); AUC = 0.811, AIC = 165.22; thus this model fits our data reasonably well.

3.6 | Predicted probability of outbreaks by logistic regression model results

The logistic regression model predicted that the Ugandan districts that lie at international borders have the highest probability of having PPR outbreaks. Just like in the negative binomial regression model, it is similarly observed that the highest probability of having PPR

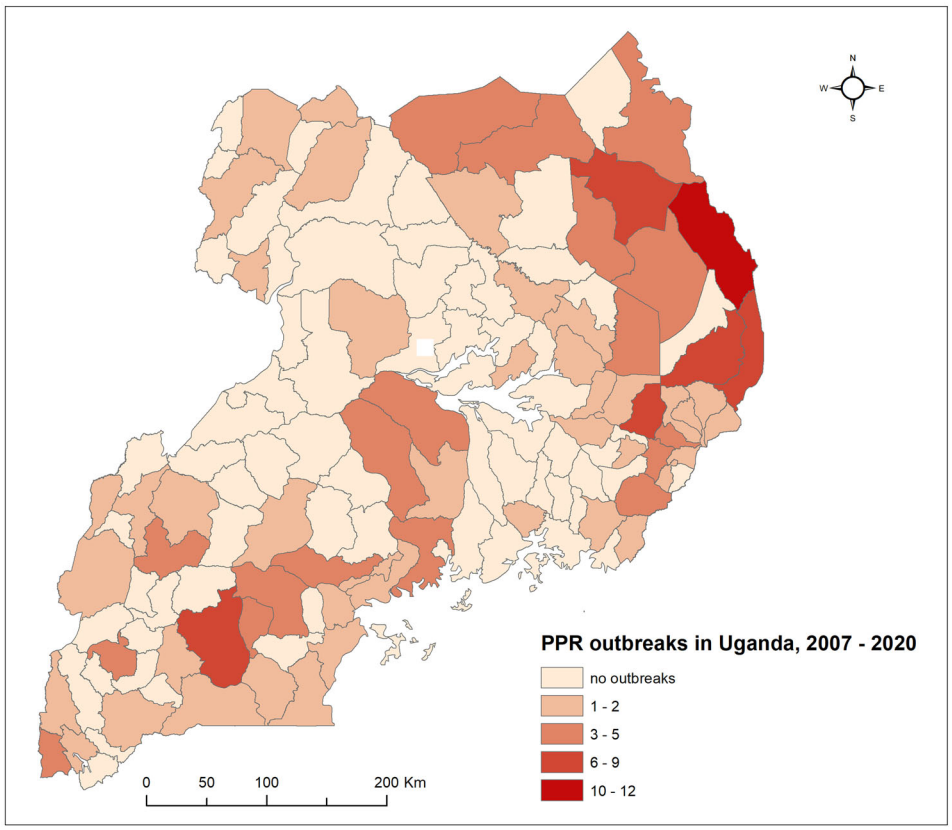

FIGURE 3 Spatial distribution of confirmed Peste des Petits Ruminants (PPR) outbreaks (2007–2020) in Uganda

TABLE 2 Logistic regression modelling of animal movement parameters as predictors of PPR outbreaks in Uganda; 2007–2020

Model	Variable *p* value	Null deviance	Residual deviance	Pseudo R^2
Outbr_bin ~ Total movements	.159	636.58	634.96	0.002
Outbr_bin ~ Livestock movement by all methods	.170	636.58	635.05	0.002
Outbr_bin ~ Livestock trekking	.122	636.58	624.24	0.019

TABLE 3 Negative binomial regression (NBR) predictors of PPR outbreaks in Uganda; 2007–2020

PPR outbreak predictor	Coefficient	Standardized coefficient	Standard error	*z* Value	Pr(>|z|)
(Intercept)	0.572		1.135	0.504	0.61431
Annual precipitation	−0.003	−0.283	0.001	−4.469	7.85e-06***
Digital elevation	0.001	0.106	0.000	1.899	0.05755
Road density	−8.321	−0.238	2.542	−3.274	0.00106**
Road length	0.005	0.200	0.001	3.048	0.00230**
Cattle density	0.005	0.107	0.002	2.134	0.03286*
Soil Water Index, June 2019	0.013	0.279	0.003	4.087	4.37e-05***
Protected area within a district	−0.001	−0.148	0.000	−2.189	0.02859*

Significance levels: ***$p < .001$, **$p < .01$ and *$p < .05$.

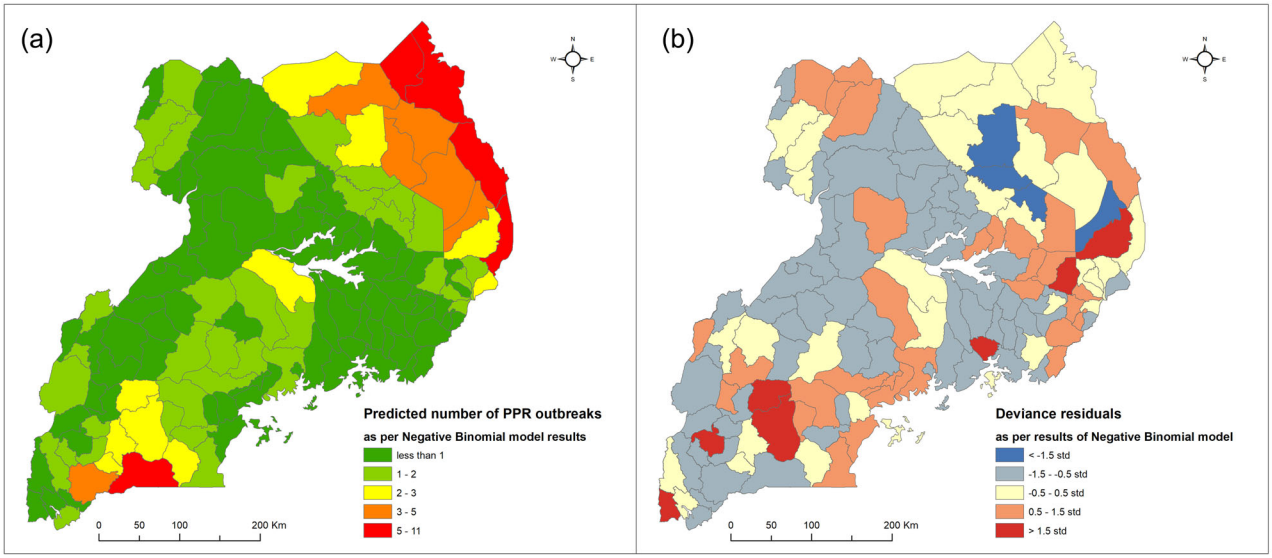

FIGURE 4 The predicted number of PPR outbreaks in Uganda as estimated by the negative binomial regression model and the distribution of the associated model residuals as visualised in ArcMap 10.7 software

TABLE 4 Logistic regression predictors of PPR outbreaks in Uganda; 2007–2020

Variable	Coefficient	Adjusted coefficient	Standard error	*z* Value	Pr (>|z|)
(Intercept)	6.408		2.514	2.549	0.01081*
Annual precipitation	−0.002	−0.852	0.001	−1.735	0.08269
Road length	0.007	1.231	0.003	2.591	0.00957**
Cattle density	0.012	1.057	0.006	2.043	0.04103*
Soil Water Index for June 2019	0.011	1.055	0.005	2.091	0.03653*
Median annual wind speed	−3.609	−1.385	1.175	−3.071	0.00213**

Significance levels: $^{**}p < .01$ and $^{*}p < .05$.

outbreaks was in the Karamoja region followed by the southwestern part of Uganda (Figure 5a). The model residuals were also randomly distributed (Figure 5b).

3.7 | Spatial autocorrelation analysis on various model inputs and outputs

The M values close to zero suggest randomness of the distribution (Figure 6). Spatial clustering of PPR outbreaks in Uganda was confirmed by testing distribution of the dependent variable (discrete total number of confirmed PPR outbreaks per district) for the NBR model that was found to be clustered with (M = 0.239, Z = 4.779 and p = .000002). Similarly, the distribution of the dependent variable (binary cases per district) for the logistic regression model was also clustered ($M = 0.143$, $Z = 2.805$ and $p = .005026$). The residuals of both models were close to random distribution with supported metrics: NBR model ($M = 0.049$, $Z = 1.058$ and $p = .289$) and logistic regression model ($M = 0.018$, $Z = 0.486$, $p = .627$).

3.8 | Space–time analysis and visualization

We identified two trend categories, 'Up Trend' and 'Down Trend' with varying degrees of confidence (90%, 95% and 99%) for the entire study period (2007–2020). The districts in the Karamoja subregion exhibited a general 'Down Trend' whereas districts around the Lake Victoria crescent (central Uganda) and southwestern Uganda exhibited a general 'Up Trend' in PPR outbreaks. There was generally no obvious pattern observed in the districts of the West Nile region and around the Lake Kyoga plains. The islands on Lake Victoria (Kalangala district) were also identified in 'Up Trend' category (Figure 7).

The 99%, 95% and 90% confidence 'Up Trend' categories consisted of 22, 19 and 10 districts respectively. The 99% 'Down Trend' category consisted of only Agago district, 12 districts in the 95% 'Down Trend' and only two districts in the 90% 'Down Trend' category (Table 5). The rest of the districts did not exhibit any significant trend.

Following the clustering pattern of PPR outbreaks through time (2007–2020), three hotspot trend categories (new, consecutive, and sporadic) were identified. Only 13 Uganda districts exhibited a

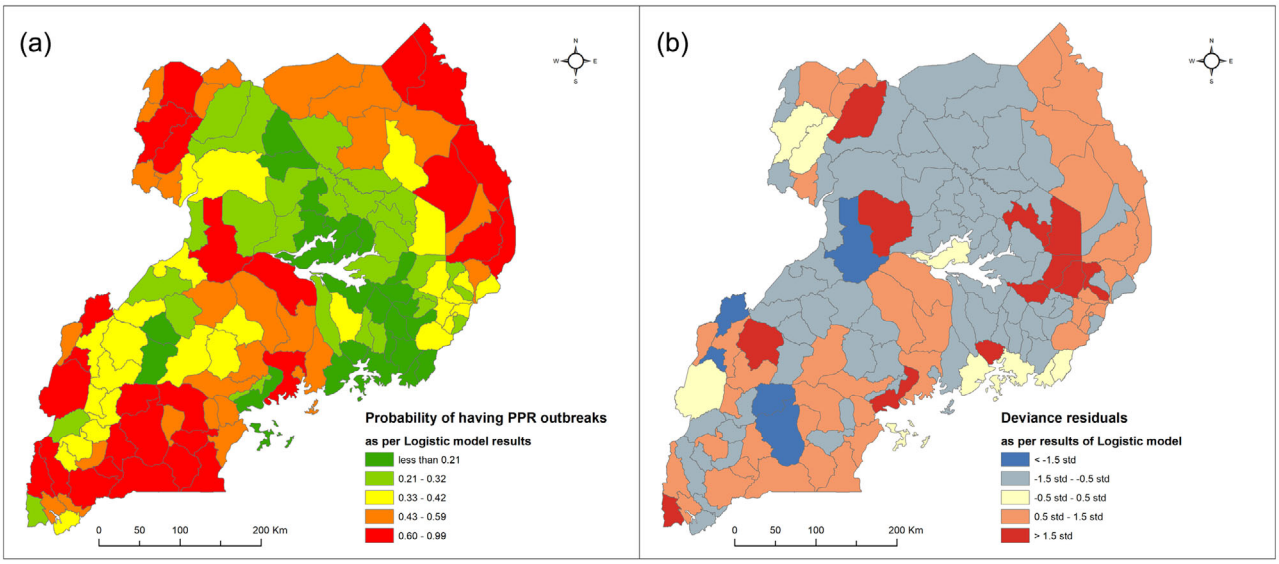

FIGURE 5 The predicted number of PPR outbreaks in Uganda as estimated by the logistic regression model and the distribution of the associated model residuals as visualised in ArcMap 10.7 software

TABLE 5 Uganda district clusters with significant (90–99%) PPR trend categories

PPR trend category (% confidence level)	Uganda district (2019)	Subregion (number of districts)
Down Trend (99)	Agago	Acholi (1)
Down Trend (95)	Kaabong, Karenga, Kotido, Abim, Napak, Kaberamaido, Kapelebyong, Lira, Kwania, Dokolo, Kitgum, Pader	Karamoja (5), Teso (2), Lango (3), Acholi (2)
Down Trend (90)	Amuria, Serere	Teso (2)
Up Trend (90)	Kabarole, Masindi, Bunyangabu, Kyenjojo, Kasese, Kamwenge, Ntoroko, Bundibugyo, Kiryandongo, Apac	Western (9), Lango (1)
Up Trend (95)	Kampala, Wakiso, Mukono, Masaka, Buikwe, Mubende, Nakasongola, Kyotera, Rukungiri, Kanungu, Ibanda, Rubirizi, Kisoro, Ntungamo, Mitooma, Buhweju, Kitagwenda, Kyegegwa, Kibaale,	Central (8), Southwestern (8), western (3)
Up Trend (99)	Kalangala, Luwero, Bukomansimbi, Mpigi, Lwengo, Lyantonde, Ssembabule, Butambala, Nakaseke, Kiboga, Gomba, Kasanda, Mityana, Kalungu, Rakai, Mbarara, Kiruhura, Sheema, Bushenyi, Isingiro, Rwampara, Kazo	Central (15), Southwestern (7)

significant trend and were categorised in at least one of the trend categories whereas the rest of the districts exhibited no significant pattern (Figure 8). Four districts of Masaka, Mubende, Gomba and Rwampara were identified as new emerging hotspots. Eight districts (Ibanda, Mbarara, Lwengo, Lyantonde, Ssembabule, Kiruhura, Isingiro and Kazo) were identified as consecutive PPR outbreak hotspots whereas only one district (Rakai) was identified as a sporadic hotspot.

4 | DISCUSSION

In this study, we present a holistic assessment of the PPR epidemiological situation in Uganda using retrospective confirmed outbreak reports, socioeconomic factors, and environmental variables. We further identified disease clusters (hotspots) and their predictors using advanced epidemiology and statistical modelling approaches.

We tested risk factors for transmission of PPRV reported in the literature against the outcomes of interest (total number of outbreaks per district or whether a district has reported an outbreak) (A. K. M. A. Rahman et al., 2021; Ruget et al., 2019). As previously reported (Ma et al., 2019), our regression models indicated a strong negative association between annual precipitation with the likelihood of PPR outbreaks implying that lower rainfall increases the chance of PPR outbreaks. During the dry season, the pastoral communities in Uganda tend to move animals over long distances within and sometimes outside the national borders in search for pastures and water for their livestock.

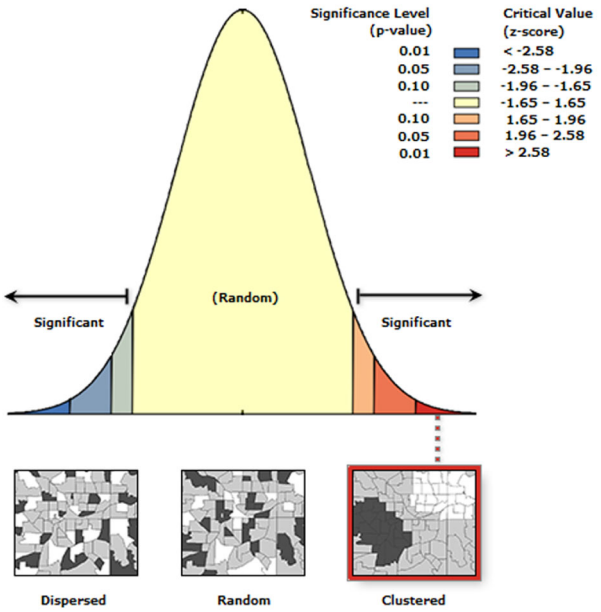

FIGURE 6 Graphical representation of acceptable regions for the measure of standard deviations and corresponding probabilities of Moran I's metrics of spatial autocorrelation

This husbandry practice increases chances of infected and naïve flocks interacting hence potentially facilitating PPRV transmission and increasing the number of outbreaks (Herzog et al., 2020). Additionally, the likelihood of animal nose-to-nose contact and therefore PPRV transmission especially at communal watering points increases during the dry season (VanderWaal et al., 2017). As well, dry seasons are associated with animal trekking for long distances in search of pastures and water which often increases stress for animals. Poor immunity compounded by inadequate/poor nutrition in the dry season will ultimately result into an increase in PPR outbreaks (Abubakar et al., 2009). It would be helpful to monitor precipitation levels and carry out vaccination exercises before onset of drought and/or at the end of the rains before susceptible animals gather at communal watering points.

Conversely, an increase in soil water index (SWI) was significantly associated with the likelihood of PRR outbreaks in Uganda. The Soil Water Index (SWI) provides an estimate of the level of moisture at different soil depths. The SWI varies significantly on small scales depending on the amounts of rainfall received, soil drainage and infiltration capacity of the soil in question (Yao *et al*., 2021). It is highly likely that as soil water index increases, the quality and volume of palatable forages increases, which in turn leads to congregation of small ruminants in such areas. Animal congregation, co-mingling and movement have previously been reported to be strongly associated with transmission of viral infectious pathogens (Kambarage & Kusiluka, 1996; VanderWaal

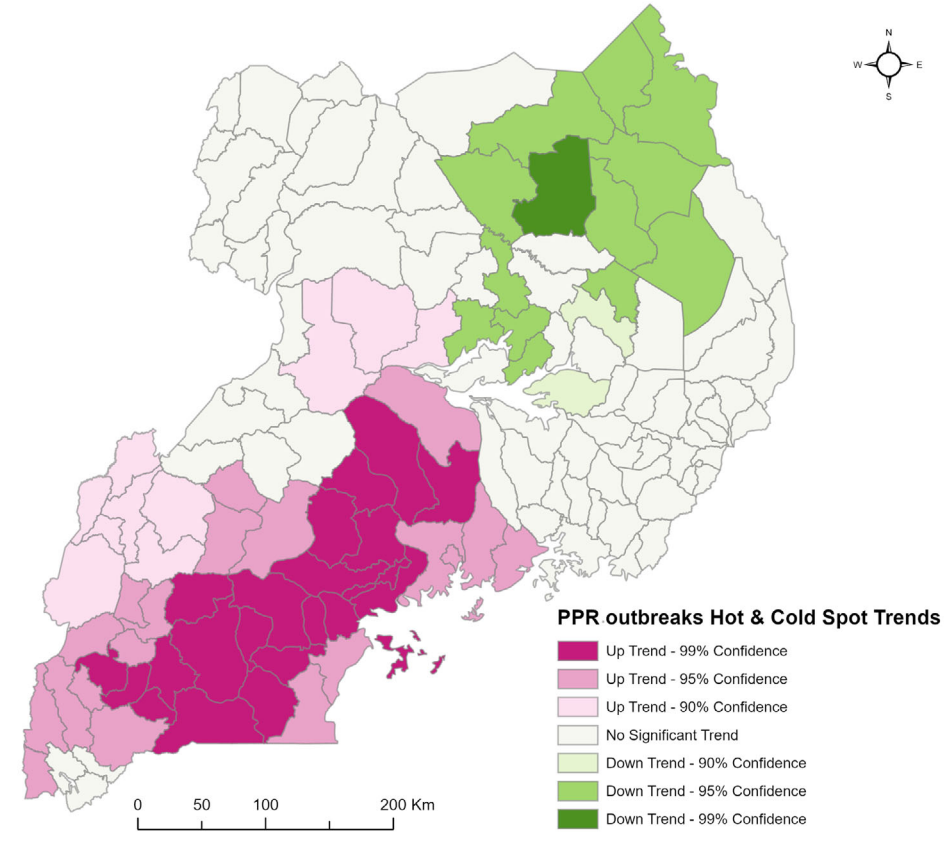

FIGURE 7 Space–time PPR hotspots and their trends across Uganda. A space–time cube data aggregation analysis, with the total number of confirmed PPR outbreaks reported per district and 1 year set as space units and time step, respectively

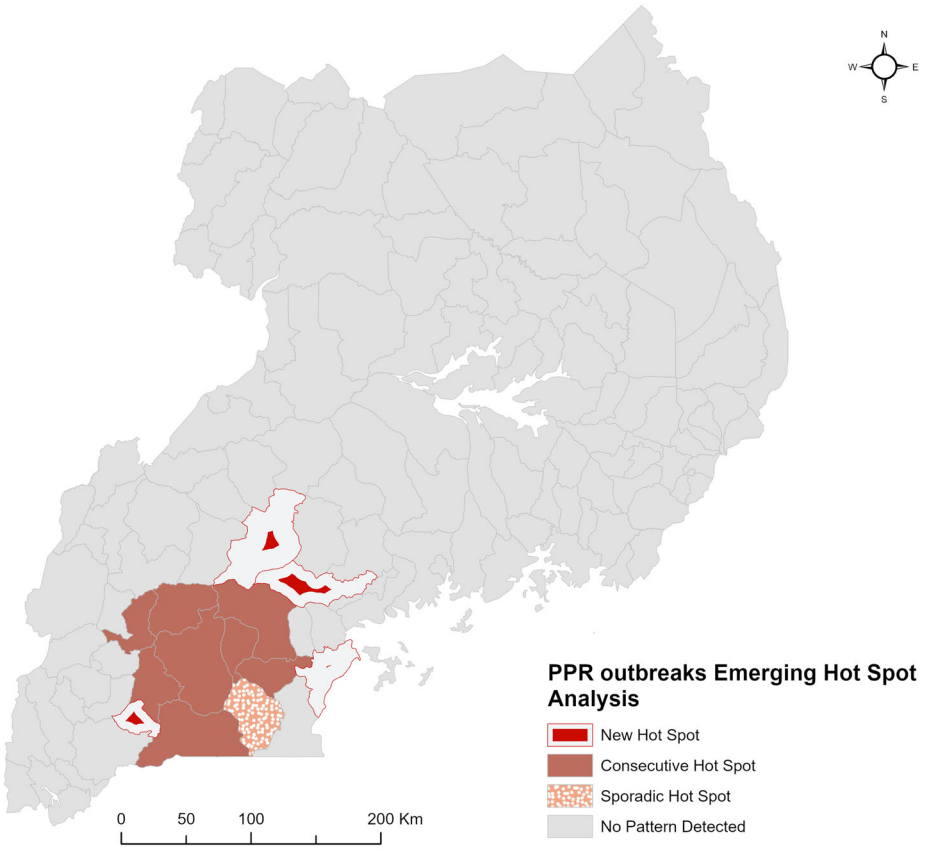

FIGURE 8 Clustering trends of PPR outbreaks in neighbouring space–time cells and hotspots. An *Emerging Hot Spot* analysis and Getis-Ord Gi* statistics analysis. Emerging hotspot trends through time; new, consecutive and sporadic PPR trends were identified following the Mann–Kendall statistics approach

et al., 2017). This could explain the significant association between the soil water index (SWI) and the likelihood of PRR outbreaks in Uganda given that PPR transmission is largely through direct contact as compared to environmental transmission (Mariner et al., 2016).

PPRV is quickly destroyed by ultraviolet light and high temperatures and thus does not survive long in the environment (Latif et al., 2016; Mariner et al., 2016). This attribute suitably explains our result of median wind speed being negatively correlated with PPR outbreaks. PPRV is majorly transmitted through direct contact between susceptible and PPRV infected animals. PPRV environmental transmission (aided by wind-propelled aerosolised virus particles) plays little or no role in PPR epidemiology as compared to increased small ruminant contact rates.

Cattle density was found to be positively correlated with PPR outbreaks. In Uganda, cattle density and small ruminant density are significantly positively correlated ($r = 0.71$, $p = 2.2e\text{-}16$, 95% CI: 0.61–0.78) (Figure S3F). Thus, the association between cattle density and PPR outbreaks could be explained by the mere fact that cattle keepers in Uganda often keep small ruminants as well. As cattle density (and indeed small ruminant density) increases in an area, the likelihood of animal congregation that improves chances of contact between flocks increases. This in turn may explain the increase in the outbreaks in such geographical areas. In addition, the production systems in which majority of Uganda's cattle populations are kept attach higher value to their livestock and are therefore more likely to report PPR outbreaks to the District Veterinary officers. Although cattle are always considered as dead-end hosts for PPR, their role in PPR epidemiology in Uganda has not been explored and should not be ignored. It is suggested by previous studies that cattle should be included as sentinels in PPR surveillance systems and also monitor their role in PPR transmission (Agga et al., 2019; Lembo et al., 2013).

The significantly positive correlation between road length and PPR outbreaks can be attributed to long distance translocation of small ruminants for sale for instance to livestock markets and for breeding purposes. The districts with longer roads are more likely to participate in long distance transportation of livestock within or outside the district boundaries which might increase the chances of importing (or exporting) a PPRV positive animal resulting into PPR outbreaks as observed in previous studies (A. K. M. A. Rahman et al., 2021). Road length signifies highways (longer road stretches) that are used for inter-district and across frontiers small ruminant movement hence fuelling PPR outbreaks as well as spread of PPR from its initial Karamoja focus to the central and southwestern Uganda foci (emerging PPR foci). Previously, highways were fewer in

Karamoja region explaining why the outbreaks took longer to expand to other districts. Road density, which signifies short distance livestock movement (Ruget et al., 2019), was negatively correlated with PPR outbreaks. Intra-district movements facilitated by dense feeder roads is important for intra-district transmission; hence playing a lesser role in inter-district PPR outbreaks that seems to describe the PPR trends in the analysed data sets.

The share of protected areas in a district was negatively associated with PPR outbreaks. Protected areas in Uganda include the land mass covered by wildlife and game reserves making up a total of approximately 4.6% of Uganda's total land mass (Munsey et al., 2019). Given that the government restricts livestock grazing in protected areas, it likely limits opportunities for contact between flocks resulting into a reduced chance of PPR outbreaks (Ruget et al., 2019). Having protected areas also minimizes contact with possible atypical wildlife hosts.

We identified diminishing PPR hotspots in the northeastern Uganda districts comprised largely the Karamoja region. This is most likely to be due to mass and ring PPRV vaccination efforts in response to outbreaks that have been undertaken in this region since 2007, leading to a herd-immunity level that affects transmission rates. At least 12 outbreaks have been confirmed in the Karamoja region resulting in an unknown percentage of small ruminant immunoprotection through natural disease challenge. There is no published literature on the actual PPR vaccine coverage in Uganda, however, with the current ring vaccination control approach following outbreaks over the past 14 years, a modest number of PPR vaccine doses has been applied in the Karamoja subregion by the Food and Agricultural Organization of the United Nation (FAO), the government of Uganda and other non-governmental organisations (Abebe, 2016). Interestingly, previous prevalence studies in this region indicate high levels (63%–85%) of seroconversion (Luka et al., 2011; Mulindwa et al., 2011) among small ruminants in Karamoja subregion. Much as there has been a significant vaccination effort in this region, it does not seem substantial enough to eliminate virus circulation, but may have been sufficient to slow down transmission. However, it may also have resulted into disease suppression and long term persistence as circulation/outbreaks would be difficult to recognise (Mariner et al., 2016), especially considering the inadequate animal disease surveillance system in Uganda.

Uptrend PPR hotspots were identified largely among districts along the cattle corridor in Western Uganda. This is a region where majority of the national livestock population are kept. In this cattle corridor, the majority of the famers communally graze their livestock, resulting into flock congregation which increases the likelihood of PPRV transmission. The observed PPR outbreak pattern is consistent with what has been reported in other East African countries with similar production systems (Mdetele et al., 2021). Over 50% and 17% of the national sheep and goat population respectively are kept in the 9 districts of Karamoja region (MAAIF & UBOS, 2009). With rampant uncontrolled inter-district animal movements supported by the recent extension of the motor way network system, it is not surprising that PPR is now spreading from northeastern Uganda (Karamoja) to southwestern and central region districts of Uganda. As a result, we identified emerging PPR hotspots in the southwestern part of Uganda categorised as new, consecutive and sporadic. The new PPR hotspot districts of Masaka, Mubende, Gomba and Rwampara have relatively high number of livestock including small ruminants per square kilometre. High density of livestock increases the likelihood of contact between infected and susceptible animals and therefore PPR transmission.

The consecutive and sporadic hotspot districts of Isingiro and Rakai respectively are characterised by communal pastoral livestock management and movement of animals across the international border, to and from the Republic of Tanzania. Transborder animal movements along this border point have previously been reported to contribute significantly to the spread and maintenance of contagious viral pathogens such as foot-and-mouth disease (Ayebazibwe et al., 2010; Di Nardo et al., 2011; Kerfua et al., 2018). This is likely the case with PPR transmission in the Rakai district sporadic hotspot and consecutive hotspot in Isingiro district. These factors increase the likelihood of contact between PPRV infected and susceptible animals. This partly explains why these districts are now consecutive and sporadic PPR hotspots. Unless targeted for control, these districts (new, consecutive and sporadic hotspots) will most likely become the new foci/epicentres for PPRV transmission.

4.1 | Study limitations

The outbreak data used in this study were based on clinical observations or outbreak reports (rather than seroprevalence data collected using robust epidemiological methods). The results and their interpretation that we report here are therefore determined using data based on the farmers' ability to primarily identify PPR outbreaks and report them to MAAIF technical personnel for confirmation. Such reports constitute just a fraction of the true PPR incidence over the study period. Additionally, there is limited capacity to accurately detect let alone investigate all the PPR outbreaks in all districts in Uganda due to majorly resource constraints. This is particularly true for Uganda where an efficient animal disease surveillance system is lacking and there are neither incentives nor penalties for livestock disease reporting or under and/no reporting respectively. The livestock disease surveillance system in Uganda suffers a number of setbacks which have affected the quality and volume of data transmitted from the lower level (livestock keeper) to the top level (disease control officers at MAAIF). These challenges include poor laboratory diagnostic services, budgetary constraints and inadequate data transmission systems precise enough to deliver data in a timely manner. Additionally, the system faces poor communication challenges among the stakeholders (Namayanja et al., 2019).

We were not able to find precise PPR vaccination data for all the districts in Uganda over time and thus we could not use vaccination data in the model. However, vaccines were applied in those places in which disease was prevalent, so, in a purely spatial model, that would come up as an association between vaccine and disease further complicating our objective of characterizing the setting. We therefore could only discuss vaccination coverage in general terms for the few regions that have somewhat vaccinated their flocks against PPR.

The key underlying assumption we employ in this study is that the parameters we used serve as a proxy for the true value of the variables, thus, allowing for spatial characterization of the settings. However, one limitation is that, because a time–space model could not be fit given limitations in the data, it is unclear whether those associations are influenced by other factors that were not measured here. Nonetheless, we believe that a purely spatial model will be helpful, novel and needed to support institution of interventions in the context of Uganda. The findings of this study provide useful information as a baseline for a more guided animal disease control interventions such as targeted vaccination and animal movement control.

4.2 | Conclusions and recommendations

The study identified three PPR disease hotspot trend categories with 90–99% confidence across different subregions in Uganda. Diminishing hotspots were identified in the Karamoja region whereas consistent, sporadic, new and emerging hotspots were identified majorly in central and southwestern districts of Uganda. The study further identified high small ruminant density, longer road length, reduced annual precipitation, high soil water index as the most important drivers of Peste des Petits Ruminants (PPR) transmission in Uganda. Findings of this study provide a basis for more robust timing and prioritization of control measures including vaccination to contribute to the global goal of control and eradication by 2030. For instance, these findings can be used to test a risk based PPR vaccination program by prioritising vaccination of small ruminants in PPR Up Trend districts. Prioritization of interventions in terms of both space and time and for example districts with uptrend, drought-prone and those with high density of small ruminants and the time of the year when the amount of rainfall is low.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Administrative clearance to access and use disease outbreak reports and laboratory data was sought from the office of the Commissioner Animal Health, Ministry of Agriculture Animal industry and Fisheries. The work described under this study was approved by the Makerere University School of Veterinary Medicine and Animal Resources Institutional Animal care and Use committee (SVAR-IACUC) (Reference number: **SVAR_IACUC/58/2020**) and the Uganda national council of science and technology (reference number: **A103ES**). This work was also approved by the ILRI Institutional Animal Care & Use Committee (Reference number: **ILRI-IACUC2021-08**) and ILRI Institutional Research Ethics Committee (Reference number: **ILRI-IREC2021-07**).

AUTHOR CONTRIBUTIONS

JN: study conceptualization and design, data acquisition and analysis, writing and reviewing. FK: data analysis, visualization, writing and editing. PL, PN, FNM: writing and reviewing. KR: grant acquisition, writing and reviewing. HK and DM: study conceptualization, writing and reviewing. AP: data analysis, reviewing. BW: study conceptualization, design, writing and reviewing. All authors have read and approved this manuscript for publication.

DATA AVAILABILITY STATEMENT

The PPR outbreak reports data may be available on reasonable request from the corresponding author.

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Chapter 4: Seroprevalence and risk factors of Peste des petits ruminants in different production systems in Uganda

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Seroprevalence and risk factors of Peste des petits ruminants in different production systems in Uganda

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ABSTRACT

Peste des petits ruminants (PPR) is a highly contagious and fatal disease of mostly domestic goats and sheep. First reported in Uganda in 2007, the extent of peste des petits ruminants virus (PPRV) exposure, geographical distribution and risk factors of its transmission and spread are not clearly understood. In this study, we used cluster random sampling methodology to select study villages from three districts representing three different production systems along Uganda's "cattle corridor". Between October and December 2022, 2520 goat and sheep serum samples were collected from 252 households with no history of PPR vaccination in the past one year. The household heads were interviewed to assess possible risk factors of PPRV transmission using a structured questionnaire. The serum samples were screened with a commercial competitive enzyme-linked immunosorbent assay (cELISA) for PPRV antibodies. The determined overall true seroprevalence of PPRV was 27.3% [95% CI: 25.4–29.1]. The seroprevalence of PPRV antibodies in different production systems was 44.1% [95% CI: 40.6–47.7], 31.7% [95% CI: 28.4–35.0] and 6.1% [95% CI: 4.4–7.9] for pastoral, agropastoral and mixed crop-livestock production systems respectively. A mixed-effects multivariable logistic regression model revealed strong statistical evidence of association between female animals and PPRV antibody seropositivity compared to males [OR= 2.45, 95% CI: 1.7–3.5, $p < 0.001$]. The likelihood of being PPRV antibody seropositive significantly increased with increasing small ruminant age. Animals older than 3 years were more than three times as likely to be PPRV seropositive compared to animals aged under 1 year [OR= 3.41, 95% CI: 2.39–4.85, $p < 0.001$]. There was no statistical evidence of association between small ruminant species and PPRV antibody seropositivity ($p = 0.423$). Village flocks that interacted with neighboring flocks daily during grazing (IRR = 1.59, 95% CI: 1.19–2.13) and watering around swamps (IRR = 1.59, 95% CI: 1.19–2.13) were highly correlated with increased number of PPRV seropositive animals as compared to flocks that were more restricted in grazing and watered around other water sources other than swamps. Flocks from pastoral and agropastoral production systems were more than 10 times more likely to have seropositive animals than mixed crop-livestock flocks. Targeting PPR control interventions (vaccination and livestock movement control) to pastoral and agro-pastoral small ruminant production systems that are very prone to PPR incursions is recommended to prevent PPRV spread to low-risk smallholder mixed crop-livestock production systems.

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1. Introduction

Peste des petits ruminants (PPR), also known as goat plague, is a highly contagious disease of domestic small ruminants (goats and sheep) caused by Peste des petits ruminants virus (PPRV) which is currently the only member of *Morbillivirus caprinae* species within the *Morbillivirus* genus of the *Paramyxoviridae* family (Postler et al., 2016).

PPR is associated with yearly economic losses of up to 2.1 billion US dollars globally (OIE-FAO, 2015). These losses result from mortalities, morbidities, cost of treatment, lost opportunities for international trade, loss in milk yield and live weight gain (Jones et al., 2016; Parida et al., 2015). Since the 1940s when the disease was first reported in West Africa, it has spread to over 70 countries in the rest of Africa and to Asia (Banyard et al., 2010). The affected countries are home to more than 80% of the global small ruminant population with over 300 million people deriving their animal protein and income from small ruminants (Banda and Tanganyika, 2021; Mazinani and Rude, 2020). Nevertheless, from the available literature, most PPR endemic countries have not sufficiently scaled up their vaccination campaigns using the commercially available effective PPR vaccines to maintain the required 80% protection levels while accounting for flock population dynamics (turnover rate, restocking frequency, movement among others) (OIE--FAO, 2015). Consequently, the disease continues to spread to new areas, causing significant economic losses. To this end, the Food and Agriculture Organisation of the United nations (FAO) and the World Organisation for Animal Health (WOAH, formerly known as OIE) have launched a global campaign to eradicate PPR by the year 2030, using vaccination as the main control measure in high-risk or endemic areas (FAO and WOAH, 2022).

Highly efficacious PPR vaccines that provide life-long protective immunity against all the four known PPRV lineages are available on the market (EFSA AHAW Panel, 2015). Moreover, small ruminants that survive the PPRV infection remain protected from severe clinical disease for at least 3 years (Baron et al., 2016). Female small ruminants that are vaccinated and or survive natural infection pass on maternal antibodies to their kids that usually offer them protection for at least 3 months (Ata et al., 1989; Balamurugan et al., 2012; Markus et al., 2019). However, there is currently no marker vaccine or diagnostic test that differentiates antibodies from vaccinated animals and those from naturally infected animals. This complicates seroprevalence estimation studies (OIE-FAO, 2015).

In Uganda, majority of livestock are kept in the “cattle corridor”, a region that runs from South-Western to North-Eastern (Fatumah et al., 2023; UBOS, 2021). These animals are generally managed under three traditional production systems that include pastoral, agropastoral and mixed crop-livestock production systems (Kambarage and Kusiluka, 1996). These production systems are defined based on the level of family dependency on livestock or livestock products for sustenance, degree of movement involved, and the type of agriculture practiced alongside livestock (Ibrahim, 1998).

Pastoral production system dominates most of northern Uganda districts, most especially the north-eastern part (Karamoja subregion). In the Karamoja subregion, more than 50% of household income is derived from livestock or livestock products with very little or no crop agriculture. Livestock are kept on a large expanse of communal land where livestock owners move animals over long distances within the region and sometimes across international borders in search of fresh pasture and water during dry periods. Pastoral systems account for more than 16% and ~50% of goat and sheep populations in Uganda respectively (Akwongo et al., 2022; UBOS, 2017).

Agropastoral production systems dominate districts in central and south-western Uganda such as Isingiro, Rakai, Sembabule, Nakasongola, Kiruhura among others. In this system, between 10% and 50% of households depend on livestock or livestock products for their livelihood (Ibrahim, 1998). Crop agriculture is practiced alongside livestock production. Livestock are often kept in fenced farms or openly grazed on fairly large expanses of land with the likelihood of periodic migration to greener areas in search of pasture and water during drought spells (Kambarage and Kusiluka, 1996).

Mixed crop-livestock production system is the commonest management system practiced in majority of the districts in Uganda. In this system, livestock production is secondary to crop agriculture with less than 10% of household income derived from livestock (Ibrahim, 1998). Small ruminants are often kept in relatively small flocks tethered on ropes or closely herded by mostly family labour (women and children) to prevent the animals from encroaching on the crop gardens (Kambarage and Kusiluka, 1996). In such communities, animals are often moved for relatively short distances with a reduced chance for direct contact between flocks.

Previous PPR studies in Uganda reported seroprevalences ranging from 60% to 85%. However, nearly all this PPRV seroprevalence literature available constitute very small studies done in the pastoral production systems (Akwongo et al., 2022; Luka et al., 2011; Mulindwa et al., 2011; Ruhweza et al., 2010). With the rather very high seroprevalence estimates, it is understood that majority of these studies mentioned that they were done following vaccination campaigns whereas the rest, although not explicitly stated, were likely conducted around PPR outbreak periods (during or after) as previously reported (Nkamwesiga et al., 2022). This is likely true because of the need to estimate extent of virus spread or to evaluate the effect of vaccination post PPR outbreaks. Additionally, several PPR outbreaks have been reported in over 50 out of 135 districts of Uganda over the past 5 years with significant uptrend PPR clustering in central and south-western Uganda (Nkamwesiga et al., 2022). These outbreaks have devastated the livelihoods of affected households, in some instances wiping out entire small ruminant flocks or forced sale / salvage slaughter of affected animals (MAAIF, 2022). With limited capacity to conduct mass vaccination and other relevant control measures, the Ministry of Agriculture Animal Industry and Fisheries (MAAIF) has distributed vaccines for field veterinarians to conduct ring vaccination around affected areas to prevent further spread (Ayebazibwe et al., 2022).

Past and recent spatio-temporal cluster analyses of PPR outbreaks, socioeconomic human activities, bioclimatic, topographic, and environmental datasets have identified small ruminant density, extensive road network, animal movement, and draught among others as key factors that drive transmission of infectious diseases such PPR (Fèvre et al., 2006; Nkamwesiga et al., 2022). However, there is still paucity of data about production system-based risk factors of PPRV spread and PPR outbreaks within the endemic districts. This makes it difficult to design production system-based control programs (such as vaccination, movement control, biosecurity and biosafety and improving restocking programs) which partly contribute to the persistence and spread of PPRV in Uganda. We undertook this study to determine the seroprevalence of PPRV across three main small ruminant production systems in Uganda [all with previous reports of PPR outbreaks except the mixed crop-livestock production system], and identify production system- and-animal-level risk factors. The results herein described will help to design production system-based PPR control programs in Uganda and other endemic countries, in line with the national and global PPR eradication campaign by 2030.

2. Materials and methods

2.1. Sampling strategy

This cross-sectional study was conducted in three Uganda districts between October and December 2022 (Fig. 1). A list of all districts along the cattle corridor was first grouped into three categories, namely, pastoral, agropastoral and mixed crop-livestock production systems based on available literature and expert opinion (Fatumah et al., 2023; UBOS, 2021). One district was selected from each category using simple random sampling methodology. The selected districts were Nakapiripirit

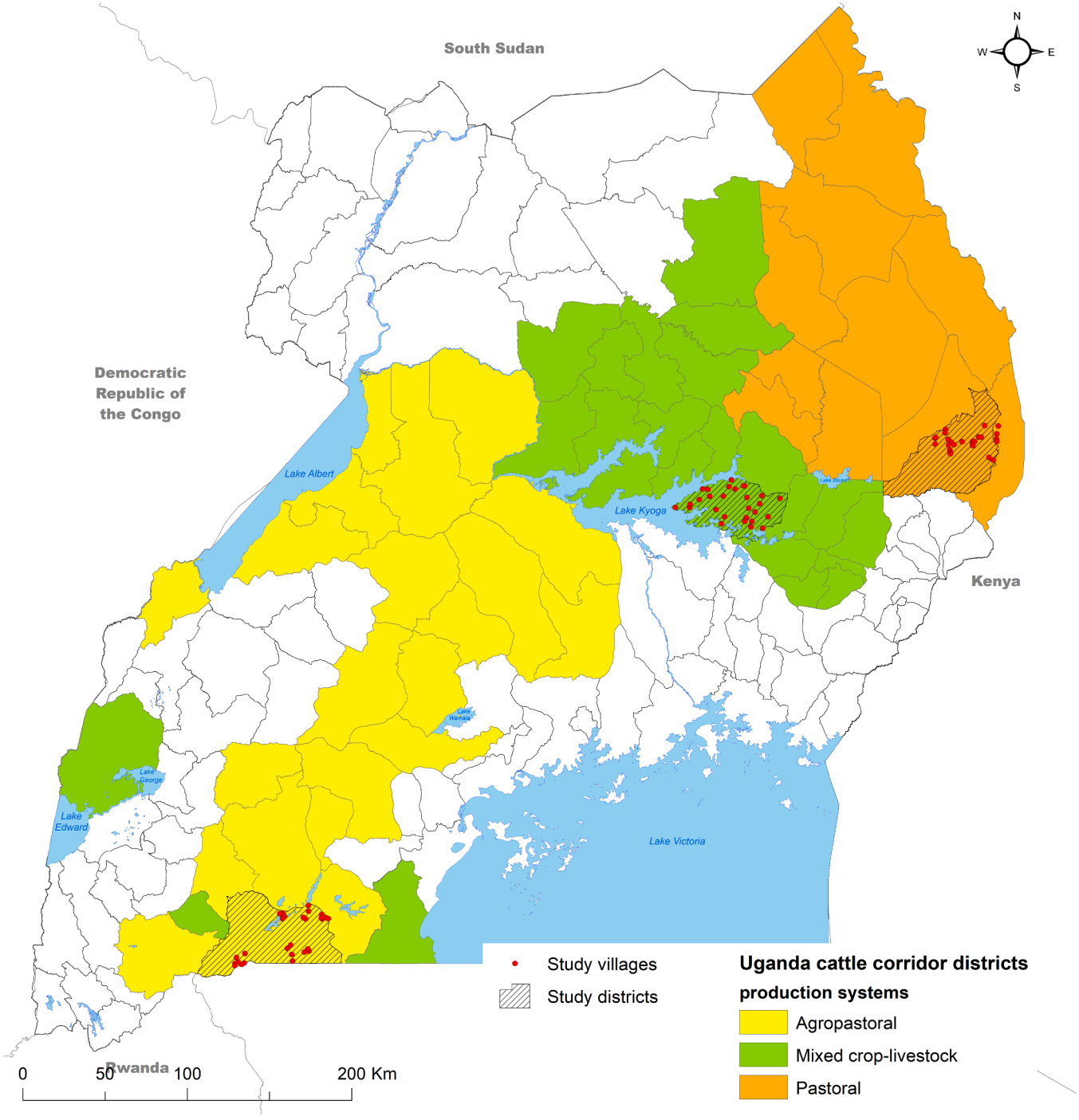

Fig. 1. Map of Uganda showing the study area. Highlighted are the major production systems that characterize districts that span the cattle corridor as demarcated based on current data. The map was generated using ArcMap 10.7 software using open-source datasets from the Uganda Bureau of Statistics.

(pastoral), Serere (mixed crop-livestock) and Isingiro (agropastoral). Study villages were selected from the complete list of villages (sampling frame) for each district provided by MAAIF (UBoS, 2009), using the cluster random sampling method as previously described (Bennett et al., 1991; Sullivan, 2007). Assuming an animal-level PPRV antibody seroprevalence of 50%, interclass correlation coefficient (ICC) of 0.029 (Waret-Szkuta et al., 2008) and design effect of 1.84, a total of 28 villages with a minimum of 30 small ruminants were required to be sampled per village. Consequently, a total of 840 small ruminants were required from each of the three districts to achieve the set precision and be able to detect risk factors if they existed. We then conducted a scoping visit to the study areas where we randomly replaced all villages where PPR vaccination had been conducted in the past 12 months with the help of district veterinary officials.

For the agropastoral and mixed crop-livestock production systems, at least three farms / households were randomly selected from a list of livestock-keeping households in the district (obtained during a scoping visit). However, in pastoral production system (Nakapiripirt district) where animals from the same village graze together, it was not necessary to select flocks as emphasis was on selection of 30 study animals from the communal village flock.

Individual animals were selected using systematic random sampling. We quickly estimated flock size and divided that number by the required animals per flock to obtain the position of the next animal to sample as

small ruminants exited the holding ground in a single file. Ten animals (5 sheep and 5 goats whenever possible) were randomly selected from each selected farm, flock or household. In case a selected household had less than 10 eligible small ruminants, we sampled all eligible animals they owned and sampled additional animals from the nearest household to make a total of 30 animals per village. Sampled animals were aged by the veterinarian taking samples based on their dentition as previously recommended (Dyce et al., 2002; Uhart et al., 2016). All the data was then aggregated at village level.

By the time of this study, no PPR outbreak had been reported from Serere district. However, from 2007 to 2020, between 10 and 12 and 1–2 laboratory confirmed PPR outbreaks had been reported in Nakapiripirit and Isingiro districts, respectively, prompting dispatch of vaccine doses sufficient for ring vaccination strategy (Ayebazibwe et al., 2022; Nkamwesiga et al., 2022).

2.2. Study population

Small ruminants (sheep and goats) of 4 months and above from flocks with no history of PPRV vaccination in the year before the study were included. All pregnant animals and clinically sick animals were excluded from the study for ethical and animal welfare reasons. All household heads (and/or caretakers of the small ruminants) of the sampled flocks were interviewed to gain insight of the epidemiological drivers of PPRV transmission.

2.3. Blood sample collection and serum extraction

In order to allow for easy access to the jugular vein, the animal handling assistant restrained the sheep/goat's body by holding the animal under its jaw and turned the head to the side, at a 30-degree angle as previously recommended (Uhart et al., 2016). Blood samples were drawn from small ruminants as previously described (Uhart et al., 2016) by Uganda Veterinary Board-licensed veterinarians. About 6 mL of jugular blood were obtained from sheep and goats into serum separator vacutainer tubes (SST) that contained a clot activator gel which allowed rapid blood clotting and serum separation. The SST tubes were serially labeled and the extra meta-data on each sample such as date, geographical position system coordinates, sex, age and species of animal recorded using Open Data Kit (ODK) on a tablet (Hartung et al., 2010). Upon separation of serum from whole blood, usually 12 h after blood sample collection, two 1.5 mL aliquots of serum were pipetted off from each blood sample into pre-barcoded cryogenic tubes. These were packed into cryoboxes and temporarily stored at − 20 °C at the district or regional laboratory before transporting them to the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) laboratory in Entebbe within one week for long term storage at − 80 °C until required for further analysis.

2.4. Household interviews

Structured farmer interview guides were used to collect data on the possible epidemiological drivers of PPRV transmission (Supplementary Table 4). These drivers included potential risk factors, production systems, water sources, possibility and frequency of contact with other flocks and wildlife, source of the animals (for restocking), distance from livestock markets, shared water sources, and vaccination status of the animals among others. The structured questionnaires were translated into respective local languages and pretested in a non-target district before implementation of this study.

2.5. Detection of PPRV antibodies

The PPRV specific IgG antibodies in serum were detected using the ID Screen® PPR (IDVet, 310 rue Louis Pasteur, 34790 Grabels, France) commercial competitive ELISA kits following the manufacturer's instructions (Libeau et al., 1995).

The cut-offs were calculated as;

$$\frac{S}{N}(\%) = \frac{OD_{sample}}{OD_{Negative\ \ control}} * 100.$$

The samples with percentage inhibition (S/N) less than or equal to 50% were considered positive, S/N % between 50 and 60 were considered doubtful whereas samples with an S/N value above 60% were considered negative. During the analysis, we considered all doubtful results as negative in the analysis since these samples were drawn from apparently healthy flocks as previously suggested (Fernandez Aguilar et al., 2020; Shyaka et al., 2021).

2.6. Estimation of true prevalence

True prevalence is traditionally estimated from the apparent prevalence using the Rogan–Gladen estimator as follows:

$$True\ prevalence = \frac{(Apparent\ prevalence + Sp - 1)}{(Se + Sp - 1)},$$

where *Se* denotes test sensitivity and *Sp* denotes test specificity (Rogan and Gladen, 1978). However, if the apparent prevalence is lower than the probability of observing a false positive (1- test specificity), the standard Rogan–Gladen estimator formular returns negative values. Also, if the apparent prevalence is greater than the diagnostic test sensitivity, the percentage true prevalence estimates will be greater than 100%. In both scenarios, the true prevalence estimates returned are not epidemiologically plausible (Reiczigel et al., 2010; Speybroeck et al., 2013). To overcome this problem, we used the fixed values of specificity (99.4%) and sensitivity (94.5%) as provided by the ELISA test manufacturers to estimate the true prevalence using Bayesian approach implemented in the R software package "prevalence" (Devleesschauwer et al., 2022).

2.7. Data analyses

All statistical analyses were done using R software, version 4.3.1 (R Core Team, 2023). We cleaned the data, determined animal-level and village-level seroprevalence and all relevant descriptive statistics. To test for animal-level risk factors, we included all the five captured animal-level factors (i.e., species, sex, age, village, and district) in a mixed effects logistic regression model using the *lme4* package in R software (Bates et al., 2015). To account for clustering and minimize the potential effects of confounding, a small ruminant "village" was set as the random effect in this model since observations were done at village level.

However, for the village level risk factors, data on a range of potential risk factors were collected, curated and screened for multicollinearity based on the variance inflation factor (VIF) to remove all the perfectly correlated variables. Variable selection for the village-level Poisson regression model was done using an automated backward model selection procedure (*stepAIC* function in the 'MASS' package in R). The precision level was set at 95% and p values < 0.05 were considered statistically significant and only the metrics in the final (best fit) model were presented.

To explore the association between incidence and the potential risk factors in a district, we tested the potential risk factors from farm/village interviews such as restocking, communal water source, frequency of contact with other flocks among others. A generalized linear Poisson regression model with log link was fit to these data using the number of positive animals per village as the dependent variable. To account for the spatial dependency of observations as a result of some villages or districts being close to each other, we conducted a spatial autocorrelation test on the residuals of the final regression model the Moran's I test (Chen, 2016).

The spatial scan statistic was computed using the Bernoulli model (Kulldorff, 1997) implemented in the SaTScan software (http://www.

satscan.org/) with default settings. To determine whether any of such clusters is statistically significant, the Bernoulli model takes binary data (positive or negative) in the form of cases and controls and identifies locations (space) where the number of observed cases tends to be more than expected (Chhetri et al., 2010). Statistically significant clusters ($p < 0.05$) were identified and visualized using ArcMap 10.7 software (ArcGIS v. 10.7, ESRI Inc. Redlands, CA, USA).

2.8. Ethical considerations

All study personnel involved in this study were protocol trained. The protocol that generated results described in this study was approved [Reference number: SVAR_IACUC/58/2020] by the School of Veterinary Medicine and Animal Resources Institutional Animal Care and Use Committee SVAR(SVAR-IACUC), Makerere University and the Uganda National Council of Science and Technology (UNCST) (reference number: A103ES). This work was also approved by the Institutional Animal Care & Use Committee (Reference number: ILRI-IACUC2021-08) and the Institutional Research Ethics Committee (Reference number: ILRI-IREC2021-07) at the International Livestock Research Institute. Additionally, the study was administratively approved by all participating district authorities (Prior Informed Consent). Animal sampling was completed by licenced veterinarians in Uganda. Written informed consent was obtained fromall participating farmers to bleed their animals, store their animal blood samples and serum therefrom as well as to interview them. All participating farmers' animals were dewormed as compensation for their time to participate inthe study.

3. Results

3.1. Village-level seroprevalence of PPRV antibodies

The village-level apparent prevalence of antibodies against PPRV ranged from 0.0% to 100.0% across the study area. All the 28 sampled flocks in Nakapiripirit district contained at least one animal positive for PPRV antibodies, resulting in 100% flock-level seroprevalence. In Isingiro district, flock-level PPRV antibody seroprevalence was 96.4% (27/28). The least number of positive flocks, (13/28), was observed in Serere district with 15 villages having no animal positive for PPRV antibodies resulting in 46.4% flock-level PPR antibody seroprevalence.

3.2. Individual animal-level seroprevalence of PPRV antibodies by district

The individual animal-level true seroprevalence of PPRV antibodies varied from 6.1% to 44.1% across the study area. Prevalence was highest (44.1%) in Nakapiripirit district, a predominantly pastoral production system, and lowest (6.1%) in Serere district where most small ruminants are tethered on ropes in a predominantly mixed crop-livestock production system (Table 1).

The spatial distribution of animal-level PPR antibody seropositivity among flocks was evenly distributed in Isingiro and Nakapiripirit districts. Majority of the villages in Nakapiripirit and Isingiro districts had animal-level seroprevalences ranging between 36.8% and 53.3% and 36.8–59.9% respectively. However, in Serere district, only Ogolai and Agola villages had high seroprevalences of 90.0% and 26.7% respectively. The rest of the 26 villages in Serere district had apparent prevalence ranging between 0% and 6.7% (Fig. 2).

Table 1
Animal-level seroprevalence of PPRV antibodies in goats and sheep (n = 2520) from Isingiro, Nakapiripirit and Serere Districts, Uganda (2022).

District	Production system	N positive [n sampled]	"Apparent" prevalence %	Estimated "true" prevalence [95% CI]
Isingiro	Agropastoral	255 [840]	30.4	31.7 [28.4 – 35.0]
Nakapiripirit	Pastoral	353 [840]	42.0	44.1 [40.6 – 47.7]
Serere	Mixed crop-livestock	52 [840]	6.2	6.1 [4.4 – 7.9]
Total		**660 [2520]**	**26.2**	**27.3 [25.4 – 29.1]**

The number of PPR seropositive animals per village (PPR cases) across the entire study site (Isingiro, Serere and Nakapiripirit districts) was found to be spatially clustered (Moran's autocorrelation statistic I = 0.302337, P = 0.001953) in two most likely clusters. The first most likely cluster was identified along the international border between Uganda and Kenya in Nakapiripirit district (log likelihood ratio = 66.96, p < 0.0001). The second statistically significant cluster was identified at the international border with Tanzania in Isingiro district (log likelihood ratio = 38.16, p = 0.0001) (Fig. 3).

3.3. Individual animal-level seroprevalence of PPRV antibodies by subcounty

The 84 randomly selected villages were distributed across 22 subcounties: Nakapiripirit (5), Isingiro (7) and Serere (10) (Fig. 4). Different sub-counties exhibited varying levels of true PPRV antibody seroprevalence. In Isingiro district, the sub-counties of Rugaaga and Bigango had the highest and lowest seroprevalence of 44.0% [95%CI: 3.2–27.0] and 6.3% [95% CI: 37–51.1] respectively. In Nakapiripirit district, the highest prevalence was recorded in Nakapiripirit town council (57.3% [95%CI: 46.4–68.0]) whereas the lowest true seroprevalence was observed in Moruita sub-county (36.3% [95%CI 30.9–41.8]). In Serere district, the highest true seroprevalence 20.4% [95%CI: 14.1–27.4] was observed in Bugondo sub-county whereas the lowest 3.4% [95%CI: 0.0–10.2] was observed in Kasilo subcounty (Supplementary table 1).

3.4. Individual animal-level seroprevalence of PPRV antibodies by village

Generally, the apparent PPRV antibody seroprevalence ranged from 0% to 96.6% whereas the estimated true seroprevalence ranged from 3.2% [95% CI: 0–9.7] to 95.7% [95% CI: 85.8–99.9]. More than 50% (15/28) of the sampled villages in Serere district had an apparent seroprevalence of 0% whereas only one village flock in Isingiro district (Kaziizi village) had an apparent seroprevalence of 0%. The lowest apparent animal-level seroprevalence at village level in Nakapiripirit was 6.0%. Ihunga village in Isingiro district, Alapat village in Nakapiripirit district and Ogolai village in Serere district had the overall highest PPRV antibody seroprevalences of 96.6%, 83.3% and 90% respectively (Supplementary table 2).

True animal-level seroprevalence in flocks was more spread out in Isingiro district than in Nakapiripirit and Serere districts. Isingiro district had more villages with seroprevalence lower than the median true prevalence value (34.0%) whereas majority of the villages in Nakapiripirit district had true seroprevalence estimates above the median value (47.5%). Over 90% of the villages in Serere district (26/18) had true seroprevalence below 10% (Fig. 5).

3.5. Animal-level risk factors of PPRV antibody seropositivity

There was no significant difference between PPRV antibody seroprevalence between goats and sheep (p = 0.423). Female small ruminants were more than twice more likely to be PPRV antibody seropositive as compared to male small ruminants (OR = 2.46, 95% CI: 1.70–3.47, p < 0.001). The likelihood of being PPRV antibody seropositive significantly increased with increasing small ruminant age. Compared to animals aged below 1 year, animals older than 3 years were more than thrice more likely to be PPRV antibody seropositive (OR = 3.41, 95% CI: 2.39–4.85, p < 0.001). Similarly, animals aged between 2 and 3 years were more than twice as likely to be seropositive whereas those aged between 1 and 2 years were nearly twice as likely to be PPRV antibody seropositive compared to the younger animals aged less than 1

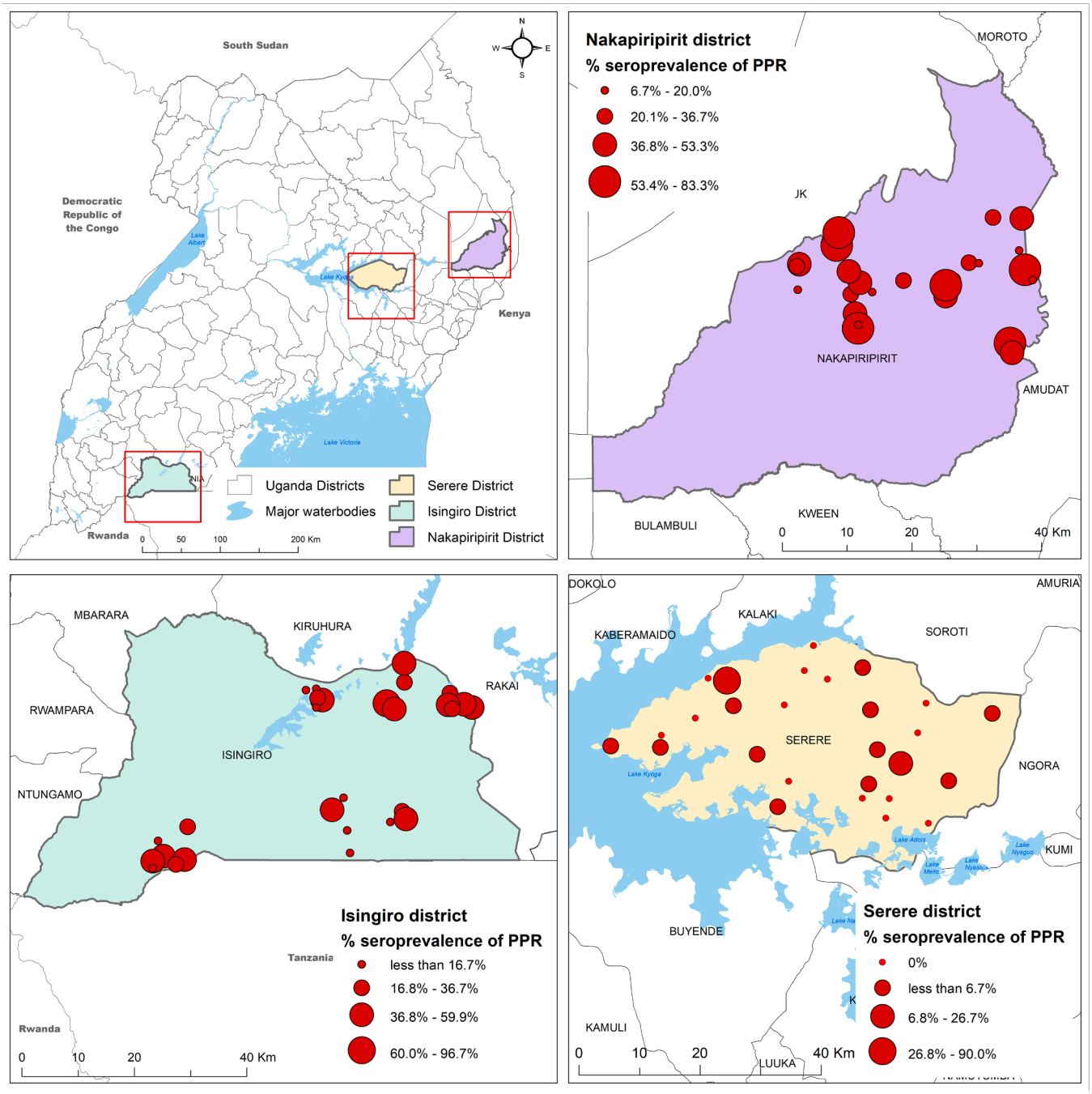

Fig. 2. Spatial distribution of animal-level PPRV antibody seropositivity among flocks in Isingiro, Serere and Nakapiripirit districts in Uganda (2022) as generated using ArcMap 10.7 software with open-source datasets.

year.

Goats and sheep from Nakapiripirit and Isingiro districts were 35 and 19 times respectively more likely be PPRV antibody seropositive than small ruminants from Serere district (Table 2).

The animal-level risk factors model random effects parameters were $\sigma^2 = 3.29$, $\tau 00_{Village} = 2.02$, ICC = 0.38, $N_{Village} = 84$, Obs = 2520, Marginal R2 / Conditional R2 = 0.337 / 0.591. The Moran's I test over the residuals of the final mixed effects regression model revealed that the data were dispersed (Moran's I = 0.031, z-score = −5.885, p-value < 0.001). The spatial clustering observed when we ran raw data (Fig. 3) disappeared after we incorporated the independent variables in the regression model. Our independent variables explain the spatial dependence that we originally found in the raw data.

3.6. Village-level risk factors of PPRV antibody seropositivity

Villages where flocks interacted with neighboring flocks daily were more likely to have PPRV antibody seropositive animals (IRR = 1.59, 95% CI: 1.19–2.13) whereas villages where flocks only interacted with other flocks less than once a month were significantly associated with reduced chances of having PPRV seropositive animals (IRR = 0.50, CI: 0.26–0.90) as compared to villages whose flocks were confined.

Villages whose water source for their animals was waterhole (IRR = 1.89, CI: 1.39–2.56) and swamp (IRR = 1.32, CI: 1.07–1.61) were strongly correlated with increased likelihood of having PPRV antibody seropositive animals compared to villages that did not use these water source types. However, using borehole as source of water for the small

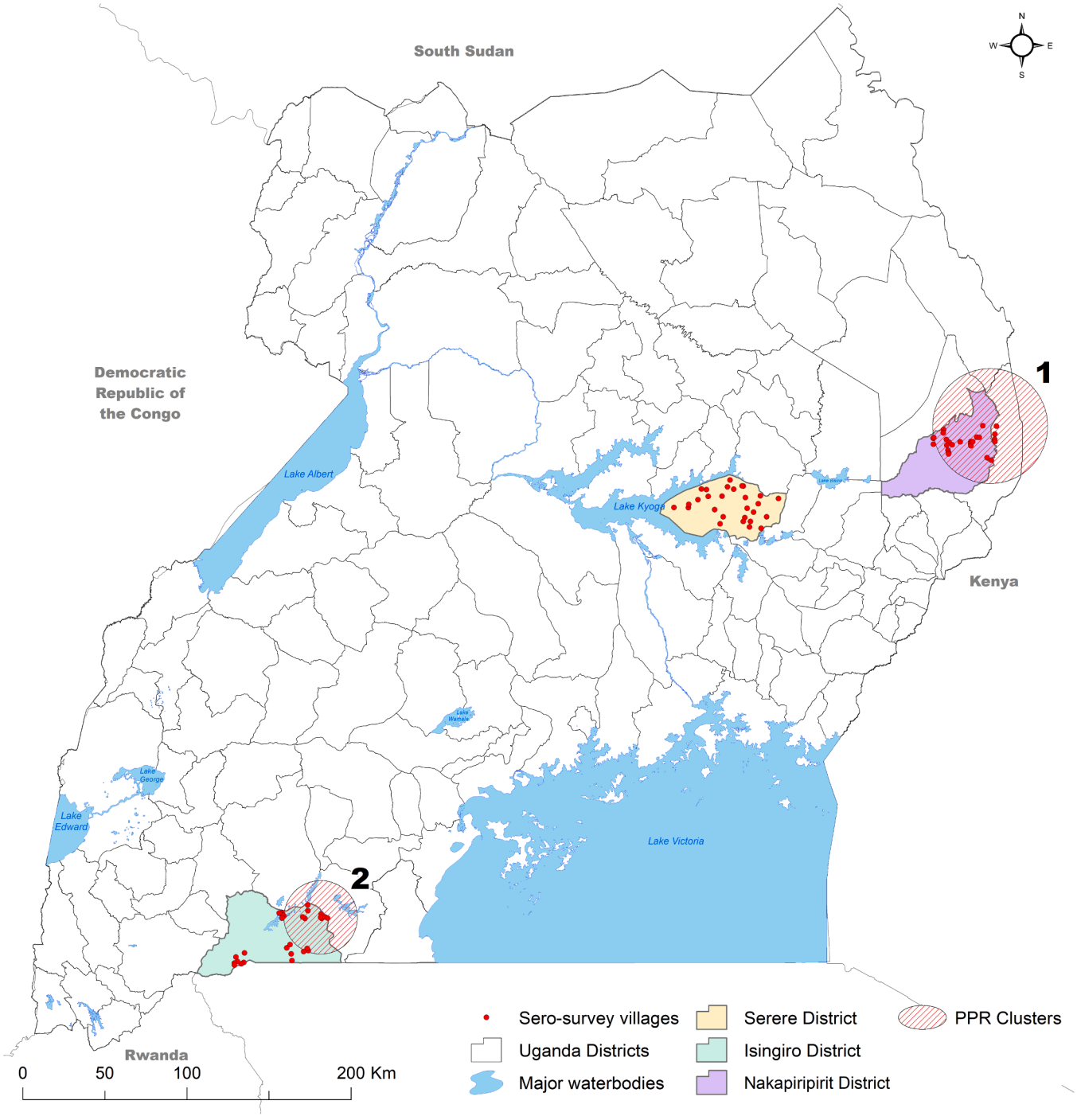

Fig. 3. Statistically significant clusters of PPR seropositivity in Isingiro, Serere and Nakapiripirit districts in Uganda (2022). The two clusters were identified using the Bernoulli method in SaTScan software and visualized in ArcMap 10.7 software.

ruminants was significantly correlated with reduced chances of having PPRV antibody seroprevalence animals (IRR = 0.55, CI: 0.43–0.69).

Villages in which households kept cattle and pigs in addition to small ruminants were 1.9 and 1.3 times respectively more likely to have PPRV antibody seropositive animals compared to villages that only kept small ruminants. Villages that maintained small ruminant flocks for at least one year were more likely to have PPRV seropositive animals than villages that purchased sheep and goats in the previous year.

Villages from Isingiro and Nakapiripirit districts were more than 10 times more likely to have PPRV antibody seropositive animals than those from Serere district. Villages where farmers reported that PPR vaccination had occurred in the past 12 months were 1.9 times more likely to be seropositive compared to villages where no PPR vaccination had not been conducted (Table 3).

4. Discussion

In this study we set out to determine the prevalence of PPRV antibodies across the three main small ruminant production systems in Uganda as well as the village- and animal-level risk factors of PPRV exposure.

The overall PPRV antibody seroprevalence estimates we report in this study are generally lower than what had been previously reported in similar settings in Uganda. The 42.0% apparent PPRV antibody

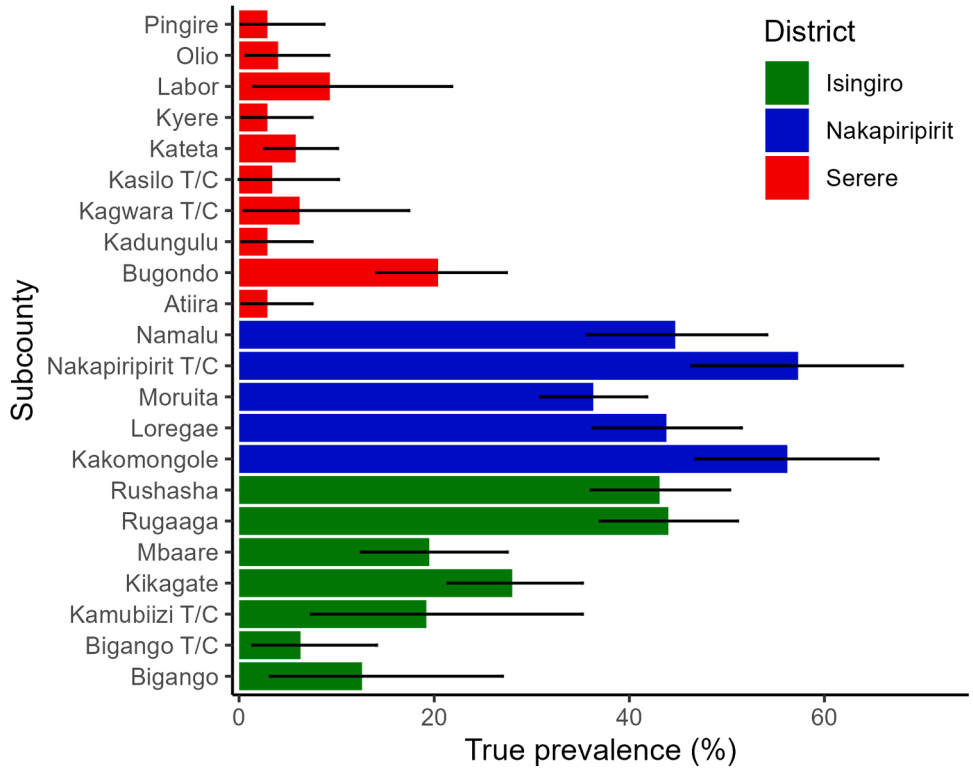

Fig. 4. True animal-level seroprevalence of PPRV antibodies in goats and sheep [n = 2520] from Isingiro, Nakapiripirit and Serere districts, Uganda (2022), as summarized at the subcounty-level. The "error bars" represent the lower and upper limits of the 95% confidence intervals from the Bayesian method.

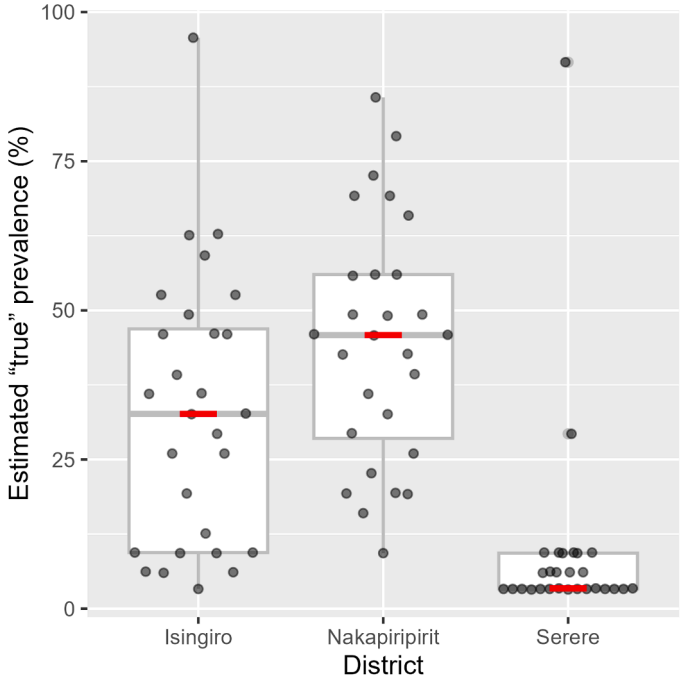

Fig. 5. Distribution of animal-level true seroprevalence of PPRV antibodies in goats and sheep [n = 2520] from Isingiro, Nakapiripirit and Serere districts, Uganda (2022), summarized by village/flock and district.

Table 2
Final multivariable mixed effects logistic regression model for animal-level risk factors of PPRV antibody seropositivity with village as a random effect [N = 2520] in Serere, Nakapiripirit and Isingiro districts, Uganda (2022).

Risk factors	n sampled [%]	n positive [%]	Odds Ratios	95% CI	p
1. **Species**					
i. Goats	1973 [78.3]	538 [27.3]	Ref		
ii. Sheep	547 [21.7]	122 [22.3]	0.87	0.63 – 1.21	0.423
2. **Sex**					
i. Male	390 [15.5]	73 [18.7]	Ref		
ii. Female	2130 [84.5]	587 [27.5]	2.46	1.74 – 3.47	< 0.001 * **
3. **Age (years)**					
i. < 1	593 [23.5]	98 [16.5]	Ref		
ii. 1–2	786 [31.2]	212 [27.0]	1.61	1.16 – 2.24	0.004 *
iii. 2–3	302 [14.3]	91 [30.1]	2.09	1.38 – 3.16	< 0.001 * **
iv. > 3	839 [30.9]	259 [30.8]	3.41	2.39 – 4.85	< 0.001 * **
4. **District**					
i. Serere	840 [33.3]	52 [6.2]	Ref		
ii. Isingiro	840 [33.3]	255 [30.4]	18.85	7.44 – 47.75	< 0.001 * **
iii. Nakapiripirit	840 [33.3]	353 [42.0]	35.15	13.94 – 88.64	< 0.001 * **

Level of statistical significance: 0 '* ** ' 0.001 '* *' 0.01 '* '

seroprevalence reported in Nakapiripirit district in the current study was lower than the overall average seroprevalence of 57.6% previously reported in the Karamoja region (Mulindwa et al., 2011). It was also lower than the 72.0%, 85.0% and 63.2% seroprevalence reported from the Karamoja districts of Nakapiripirit, Kotido and Moroto, respectively (Mulindwa et al., 2011). The study by Mulindwa et al. (2011) was conducted following the first major reported PPR outbreak in the Karamoja region in 2007, a situation that might have led to overestimation of PPRV seroprevalence. Additionally, Mulindwa et al. (2011) set out to

Table 3
Final multivariable Poisson regression model for village-level risk factors of PPRV antibody seropositivity [N = 84] in Serere, Nakapiripirit and Isingiro districts, Uganda (2022).

Risk factors	No. villages [%N]	Estimate	Std. Error	IRR	95% CI	p-value
Other flock contact frequency						
Never	20 [23.8]	Ref				
Daily	61 [72.6]	0.46	0.15	1.59	1.19 – 2.13	0.002**
< once a month	3[3.6]	-0.69	0.31	0.5	0.26 – 0.90	0.028*
Water source¶						
Communal	68 [81.0]	-0.90	0.15	0.41	0.30 – 0.54	< 0.001***
Swamp	25 [29.8]	0.27	0.10	1.32	1.07 – 1.61	0.009**
Borehole	44 [52.4]	-0.61	0.12	0.55	0.43 – 0.69	< 0.001***
Other livestock owned¶						
Cattle kept	72 [85.7]	0.65	0.15	1.91	1.43 – 2.56	< 0.001***
Pigs kept	31 [36.9]	0.26	0.12	1.30	1.03 – 1.64	0.027*
Restocking¶						
Goats purchased	25 [29.8]	-0.30	0.11	0.74	0.60 – 0.93	0.008**
Sheep purchased	16 [19.0]	-0.38	0.14	0.69	0.52 – 0.90	0.008**
Goats born within	44 [52.4]	0.65	0.15	1.92	1.44 – 2.57	< 0.001***
Other flock contact	58 [69.0]	0.42	0.27	1.52	0.87 – 2.53	0.124
Vaccination status¶						
PPRvaccinated	19 [22.6]	0.66	0.11	1.93	1.56 – 2.38	< 0.001***
District						
Serere	28 [33.3]	Ref				
Isingiro	28 [33.3]	2.37	0.25	10.65	6.65 – 17.40	< 0.001***
Nakapiripirit	28 [33.3]	2.41	0.21	11.14	7.49 – 16.90	< 0.001***

Level of statistical significance: 0 '* ** ' 0.001 '* *' 0.01 '* ' 0.05, R^2 Nagelkerke = 0.992, IRR= incidence rate ratio, Std. Error = standard error, ¶ These were binary categorical variables (yes or no); only "yes" is tabulated for ease of visualization, otherwise "no" is the reference category.

collect 354 samples but for some reason they were only able to sample a total of 280 animals in the four study districts, which might have further underpowered their study leading to potential overestimation of prevalence.

Our PPRV seroprevalence estimate from the pastoral production system was lower than 55.26% (Luka et al., 2011) previously reported from a sero-monitoring study and 51.4% (Akwongo et al., 2022) reported from a study that only focused on communal protected kraals as primary sampling units. Following vaccination, the antibody prevalence is expected to be higher than expected in apparently healthy flocks whereas protected kraals maximise the chance of nose-to-nose contact between small ruminants and thus increasing their likelihood of exposure to PPRV. The lower animal-level prevalence reported in this study could therefore partly be explained by a larger and more representative sample as well as a shift in time and dynamics in small ruminant flocks because of fast small ruminant enterprise turnover. The recent reduction in the number of PPR outbreaks in the Karamoja region could have resulted in less PPRV exposure and hence a reduction in PPRV seroprevalence (Nkamwesiga et al., 2022).

The PPRV seroprevalence of 30.4% for Isingiro district reported in this study was higher than 22.2% reported from a convenient sample of sheep from communities around the wildlife-livestock interface in Kasese district, southwestern Uganda (Fernandez Aguilar et al., 2020). In southwestern Uganda, Isingiro district, an agropastoral production system (fenced grazing with occasional transhumance), experience extreme drought seasons each year. Drought seasons in turn result in informal animal movements into other neighboring districts in Uganda (Nkamwesiga et al., 2022) and across the international border, in northern Tanzania with reported PPRV antibody seroprevalence ranging from 21% to 78% (Idoga et al., 2020) which potentially increases the risk of small ruminant exposure to PPRV. There has also been evidence of animal movement across international borders for purposes of trade and other social functions such as traditional weddings which also increase the risk of disease introduction into previously free areas (Wieland et al., 2020).

Serere district [proxy for mixed crop-livestock system] in the Teso subregion in Eastern Uganda is one of the districts where PPRV outbreaks had never been reported by the time of this study. Consistent with a previous small study (Ruhweza et al., 2010), we found a very low (6.2%) PPRV antibody seroprevalence in Serere district. In Teso subregion, small ruminants are often tethered to restrict them from grazing on crops which strongly limits animal co-mingling and therefore the risk of PPRV transmission; thus, explaining the low PPR seroprevalence levels. Owing to the very low seroprevalence estimates in Serere district, there is an urgent need to vaccinate flocks in the mixed crop-livestock production systems to protect them from future PPR outbreaks which often negatively impact livelihoods.

Generally, the seroprevalence of PPR was significantly higher in small ruminants older than one year of age than in those under one year of age. This observation is consistent with previous studies in Uganda and elsewhere (Akwongo et al., 2022; Torsson et al., 2017). Older small ruminants are more likely to have been exposed to PPRV during the course of their lives than those below one year, especially in PPR endemic countries such as Uganda. Additionally, older animals are more likely to have been exposed to vaccination against PPR especially in endemic areas with vaccination campaigns. Being a female small ruminant was identified as a significant animal-level risk factor for PPRV antibody seropositivity. This is in line with previous studies that have suggested female small ruminants have a higher risk of being PPRV antibody seropositive than male small ruminants (Kihu, Gachohi et al., 2015; Megersa et al., 2011; Torsson et al., 2017). It was suggested that female ruminants, owing to their key role in flock multiplication, are often kept for longer times at the farm which increase their likelihood of exposure to PPRV; thus, a higher risk of being seropositive than male small ruminants. However, this relationship could also be spurious because the average number of males is usually much lower than that of females. In our case, males represented only 15.5% (230/2520) of the entire sample.

We found that daily flock contact with neighboring flocks was strongly associated with increased PPRV antibody seropositivity within flocks. On the other hand, flocks that interact less frequently (less than once a month) were associated with decreased likelihood of having PPRV seropositive animals. This can be explained by the fact that PPR

mode of transmission is through direct contact between susceptible and infected animals and therefore the higher the contact frequency, the higher the chances of PPR transmission between flocks (Ekwem et al., 2021).

Flocks from villages where animals are watered at swamps were more likely to have PPRV seropositive animals compared to those that do not have swaps in their areas. Swamps are usually communal watering points usually attracting all animals in a village to drink water which in turn increases chances of interacting with PPRV infectious flocks. This is consistent with previous studies that have reported communal water sources as significant risk factors for infectious disease transmission especially those that require direct contact (Ekwem et al., 2021; VanderWaal et al., 2017). Conversely, villages whose water source was borehole were significantly associated with reduced chances of having PPRV seropositive animals. This is partly because boreholes are more restricted and are more likely restrict animal congregation hence boreholes being a protective factor.

In Uganda, households that keep cattle are the ones that also keep majority of the small ruminants. Moreover, keeping cattle was associated with an increased number of PPRV antibody seropositive animals. Considering that cattle are "dead-end" hosts for PPRV (Herzog et al., 2020), it is highly unlikely that they contribute to PPRV antibody seropositivity in small ruminants. Interestingly, villages where small ruminants were kept in addition to pigs were more likely to have PPRV seroprevalence as compared to villages where no pigs were kept. Pigs have previously been linked to shedding of PPR virus (Schulz et al., 2018), although experimentally, their role in the field epidemiology of PPR needs to be investigated further.

We also found that villages that maintained small ruminant flocks for at least one year (without any foreign introduction via purchase or gift) were more likely to have PPRV seropositive animals than villages that purchased sheep and goats in the previous year. Maintaining the animals in one area for a long period increases the chances of exposure to PPR virus especially in endemic districts whereas purchase of animals to improve breed or increase flock size could introduce naïve animals into the flock, depending on the source of animals and the status of their vaccination. Village where farmers reported that PPR vaccination had occurred in the past 12 months were 1.9 times more likely to be seropositive compared to villages where no PPR vaccination had been conducted. This was likely so because there are veterinarians that carry out private vaccination especially for commercial small ruminant farms, it was the reason we included this question in the questionnaire to try and explain some of the results.

Villages from pastoral and agropastoral production system districts were more than 10 times more likely to have PPRV antibody seropositive animals than those from mixed crop-livestock production system district. Transhumant pastoralists like those in Nakapiripirit district, Karamoja region, tend to move their flocks over long distances in the dry season to water and graze them at communal watering points and pasture fields respectively (Mbyuzi et al., 2014). Animal movements in search of pastures and water are maximal and semi-maximal in pastoral and agropastoral production systems. These two small ruminant production systems have been significantly associated with PPR seropositivity, as reported elsewhere (Fournié et al., 2018; Mdetele et al., 2021). Communal grazing and communal watering of small ruminants increases the likelihood of effective nose-to-nose contact between animals and therefore promotes PPRV transmission (Herzog et al., 2019). Additionally, animal movement, especially for trade purposes, has previously been linked to an increased potential for the spread of infectious diseases (Hasahya et al., 2023). Moreover livestock restocking programs in Uganda by different governmental and non-governmental organizations rarely adhere to strict laboratory screening and/ vaccination guidelines which potentially leads to introduction of infectious diseases such as PPR into naïve flocks, as has been the case with other livestock diseases (Selby et al., 2013).

There were two statistically significant spatial clusters of PPRV infection across the study area (Fig. 3). The spatial clusters of disease are epidemiologically defined as a set of interconnected regions which attains the maximum likelihood ratio as identified by spatial scan statistic as the most likely cluster (MLC) (Tango, 2021). The first cluster was identified around villages in Nakapiripirit district at the international border with Kenyan west Pokot pastoral communities in Turkana county, Kenya, which has previously been associated with high PPRV antibody seroprevalence of 40% and 36% in goats and sheep respectively (Kihu, Gachohi et al., 2015; Kihu, Gitao et al., 2015). The second statistically significant likely cluster was identified around Isingiro district, at the international border with Tanzania. These statistically significant spatial disease clusters are consistent with a previous study that documented confirmed PPR outbreaks over a 14-year period in Uganda (Nkamwesiga et al., 2022). International borders are associated with small ruminant comingling during both official and illicit livestock movement for international trade and in search of pasture and water. These international borders are both occupied by pastoral communities that freely move livestock across frontiers during the dry season.

5. Study limitations

The unavailability of a serological test that differentiates naturally infected animals from those vaccinated against PPR suggests that we could have inadvertently included previously vaccinated animals in the study, possibly leading to overestimation of seroprevalence in some areas. This likelihood is further aggravated by the absence of a livestock identification and traceability system in Uganda which makes it difficult to trace vaccinated animals. Nonetheless, we worked with the area veterinarians to select animals from households that had not participated in PPR vaccination exercise for at least 12 months. Also, the fact that PPR vaccination coverage in Uganda accounts for less than 10% of the total small ruminant population gives us confidence that the estimates from this study are reliable and can be used to guide interventions. There was generally poor record keeping at flock level in livestock keeping communities which could have introduced recall bias especially at village level. However, we made an effort to triangulate our findings with key stakeholders especially district veterinary officials to reduce the effects. This coupled inherent variation in production types per village across districts, these results may not be perfectly generalisable to all districts in Uganda. However, this study provides a starting point to initiation of production system-based interventions.

6. Conclusion

Transhumant pastoral production system was associated with the highest PPR antibody prevalence while smallholder mixed crop-livestock production systems where goats are often tethered reported the lowest PPRV seroprevalence. Agro-pastoral production system presented with mid-range risk of PPR seropositivity. Animal sex (female / male), age (in favour of older animals; >1 year) were the animal-level risk factors of PPR seropositivity while rearing of cattle and pigs, communal water sources and frequency of contact between flocks were production system-based risk factors of PPR seropositivity. Targeting PPR control interventions (vaccination and livestock movement control) to and from pastoral and agro-pastoral small ruminant production systems that are prone to PPR incursions is recommended to prevent PPRV spread to low-risk smallholder small ruminant production systems.

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CRediT authorship contribution statement

JN: study design, data collection and analysis, writing, and reviewing; FK, DPN: data analysis and reviewing; PL: writing, and reviewing; KR: grant acquisition, study conceptualization, study design and reviewing, BW: study conceptualization and reviewing; HK and DM: study design, writing, and reviewing; AP: data analysis, reviewing; all authors have read and approved this manuscript for publication.

Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.106051.

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Chapter 5: Detection and Characterisation of Peste des petits ruminants virus and other co-infections from suspected outbreaks in Uganda 2020-2022

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Detection and Characterisation of Peste des petits ruminants virus and other co-infections from suspected outbreaks in Uganda 2020-2022

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Abstract

Peste des petits ruminants virus (PPRV) is the causative agent of the transboundary animal disease Peste des petits ruminants (PPR) in both domestic and wild small ruminant hosts. In this study we used a panel of molecular diagnostic techniques to test for the presence of nucleic acid material of five different small ruminant disease pathogens. The studied pathogens included PPRV, Foot and mouth disease virus (FMDV), Goat pox virus (GTPV), *Parapoxvirus ovis* (ORFV) and *Mycoplasma capricolum* subspecies *capripneumoniae* (CCPP) from 18 suspected PPR outbreaks across 15 districts in Uganda between 2020 and 2022. Additionally, samples that tested positive for PPRV using RT-PCR were sequenced using the Sanger method. PPRV nucleic acid material was detected in samples from 61.1% (11/18) of the suspected PPR outbreaks. At least one of the assayed disease pathogens was detected in 83.3% (15/18) of all reports. A range of co-infections were detected, with PPRV and ORFV being the commonest at 4.4% (20/451), followed by PPRV-CCPP and ORFV-GTPV both at 0.4% (2/451). The least common coinfection was PPRV and GTPV at 0.2% (1/451). Nucleotide sequencing of the partial PPRV nucleoprotein and fusion gene amplicons revealed that the viruses belonged to PPRV lineage III. Nucleotide sequences from majority of the districts clustered very closely with recently characterised lineage III sequences from Burundi. Additional sequences from Kazo and Kasese districts in Western Uganda showed 100% similarity to the previously reported lineage III sequences from Tanzania. This study confirms that putative clinical outbreaks were indeed caused by PPRV but co-circulation of multiple disease pathogens with PPRV could potentially complicate diagnosis and should therefore be incorporated into the PPR national surveillance and control plans. Moreover, the very high similarity between PPRV nucleotide sequences from this study and those from neighbouring countries points to transboundary movement of livestock, which should also be considered if PPR and other important small ruminant diseases are going to be effectively controlled.

Key words: co-infection, PPRV, Parapox ovis, *Mycoplasma capricolum*, Goat pox virus, Foot and mouth disease virus

Introduction

Peste des petits ruminants (PPR) is a transboundary animal disease that infects domestic small ruminants (goats and sheep) and, to some extent, wild ruminant species including African buffalo (*Syncerus caffer)*, gazelle (Gazella spp.), waterbuck (*Kobus ellipsiprymnus)* and many others. It is caused by Peste des petits ruminants virus (PPRV), classified under the family *Paramyxoviridae*, genus *Morbillivirus*, and is the only member of the *Morbillivirus caprinae* species (Schoch et al., 2020).

Clinical PPR progression in domestic small ruminants typically follows five different disease stages including the incubation period, which typically lasts for two to six days; the febrile phase, marked by fevers exceeding 40 °C; the mucosal phase, involving discharges from the nose and eyes and erosion of the oral cavity (tongue, lips, and gums); and the diarrheal stage, typified by foul-smelling diarrhea that stains the affected animal's hindquarters (Diallo, 1995). The recovery stage, which occurs in non-fatal cases, is the last stage of the illness during which animals that survive acquire lifelong immunity that often lasts at least three years (Balamurugan et al., 2014; Diallo, 1995).

It may be difficult to observe all typical PPR clinical symptoms under epidemic conditions, particularly in endemic settings due to different levels of disease modulation through immunity of previously exposed or vaccinated animals in flocks (Taylor, 1984). Furthermore, PPR is frequently misdiagnosed as other small ruminant respiratory infectious diseases because of how challenging it is to distinguish its clinical presentation from other PPR-like diseases (Santhamani et al., 2016). Contagious ecthyma (Orf, *Parapoxvirus ovis*), Sheep and goat pox (GTP, Goat pox virus), Bluetongue (BT, Bluetongue virus), Foot and mouth disease (FMDV, Foot and mouth disease virus), Contagious caprine pleuropneumonia (CCPP, *Mycoplasma capricolum* subspecies *capripneumoniae*) and pasteurellosis (*Pasteurella multocida, Mannheimia haemolytica*) are among the small ruminant diseases with comparable symptoms for the differential diagnosis of PPR (Balamurugan et al., 2014; Diallo, 1995). There is an increasing demand for molecular diagnostic tests that simultaneously detect as many disease-pathogen combinations to be included in routine PPR surveillance activities to address other important small ruminant diseases with negative economic impact on the small ruminant production enterprise (Settypalli et al., 2016).

The small ruminant production venture is preferred by smallholder farmers because they have a quick production cycle, are easy to de-stock and restock, and also require relatively low capital investment to kickstart the venture (Devendra, 1994). Consequently, smallholder farmers depend on the small ruminants for their livelihood, especially as a quick source of money to take care of medical bills, children's school fees, settling of debts (traditional fines, dowry among others), and animal-source proteins for the households (FAO, 2013). The small ruminant production enterprise is now threatened by PPRV and other small ruminant diseases. Considering the dire effects of PPR on the small ruminant industry and the livelihood of world's poor, the Food and Agriculture Organisation of the United Nations (FAO) and the World Organisation for Animal Health (WOAH), launched a global campaign to eradicate the disease by 2030 (FAO & WOAH, 2022).

In naïve populations, PPR causes morbidity and case-fatality rates between 80 and 100% whereas in endemic settings, morbidity and mortality rates often range between 10 and 100% (Albina et al., 2013). Global yearly economic losses resulting from PPR are estimated to be between USD 1.5 and 2.1 billion (Jones et al., 2016). These costs result from lost opportunities for international trade, decline in body condition, expense of control methods such as vaccination, reduction in milk yield among others (Jones et al., 2016). In Uganda, PPR was first reported in 2007 in Karamoja subregion, an outbreak attributed to loss of over 500,000 small ruminants estimated at about USD 30 million (Luka et al., 2012).

The severity of the disease depends on several animal-level factors, such as age, species, breed, immune status, and other factors such as the virulence of the virus lineage involved (Banyard et al., 2010). The PPRV has four distinct lineages (I – IV) that are endemic in different geographical areas; lineages I and II are predominantly West African lineages whereas lineages III and IV are predominantly eastern Africa and Asian lineages respectively (Banyard et al., 2010). Lineage I was the first PPRV lineage observed in Côte d'Ivoire in the 1940s, spread across West Africa to Senegal until 1969 when it was reportedly replaced by lineage II. However, PPRV lineage I, was unexpectedly detected in Mali, West Africa, in 2014 after being considered extinct (Tounkara et al., 2021). Since the 1970s, lineage II has been the dominant and almost the only PPRV lineage across West Africa. In Africa, the Asian lineage IV was first detected in Nigeria in 2008 and has continued to rapidly expand to the rest of western and northern African countries and to some extent central and eastern African countries (Kwiatek

et al., 2011). Lineage III on the other hand, has been the predominant lineage in eastern Africa having first been reported in Sudan in the 1970s. PPRV lineage III appears to be replaced by the Asian lineage IV, especially in the case of Ethiopia and Sudan (Kwiatek et al., 2011). PPRV lineage III has been the only lineage linked to PPR outbreaks in Uganda for the past 15 years, with lineage IV being linked to just one outbreak that occurred in the Karamoja subregion in 2007 (Luka et al., 2012). Uganda is a landlocked country bordered by the Democratic Republic of the Congo (DRC), Kenya, Tanzania, Rwanda, and South Sudan. The majority of these nations have formally acknowledged the existence of PPR through genetic or serological means. With PPRV lineage III being the most prevalent in eastern Africa, it has been shown to infect small ruminants in Kenya, Tanzania, and the Democratic Republic of the Congo (Dundon et al., 2020).

Among the countries bordering Uganda, PPR was first officially reported in Kenya in 2007 in the West Pokot County that borders Karamoja subregion in Uganda (Gitao et al., 2014). Following PPR confirmation in Kenya, an outbreak of PPR was also confirmed in Karamoja subregion in Uganda later that year (Luka et al., 2012). The disease outbreaks in both Kenya and Uganda were confirmed to have been caused by PPRV lineage III much as Luka et al. (2012) erroneously classified the viruses in the Karamoja region outbreak as lineages I, II and IV. Upon reanalysis of the nucleotide sequences generated from the Luka et al. (2012) study, the viruses were classified as lineage III and IV. The PPRV lineage IV reported by Luka et al. (2012) in Uganda was genetically more closely related to isolates from Nigeria and India which suggest a potential laboratory contamination of the isolates since this lineage has not been reported in Uganda ever again since 2007 (Dundon et al., 2020).

In Tanzania, serological evidence of presence of PPRV in the country was first officially reported in 2008 with molecular confirmation in 2014 (Kgotlele et al., 2014). PPRV lineage II and III have both been reported in Tanzania, however from the recent studies, it seems only lineage III has been confirmed by partial and or whole genome sequencing as the available lineage II sequences from Tanzania share a striking resemblance to the Nigeria 75/1 vaccine strain suggesting potential laboratory contamination (Dundon et al., 2020). Along the same southern border, only serological evidence of PPR presence was recently reported from Rwanda (Shyaka et al., 2021). Nonetheless, PPR is very likely to circulate in Rwanda given that confirmed PPR cases have been documented in all surrounding nations, including

Uganda, Burundi, and the Democratic Republic of Congo.

Historical data indicates that PPRV lineage IV, which was subsequently linked to PPR outbreaks in neighboring Gabon and Nigeria, was the cause of the first official PPR outbreaks in the Democratic Republic of the Congo (DRC) between 2008 and 2012. However, more recent studies in DRC, have confirmed circulation of PPRV lineages II and III (Mulumba-Mfumu et al., 2021; Tshilenge et al., 2019). At the Uganda's northern border lies South Sudan, a country where PPRV lineage IV has previously been reported to circulate since 2011 (Dundon et al., 2020).

The communities around Uganda's international borders are interconnected by human socio-economic activities such as livestock trade, shared communal grazing, and social exchanges of animals during marriage, among others. The shared international borders and the overlapping epidemiological characteristics around these border areas warrant an epizone approach to effectively control PPR at regional level (WOAH & FAO, 2022).

Characterising PPRV needs to be an ongoing process that includes not only monitoring the genetic changes made by the virus over time (evolution), but also attempting to map the disease's temporal spread throughout various geographic areas (both within and across national borders). To help in this attempt, the surveillance strategies of each impacted country (including Uganda) must include characterization of PPR viruses causing outbreaks. The aim of this study therefore was to determine the PPRV lineages and potential differential pathogens responsible for all putative PPR outbreaks reported in Uganda between 2020 and 2022.

Materials and methods

Sample and metadata collection

All ocular or nasal swab samples and tissues were collected by staff of the Ministry of Agriculture Animal Industry and Fisheries (MAAIF) either through active surveillance activities or outbreak investigation following field reports of PPR-like disease. Aliquots of swab samples in either dry vials or vials containing Phosphate buffered saline (PBS) were then shipped on dry-ice to the Institute of Diagnostic Virology (IVD) at the Friedrich-Loeffler-Institute, Greifswald - Insel Riems, Germany for analysis.

Nucleic acid extraction and PCR amplification

Nucleic acid material (DNA & RNA) was extracted from the samples using the NucleoMag VET kit (Macherey-Nagel, Düren, Germany) and the half-automated King Fisher platform (King-Fisher Flex magnetic particle processor, Thermo Fisher Scientific, Waltham, MA, USA). Real time RT-PCR detection was performed in a CFX 96 real-time PCR cycler (Bio-Rad, Hercules, CA, USA). Briefly, amplification reactions were performed in final volumes of 12.5 µL containing 1.25 µL of RNase-free water, 6.25 µL of a 2× RT-PCR buffer, 0.5 µL of 25× RT-PCR enzyme mix, 1.0 µL of 15 µM specifics primer and 5 µM probe mix (Polci et al., 2015) and 1.0 µL of an internal heterologous EGFP-based control system (Hoffmann et al., 2006) that was co-detected in all RT-qPCR runs using the HEX channel. After completion of the PCR run, threshold-crossing values (Ct) were assigned to each sample in the exponential phase of the amplification plot of each cycle as previously described (Batten et al., 2011). Genome copy numbers were calculated using the droplet PCR (QX200 Droplet Digital PCR System, Bio Rad, Hercules, CA, USA) based on the generated PPRV standard series.

Nucleotide sequencing and analysis

Partial *Nucleoprotein* (N) gene (362 nucleotides) and *Fusion* (F) gene (412 nucleotides) PCR products from all samples with low PPRV RT-PCR ct-values (≤ 35) were sequenced using the commercial BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, Massachusetts, United States). The sequencing products were resolved on an automated DNA sequencer (Applied Biosystems, Waltham, Massachusetts, United States). The raw sequences were curated using FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) and phylogenetic analysis was done using MEGAX software (Kumar et al., 2018). Consensus sequences were generated from a set of identical sequences from each district to represent potentially separate virus populations in each outbreak.

Ethical considerations

The protocol that generated results described in this study was approved [Reference number: SVAR_IACUC/58/2020] by the School of Veterinary Medicine and Animal Resources Institutional Animal Care and Use Committee SVAR (SVAR-IACUC), Makerere University and the Uganda National Council of Science and Technology (UNCST) (reference number: A103ES). Furthermore, this work was endorsed by the Institutional Animal Care & Use Committee (Reference number: ILRI-IACUC2021-08) and the Institutional Research Ethics Committee (Reference number: ILRI-IREC2021-07) at the International Livestock Research Institute. Additionally, the study was administratively approved by all participating district authorities (Prior Informed Consent). Shipment of samples from Uganda to Germany is compliant with Access and Benefits Sharing Agreement (Nagoya protocol) (UNCST reference number: A139ES).

Results

A total of 451 swab samples were collected from 15 districts in 18 different suspected PPR outbreaks reported between January 2020 and September 2022. 19.1% (86/451) of the samples tested positive for PPRV whereas 8.9% (40/451), 2.7% (12/451) and 0.4% (2/451) tested positive for ORFV, GTPV and CCPP respectively. None of the 451 samples tested positive for foot-and-mouth disease virus RNA (**Table 1**).

While there were no animals that tested positive for PPR in the Gulu, Luwero and Sheema suspected PPR outbreaks, 5.9% (4/68), 9.1% (1/11) and 66.7% (2/3) of the animals respectively tested for ORFV. The suspected PPR outbreak in these three districts might have actually been an ORFV outbreak. Only Sembabule district had animals infected with more than 2 of the tested pathogens (**Table 1**).

Table 1: Summary of the 451 samples tested for PPRV and other coinfections across 15 districts.

			No. positive [% positivity]			
S/N	**District**	**No. sampled**	**PPRV**	**ORFV**	**GTPV**	**CCPP**
1	Amudat	48	0[0]	0[0]	0[0]	0[0]
2	Gulu	68	0[0]	4[5.9]	0[0]	0[0]
3	Isingiro	20	2[10.0]	1[5.0]	0[0]	0[0]
4	Kyankwanzi	20	11[55.0]	0[0]	0[0]	2[10.0]
5	Kasese	4	4[100.0]	0[0]	0[0]	0[0]
6	Kazo	1	1[100.0]	0[0]	0[0]	0[0]
7	Luwero	11	0[0]	1[9.1]	0[0]	0[0]
8	Lyantonde	6	5[83.3]	2[33.3]	0[0]	0[0]
9	Moroto	13	1[7.7]	2[15.4]	0[0]	0[0]
10	Moyo	34	6[17.7]	0[0]	0[0]	0[0]
11	Nakasongola	39	30[76.9]	9[23.1]	0[0]	0[0]
12	Rakai	70	10[14.3]	3[4.3]	0[0]	0[0]
13	Sheema	3	0[0]	2[66.7]	0[0]	0[0]
14	Ssembabule	101	15[14.8]	14[13.9]	12[11.9]	0[0]
15	Wakiso	13	1[7.7]	2[15.4]	0[0]	0[0]
	Total	**451**	**86[19.1]**	**40[8.9]**	**12[2.7]**	**2[0.4]**

About 5.5% (25/451) of the sample animals were infected with more than one disease problem. All animals that tested positive for any of the co-infections also tested positive for PPRV. In addition to being positive for PPRV, GTPV and ORFV, two animals from Sembabule district tested positive for 3 disease coinfection combinations. The commonest co-infection was PPRV and ORFV at 4.4% (20/451) followed by PPRV-CCPP and ORFV-GTPV at 0.4% (2/451). Only one animal out of 451 was infected by both PPRV and GTPV. No other infection combinations were detected among the tested samples (**Table 2**).

Table 2: Coinfections of Peste des petits ruminants

		Coinfection combination [%]						
District	**n_sampled**	**PPRV* ORFV**	**PPRV* GTPV**	**PPRV* CCPP**	**ORFV* GTPV**	**ORFV* CCPP**	**GTPV* CCPP**	**ALL_4**
Amudat	48	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Gulu	68	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Isingiro	20	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Kyankwanzi	20	0[0]	0[0]	2[10]	0[0]	0[0]	0[0]	0[0]
Kasese	4	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Kazo	1	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Luwero	11	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Lyantonde	6	2[33.3]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Moroto	13	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Moyo	34	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Nakasongola	39	9[23.1]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Rakai	70	1[1.4]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Sheema	3	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Ssembabule	101	8[7.9]	1[1]	0[0]	2[2]	0[0]	0[0]	0[0]
Wakiso	13	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]	0[0]
Total	**451**	**20[4.4]**	**1[0.2]**	**2[0.4]**	**2[0.4]**	**0[0]**	**0[0]**	**0[0]**

Most outbreaks were reported between July 2020 and July 2021. In the majority of PPRV affected flocks, the percentage positivity was between 75 -100% whereas in ORFV infected flocks, the percentage positivity was below 30%. The commonest coinfection was PPRV and ORF observed in 7 out of 15 districts (**Figure 2**).

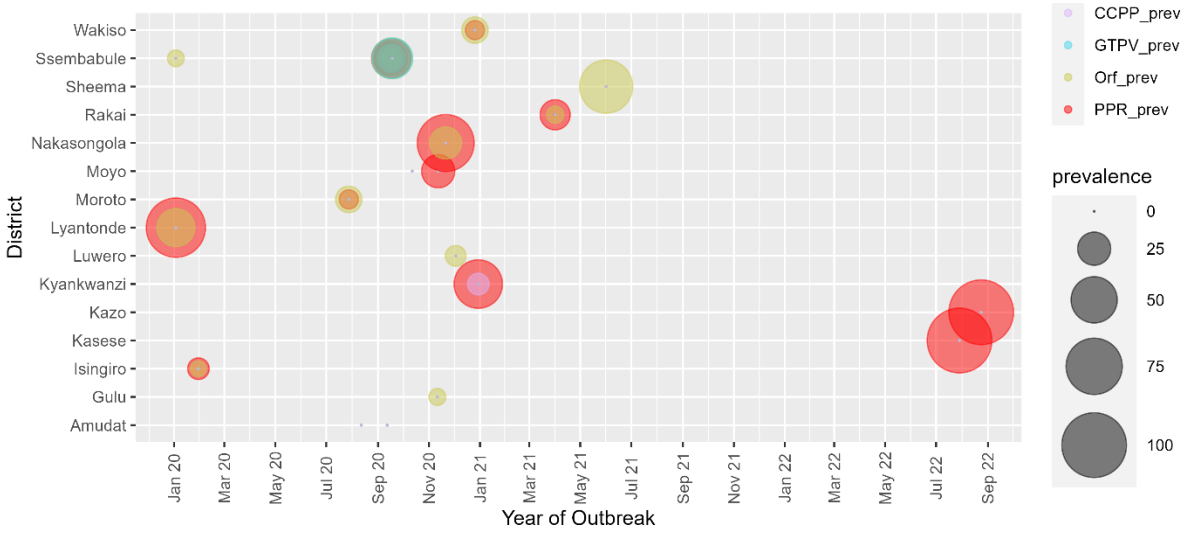

Figure 2: PPRV coinfections in different districts between 2020 and 2022. Each disease pathogen is represented by an individual color code. The graphic was developed using ggplot2 function in R software version 4.3.3

Sequencing results

All the sequences from this study were identified as PPRV lineage III based on both F and N genes. All N gene sequences from each district were identical to one another, except for Nakasongola district where sequences could be grouped into 3 different sets based on single nucleotide changes on position 111. The percentage identity of N gene sequences from this study ranged between 98.2 to 99.7% (**Table 3**)

Table 3 Quality and percentage similarity of the N gene sequences from this study and those in GenBank.

District	Species	Average Ct	No. of sequences	% Identity	Lineage	Country of origin	GeneBank Number
Lyantonde	Caprine	25.0	5	99.6	III	Burundi	MK686066
Isingiro	Caprine	24.3	1	99.7	III	Burundi	MK686066
Kasese	Caprine	22.8	4	98.2	III	Tanzania	MZ322753
Kazo	Caprine	20.63	1	98.3	III	Tanzania	MZ322753
Kyankwanzi	Goat	30.55	5	99.7	III	Burundi	MK686066
Moroto	Caprine	27.16	1	99.4	III	Kenya	KM463083
Moyo	Caprine	26.4	5	99.6	III	Burundi	MK686066
Nakasongola	Goat	28.5	27	99.6	III	Burundi	MK686066
Rakai	Goat	29.3	7	99.6	III	Burundi	MK686066
Ssembabule	Caprine	27.2	7	99.3	III	Burundi	MK686066
Wakiso	Sheep	31.3	1	99.7	III	Burundi	MK686066

Phylogenetic analysis of the N gene sequences revealed clustering of consensus sequences from different districts into three distinct clades. The consensus sequences from Kazo and Kasese districts clustered together with lineage III sequences from Tanzania. There rest of the sequences from this study clustered in a clade that was more closely related to lineage III sequences from Burundi than a lineage III sequence from Moroto district (this study) (**Figure 3**).

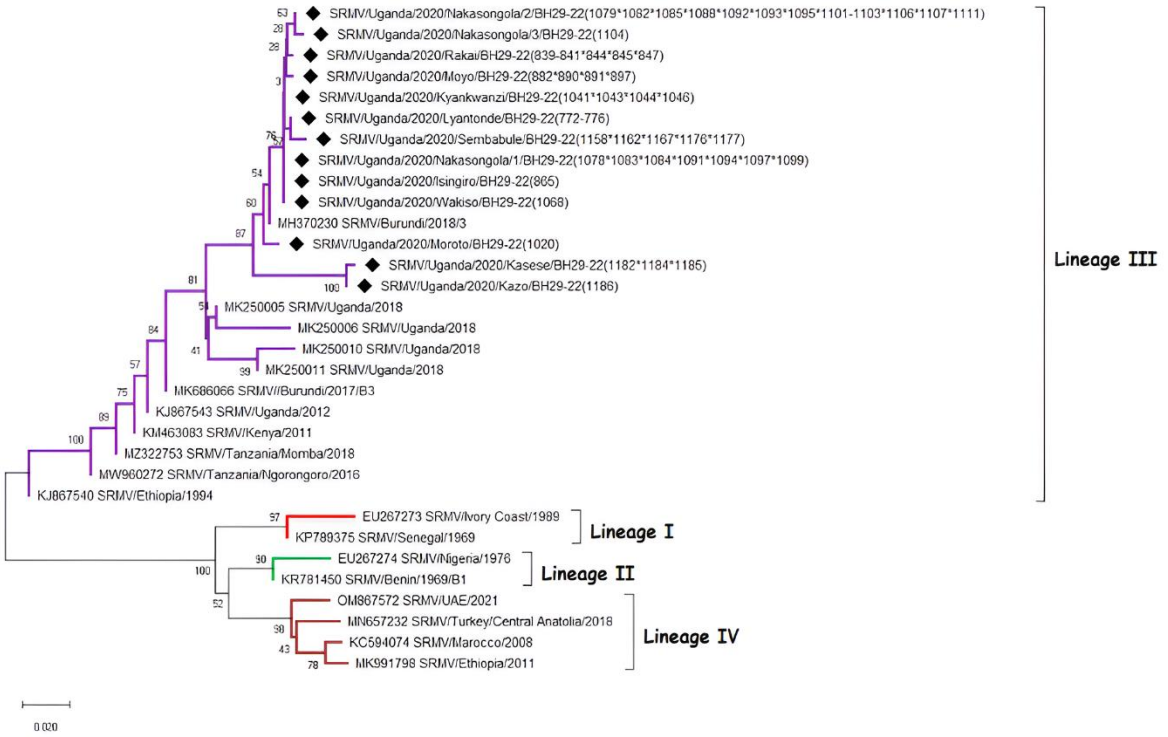

Figure 3: Phylogenetic tree of N gene sequences from this study and related nucleotide sequences from GenBank as constructed by maximum likelihood method. The confidence levels of the association between the paired sequences as assessed via boot strap statistical analysis are indicated by the numbers below and above the branch points. The branch lengths of the scaled-up tree are expressed in terms of the number of nucleotide substitutions per site.

Majority of the F gene sequences from this study clustered with lineage III F gene isolates from Burundi. Similarly, samples from Kasese and Kazo clustered together with lineage III sequences from Tanzania. The percentage nucleotide identity of the F gene sequences ranged between 99.8 – 100% (**Table 4**).

Table 4: Comparison between F gene sequences from this study and those in GenBank

District	Species	Average Ct	No. of sequences	% Identity	Lineage	Country of origin	GeneBank Number
Lyantonde	Caprine	25.0	5	100	III	Burundi	MK686066
Isingiro	Caprine	22.8	1	100	III	Burundi	MK686066
Kasese	Caprine	27.2	4	99.8	III	Tanzania	MZ322753
Kazo	Goat	31.3	1	99.8	III	Tanzania	MZ322753
Kyankwanzi	Sheep	27.16	5	99.9	III	Burundi	MK686066
Moroto	Goat	30.55	1	100	III	Burundi	MK686066
Moyo	Caprine	20.63	5	100	III	Burundi	MK686066
Nakasongola	Goat	28.5	25	99.9	III	Burundi	MK686066
Rakai	Caprine	24.3	7	100	III	Burundi	MK686066
Ssembabule	Goat	29.3	4	100	III	Burundi	MK686066
Wakiso	sheep	26.4	1	99.8	III	Burundi	MK686066

Phylogenetic analysis of Fene sequences revealed similar clustering patterns as those of the N gene. Notably, there were three different groupings of the sequences with samples from Kazo and Kasese clustering with those from Tanzania. The majority of the sequences showed a closer genetic relationship to previous isolates Burundi in 2018 than the ones isolated in Uganda in 2018 (**Figure 4**).

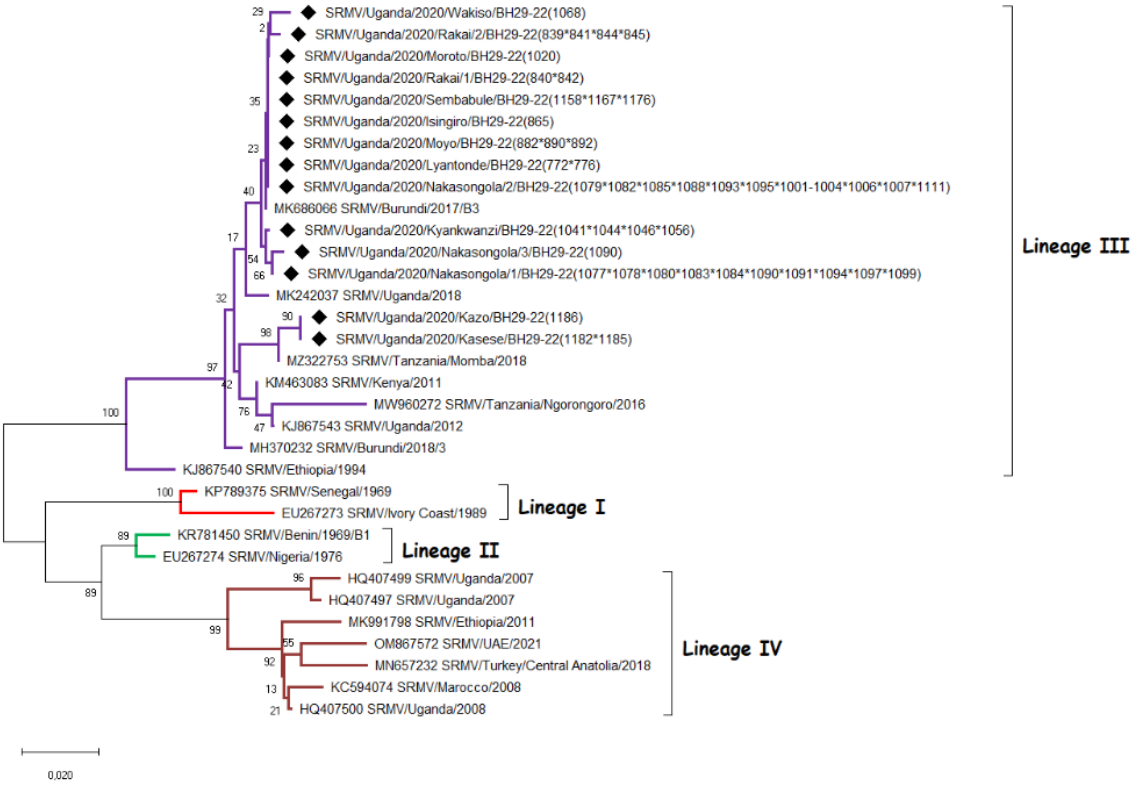

Figure 4: Phylogenetic tree of F gene sequences from this study and related nucleotide sequences from GenBank as constructed by maximum likelihood method. The confidence levels of the association between the paired sequences as assessed via boot strap statistical analysis are indicated by the numbers below and above the branch points. The branch lengths of the scaled-up tree are expressed in terms of the number of nucleotide substitutions per site.

Discussion

In this study, we analysed 451 ocular swab samples from 18 suspected PPR outbreaks across Uganda between January 2020 and September 2022. The samples were subjected to a WOAH recommended PPR molecular detection assay. Additionally, the samples were subjected to a panel of molecular diagnostic tests for detection of FMDV, GTPV, ORFV and CCPP. The samples were positive for at least one of the assayed disease pathogens, except FMDV. As the goal of the global eradication campaign is set for 2030, PPR control efforts are intensified in Uganda. However, in some cases, samples from suspected PPR outbreaks test negative for PPRV and there is usually no other disease pathogen tested. This complicates the relationship between the disease investigators and the livestock farmers as it is not enough to tell the farmers that the animals are negative for PPR. In this study, we provide evidence and clues that in addition to PPR, there are other important small ruminant diseases that need to be simultaneously targeted or at least included in the diagnostic testing and/or surveillance routines.

All the PPR positive samples sequenced from this study belonged to PPRV lineage III. Moreover, for the last 15 years, only PPRV lineage III has been reported from outbreaks in Uganda (Muniraju et al., 2014; Nkamwesiga et al., 2019). It is reasonable to conclude that PPRV lineage IV has since disappeared, or its earlier description was indeed a laboratory contamination as has previously been suggested (Dundon et al., 2020). Phylogenetic analysis of the nucleotide sequences from this study revealed close relatedness among the virus isolates from majority of the districts around Uganda to those reported in neighboring countries such as Burundi and Tanzania (Kinimi et al., 2021; Niyokwishimira et al., 2019). This strongly suggests interconnectedness of the districts through animal movements for different purposes such as trade, social exchanges and breeding as previously suggested (Dundon et al., 2017).

The analysis from this study suggests that there are at least three separate virus transmission systems across different regions in Uganda as districts from the same region tend to have an increased chance of exchanging animals including those infected with PPRV. Most of the sequences from this study were closely related with the recent isolates from Burundi, a result that resonates well with a recent report of trade of animals for breeding purposes from Bushenyi district in Western Uganda to Burundi that coincided with the first PPR outbreak in

Burundi (Dundon et al., 2020).

The PPRV lineage III isolates from Kazo and Kasese districts in southwestern Uganda shared a very close relationship with each other, but most interestingly, they shared a close relationship with isolates from Ngorongoro, northern Tanzania. This relationship strongly affirms the importance of nucleotide sequencing in tracking the transboundary movement of diseases through human socioeconomic activities that must be incorporated in endemic countries' disease control programs to be able to reduce transmission and spread of PPR.

The commonest co-infection detected was PPRV and ORFV. The Parapoxvirus ovis, sometimes referred to as contagious ecthyma, sore mouth, or scabby mouth is a DNA virus that belongs to the Poxvirus family and parapoxvirus genus, very common in most areas where small ruminants are kept. Even though it can infect humans and usually causes a self-limiting skin infection, this virus mainly affects sheep and goats. Typically, animals infected with ORF virus get scabby sores (lesions) in their mouth, on their snout, and around their lips. Although the disease is largely believed to cause a self-limiting disease, it causes serious mortalities in naïve kids and labs (Bala et al., 2018). Coupled with other coinfections such as PPR that are associated with more serious pathologies to the animals, Orf can exacerbate the already constrained small ruminant production venture. Detection of PPRV and ORFV coinfection in this study contributes to the limited body of literature that has previously reported PPRV and ORFV coinfection in PPR endemic countries (Saravanan et al., 2007).

Although it is the first time to report co-infection of PPRV and GTPV in Uganda, it has previously been reported in Nigeria under relatable field conditions (Akanbi et al., 2020). In most PPR endemic countries, clinical diagnosis and indeed PPR disease prognosis are often complicated by coinfections such as CCPP that we detected in this study. Comparable percentage positivity of the animals that tested positive for CCPP in this study were previously reported in the neighboring Tanzania (Kgotlele et al., 2019). CCPP causes significant global economic losses of more than USD 500 million annually although studies documenting the extent of spread and risk factors are still limited across the globe (Ahaduzzaman, 2021; Iqbal Yatoo et al., 2019).

Our goal in this study was to determine the lineages of the PPRV and other co-infections based on reports of potential PPR outbreaks in Uganda. Only 26.7% (4/15) of the outbreaks were linked to diseases other than PPR, while over 73.3% (11/15) of the districts that reported PPR-like sickness in this study had at least one animal testing positive for PPR.

Conclusion

This study confirmed PPRV, CCPP, ORFV and GTPV coinfection among animals from flocks exhibiting PPR-like clinical signs. This observation further complicates field clinical diagnosis of PPR especially in countries without extensive laboratory diagnostics capacity such as Uganda. In conclusion, there is need to incorporate the detected coinfections in the panel of molecular diagnostics in Uganda to be able to achieve the target of effective PPR control of PPR and other small ruminant diseases. Furthermore, this study reports for the first time coinfection of other important small ruminant diseases together with PPRV, an observation we believe will improve preparedness for proper disease management options such as chemotherapeutic treatment and vaccination to simultaneously target different disease pathogens. Animal movement control especially at international borders needs to be strengthened to reduce the likelihood of importing or exporting PPR infected small ruminants.

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Author contribution

JN: study design, data collection and analysis, writing, and reviewing; MM: laboratory screening of the samples, sequencing of the samples, phylogenetic analysis, writing and reviewing; PL: outbreak investigation, sample and data collection, and reviewing; KR: grant acquisition, study conceptualization, study design and reviewing, HK and DM: study design, writing, and reviewing; KO, BH and KD: laboratory analysis, writing and reviewing; all authors have read and approved this manuscript for publication.

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Chapter 6: Network analysis of small ruminant movements in Uganda: implications for control of infectious diseases with a special focus on Peste des petits ruminants

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Social network analysis of small ruminant movements in Uganda: Implications for control of small ruminant infectious diseases with a special focus on Peste des petits ruminants

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Abstract

Domestic animals are moved for reasons that are mutually beneficial to the animal and the farmer. Some examples include the need for fresh grazing grounds and watering points, or the need to access livestock markets for income to sustain farmers' livelihoods. However, livestock mobility is a key risk factor for the transmission of infectious diseases, especially those that have no known arthropod vectors and rely on close contact between animals for their transmission. Contact tracing of individual animals and flocks is very challenging, especially in most low-income countries, due to a lack of efficient livestock traceability systems. Despite these challenges, low-income countries, such as Uganda, issue paper-based animal movement permits (AMPs) to ensure only clinically healthy animals are moved following a physical inspection. In this study, we used national ~9-years (2012—2020) small ruminant movement data obtained from archived AMPs in Uganda to describe small ruminant movement networks. We identified districts which warrant attention as targets of control interventions using standard social network analytic approaches. We identified Lira, Kaberamaido, Nabilatuk, Mbarara, Kiruhura, Kampala and Wakiso as districts with the highest degree (in and out-degree) and betweenness among other centrality measures. Results suggest these districts could be the most important bridges connecting the various regions of

the country. Tailoring control interventions to such districts with high in-coming and high out-going shipments, or bridges, would accelerate the nation's ability to timely detect outbreaks, prevent or mitigate further spread, and contain diseases in their original foci, respectively. We also identified areas for active surveillance, vaccination, quarantine and biosecurity measures depending on prevailing circumstances. These findings will be used to guide the national small ruminant infectious diseases control strategies and subsequently contribute to national and global initiatives, such as the 2030 Peste des petits (PPR) eradication program.

Background

Livestock mobility is one of the major epidemiological risk factors for the transmission and spread of infectious diseases of veterinary and public health concern (Fèvre et al., 2006a). Livestock are often moved from place to place for purposes of trade, social functions, and flock breed improvement within and outside international boundaries (MAAIF, 2022). In Uganda, most animals are moved between districts from within the country and, to a lesser extent, across the frontiers through legal and illicit trade, especially at porous international borders (Medley et al., 2021; Mugezi et al., 2020).

Seasonal variations strongly influence livestock movements across districts in Uganda, as well as across international borders. During the dry season, animals from semi-arid areas are moved in search for fresh pastures and water (Ekwem et al., 2023). The majority of livestock in Uganda are moved from rural to urban and peri-urban areas for slaughter owing to the increased demand for livestock and livestock products (González-Gordon et al., 2023). Additionally, livestock are borrowed or gifted between households for social functions and social security; sometimes these transactions occur across international borders, especially in communities that occupy areas that lie across international borders (Iyer, 2021; Mugezi et al., 2020; Okello et al., 2015).

In Uganda, there are more small ruminants (21.8 million total estimated; 17.4 m goats and 4.4 m sheep) than there are cattle (14.5 million). This is due to small ruminants' ability to multiply quickly, making them easier to convert to cash for farmers to take care of urgent basic needs, like children's school fees, food, healthcare and other livelihood needs (UBOS & MAAIF, 2024). However, the high burden of livestock diseases in Uganda directly affects livestock productivity and the general livelihoods of the livestock keeping communities (Byaruhanga et al., 2017; Vudriko et al., 2021). Furthermore, the risk of zoonotic disease transmission is also very high because of the close contact that occurs between livestock and their caregivers during grazing, watering, milking and transportation events. These activities, coupled with poor biosecurity measures, especially in districts with high levels of livestock mobility, further aggravate the risk of zoonotic transmissions (Birungi et al., 2021; Nyakarahuka et al., 2023; Wolff et al., 2017).

Even though livestock movement positively contributes to the livelihoods of farmers and all those involved in the livestock value chain, its contribution to transmission and spread of infectious livestock diseases (within and beyond national borders) cannot be ignored. For example, the first confirmed outbreak of Peste des petits ruminants (PPR) in Burundi during the year 2018 coincided with movement of an exotic breed of goats from western Uganda to Burundi through Tanzania in a project aimed at improving goat production in Burundi (Dundon et al., 2020; Niyokwishimira et al., 2019). Movement of infected animals between premises,

such as farms and livestock markets, was also reported as one of the most important factors that contributed to the first outbreak of foot and mouth disease in Uruguay (Iriarte et al., 2023). Infectious diseases such as PPR have no known arthropod vector and rely heavily on the close contact between susceptible and infectious small ruminants for their successful transmission. Unrestricted animal movements are a potential threat to the introduction and re-introduction of such diseases across geographical areas in the shortest time possible (Dekelaita et al., 2023; Fèvre et al., 2006b).

Animal mobility data, if well collected through accurate animal traceability systems, can be an integral tool in the management and control of livestock infectious diseases. However, in most African countries, including Uganda, no such systems exist. Instead, paper-based animal movement permits (AMPs) are issued for animals relocating for commercial purposes but less so for local animal movements purposes such as grazing and watering (Chaters et al., 2019). Animals are trekked for relatively short distances, such as to nearby markets within districts, or for longer distances on trucks for different purposes including trade, breeding, and slaughter. In such resource-constrained settings, AMPs are often issued to generally ensure that 1) only healthy animals are moved, and 2) to collect government taxes as a modest amount of money is levied on every individual animal moved (MAAIF, 2022). The AMP data are often incomplete because of either non-compliance, non-vigilance of attending veterinarians, or due to poor archiving methods, which result in unprecedented gaps in the data that are difficult to account for (Chaters et al., 2019). Missing data in these AMPs with long periods of no animal movements recorded make it difficult to determine whether these are true observations (i.e., no animals were moved during these periods), or whether movements were simply not recorded (Chaters et al., 2019). Nonetheless, data from AMPs have previously been incorporated into the social network analytic workflows to identify critical areas for surveillance and targeted control strategies (Chaters et al., 2019; Ekwem et al., 2023).

According to Guinat et al. (2016), the relationship between "actors" or "nodes" and how they are connected to one another (or “edges”) may help forecast the spread of diseases and, at its core, should offer information about the scale of prospective epidemics. This information may also be used to enhance surveillance and control tactics. Social network analysis (SNA) methodologies allow for the identification of important (“central”) nodes (e.g., epidemiological units such as individuals, farms, etc.). In the context of this study, we describe the relationship between nodes through their observed “edges” (i.e., observed animal shipments, frequency of shipments, number of animals moved per shipment, etc.) in an animal movement network of small ruminants within Uganda. Through SNA we describe important players’ influence and “centrality” in the larger movement network by examining the level of “connectedness” each

node is suggested to be by use of standard SNA metrics, as previously described (Dubé et al., 2009; Martínez-López et al., 2009; VanderWaal et al., 2016).

Previous studies in Uganda used recent (2019-2021) animal movement permits that may have had interruptions caused by the COVID-19 lockdown periods and, consequently, only considered a few years to describe animal movement networks (González-Gordon et al., 2023; Hasahya et al., 2023). In this study, we set out to conduct network analyses to describe small ruminant (goats and sheep) movement networks over the approximate 9-year period (2012 — 2020) to understand the flow of small ruminants across the four different regions of Uganda (Central, Northern, Eastern and Western) and their respective subregions. This work, in turn, identifies the critically important districts in each region which should be targeted for small ruminant surveillance and other relevant control interventions to minimize or even block transmission of infectious small ruminant diseases for example PPR.

Materials and Methods

Data source

This study was primarily based on archived animal movement permits for the past 9 years (2012—2020) retrieved from Uganda's Ministry of Agriculture Animal Industry and Fisheries (MAAIF). These permits were digitized in Microsoft Excel to capture key movement attributes such as the district of origin and destination, date on which animals were moved, number and species of livestock moved, as well as mode of movement or transportation, and purpose for the recorded movement. By the time of the study data endpoint (2020), Uganda had 137 districts distributed across 10 subregions with related within-region socioeconomic and demographic characteristics (**Fig.1**).

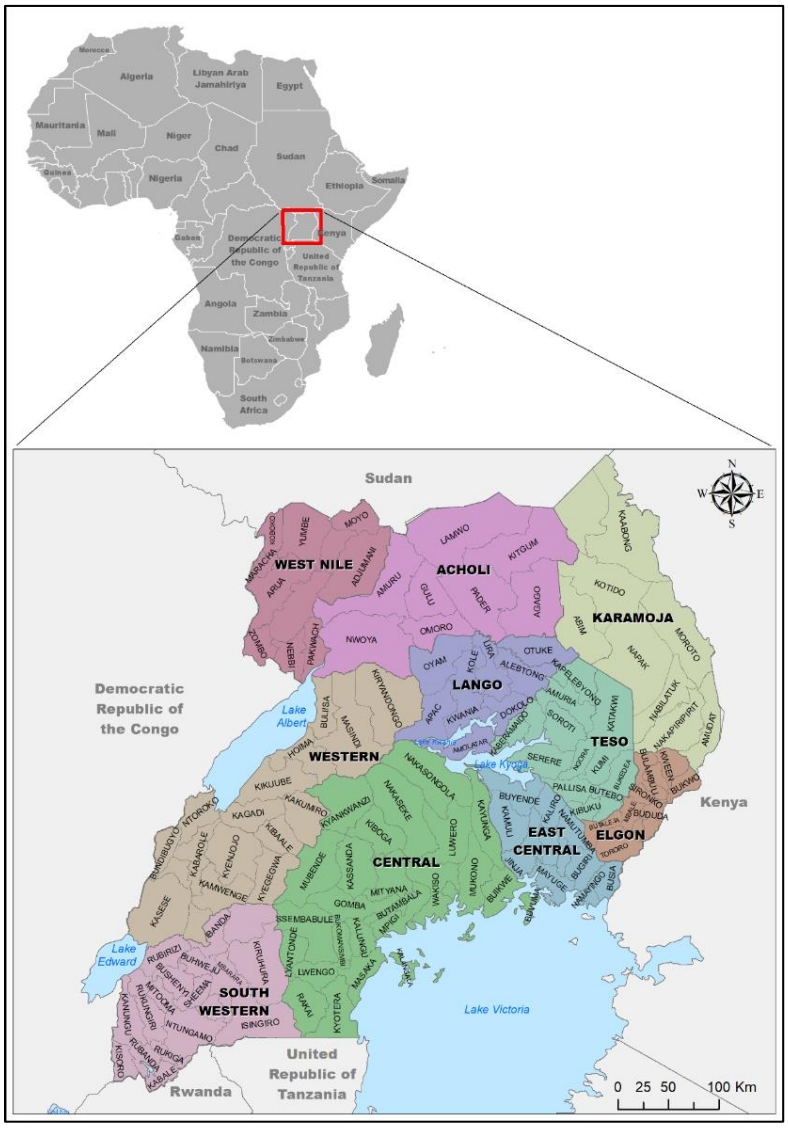

Fig. 1: Map of Uganda showing the different administrative districts and subregions. The map was adapted from a study (Nkamwesiga et al., 2022).

Data Analysis

In the following network analysis, the individual districts are referred to as “nodes” where connections between districts (i.e., “edges”) represent the event of an animal shipment between two districts. Here, a directed network graph illustrates the interconnectedness between districts by means of animal movements from districts of origin to associated destinations. In contrast, an undirected network is a graph where there is no explicit trajectory or direction in the movement; edges could be bidirectional and are often represented with no arrows.

The relative importance of districts or groups of districts was described using social network centrality measures. Network centrality measures are a critical tool for quantitatively describing the relative importance of either, 1) a given node or individual to other nodes (i.e., node-level centrality), or 2) group of nodes (graph-level centrality) in a network. Different centrality metrics have been developed and can be implemented depending on whether the graph is directed or not. However, it is important to note that a node can be “highly central” by one measure and yet have very low centrality by another measure or definition. For instance, though *degree* centrality (see **Table 1**) gives a glimpse of how many unique neighbors a node has in an observed network, it does not necessarily comprehensively reveal how “important” that node is to other nodes, or the whole network. The interpretation of centrality measures strongly relies on the understanding and context of the analysis at hand. Some of the terminologies this study used to describe the small ruminant movement network are summarized in **Table 1**.

Table 5: Some of the key measures of centrality used to describe network graphs.

Parameter	Description	Reference
	General Network Terminologies	
Node	The unit of interest in the network analysis for example individual entities, farms, districts etc.	(Wasserman & Faust, 1994).
Edge	Connection between two nodes in the network graph	(Wasserman & Faust, 1994).
Static network	Snapshot of a network that contains all nodes and edges for a given duration of observation time	(Farine, 2018)
Dynamic network	Captures the structural changes in both edges and nodes over time	(Farine, 2018)
	Node-level Metrics	
Degree	The total number of edges connected to a given node.	(Golbeck,

		2013)
In-degree	The total number of connections directed to a given node	(Wasserman & Faust, 1994).
Out-degree	The total number of connections directed away from a given node	(Wasserman & Faust, 1994).
Closeness centrality	This is a measure of how near a given node is to other nodes computed as the mean length of the shortest paths from one node to each other nodes.	(Golbeck, 2013)
Betweenness	This quantifies the number of times a given node acts as a bridge between two other nodes along the shortest path.	(Gómez, 2019)
Eigenvector Centrality	Measures the importance of a particular node proportional to centrality scores of all its neighbors: a node is important if its neighbors are important.	(Gómez, 2019)
PageRank	PageRank's logic is comparable to that of eigenvector centrality, except it makes a significant distinction: the importance of a node's connection to an important source varies depending on how many or how few links that source has. If there are a lot of links, the source is penalized because its importance is a bit diluted.	(Golbeck, 2013)
Hubs and authority	The Hub score is an efficient measure of the node's ability to send out links whereas the authority score is associated with the node's ability to receive links. Just as in the eigenvector centrality, the importance of a hub or authority is hugely dependent on the corresponding connection. According to inventor of the method: "*A node is an authority if it is linked to by hubs; it is a hub if it links to authorities."*	(Gómez, 2019; Kleinberg, 1998)
	Network-level Metrics	
Diameter	The largest geodesic distance in the network, i.e., the highest number of edges in the shortest path between two nodes	(Wasserman & Faust, 1994).
Assortativity	Tendency of nodes to connect more with nodes that share similar characteristics	(Wasserman & Faust, 1994).

An analysis of variance (anova) was used to compare the different measures of centrality by

subregion. Respective model residuals were tested for homogeneity of variance normality using the Levene and Shapiro Tests, respectively. Finally, the Kruskal-Wallis test with Bonferroni correction was used to compare the metrics that were not normally distributed (Kruskal & Wallis, 1952). The quantity of small ruminants moved, and the frequency of movement transactions were summarized and visualized using *ggplot2* function in R software version 4.4.1 (R Core Team, 2023).

Modelling approach

Two tables were prepared from the digitized AMP spreadsheets using the *dplyr* package from the *tidyverse* collection of packages (Wickham et al., 2019). One table (i.e., the *nodelist*) contained information on the unique districts that appeared as either an origin or destination district along with district-level attributes, such as region, subregion, and the estimated small ruminant population. The second table (i.e., the *edgelist*) contained details specific to the event or date on which small ruminants were moved between two districts in Uganda. The *edgelist* described the number of small ruminants moved, frequency of small ruminant movements, as well as the purpose and mode of transportation. All movement records were aggregated and summarized to describe monthly origin-destination transactions.

A static network was constructed from the prepared node- and edge-lists using the R software package network (Butts, 2008). Each district's earliest onset and latest terminus time (i.e., the earliest and latest month each district was available to move or receive animals) was obtained from observed AMP movement dates. A dynamic network was then constructed with discrete monthly time-steps using the R package *networkDynamic* (Butts et al., 2023).

Network visualizations and centrality measures

Static networks were visualized using the *igraph* package (Csárdi et al., 2024). The *intergraph* package (Bojanowski, 2023) was used to convert the network objects into *igraph* objects before computing node-level metrics. Node-level metrics such as degree, indegree, outdegree, closeness, betweenness *eignenvector* centrality, authoritative score, and PageRank (**Table 1**) were computed using their corresponding functions implemented in the *igraph* package. Node-level attributes of the dynamic network such as backward and forward reachability among other time series metrics were computed using the *tSnaStats* function from the R software package *tsna* (Bender-deMoll & Morris, 2021). Plots were generated using igraph while data were wrangled using *tidyverse* and visualized using *ggplot2* packages (Wickham et al., 2019).

Results

The retrieved animal movement permits (AMPs) contained records from 18th January 2012 to 12th March 25, 2020. The AMP records spanned 95 monthly-time steps across 94% (127/135) of all districts in Uganda with 2,642 unique transactions accounting for more than 200,000 small ruminants moved across Uganda. The total number of small ruminants moved (both in- and out-bound) per month generally increased over time from 2012 to 2020 across the different regions of Uganda. More specifically, a gradual increase in small ruminant movements were observed between January 2012 through January 2016 before a subsequent sharp rise around March 2016. The total number of animals moved continued to increase, resulting in a second sharp rise in animal movements around December 2017. The frequency of small ruminant movements followed a general increasing trend over time (**Figure 2**).

The highest volume of animals was consistently moved from 2018 to 2020. The Western region demonstrated the highest number of small ruminant movements with three temporal spikes attributed to this region across the study period. About 4,000 small ruminants were moved from the western region around June 2016, whereas over 6,800 small ruminants were moved from the western region around December 2017 and June 2020 respectively (**Figure 2a**). Generally, districts from the central region of Uganda dominated the receiving of small ruminants from the rest of the regions in Uganda. Mid 2016, 2017, 2018 and 2020 were the years in which the central region received the highest number of small ruminants. In terms of receiving animals, the central region was followed by the western and northern regions (**Figure 2b**).

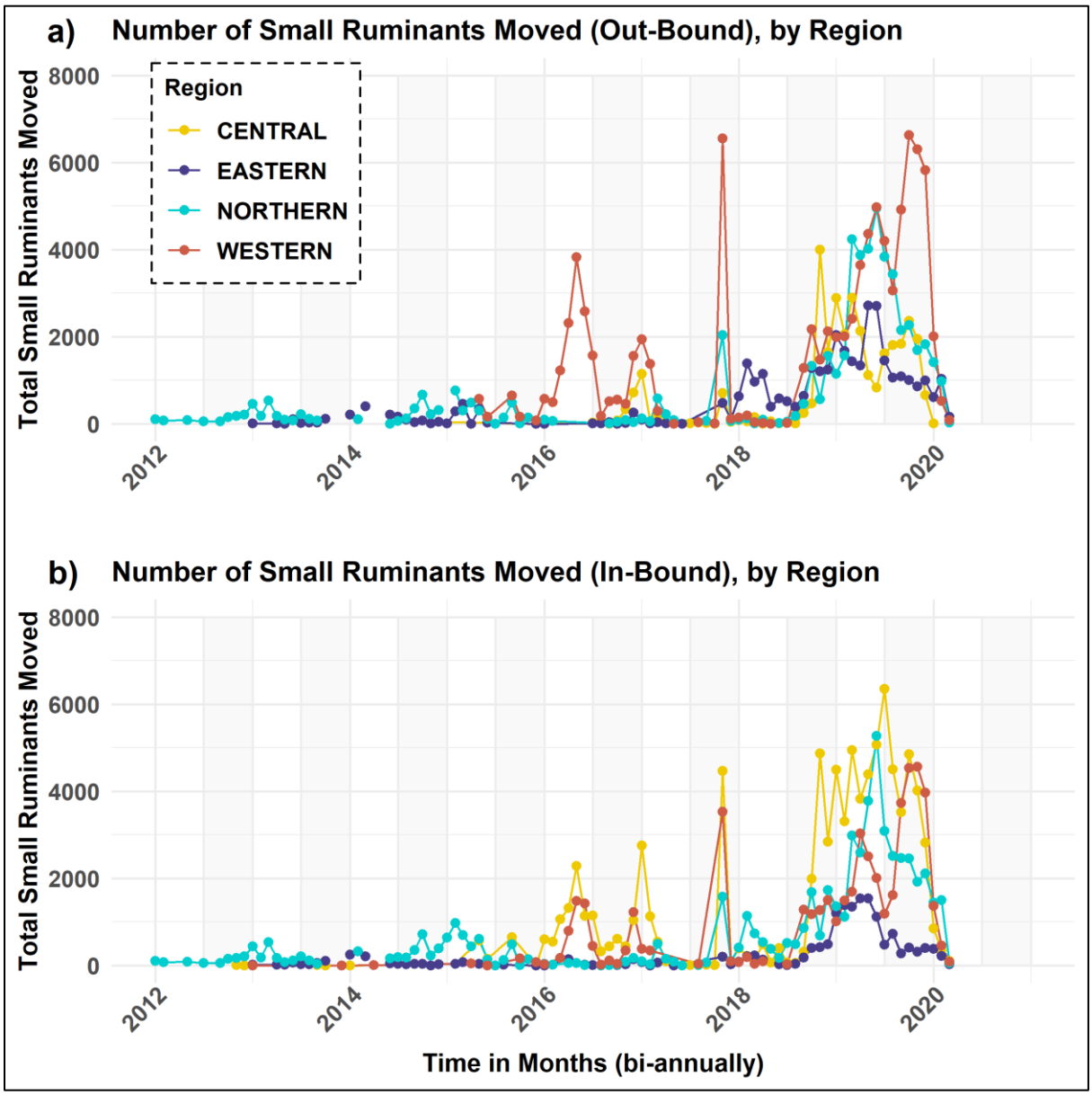

Fig. 2 The total number of small ruminants moved from all 127 districts for a period of 95 months. Panel a) shows the quantity of monthly out-bound small ruminant moved between districts across the four regions of Uganda. Panel b) shows the total number of out-bound small ruminants. The movements were aggregated by a triplicate-variable containing origin—destination—month temporal windows and plotted on a bi-annual scale.

The number of out-bound shipments within and between regions changed drastically over time with the initial shipments between the northern and eastern region albeit on a small scale around the year 2014. Between 2012 and 2015, out-bound small ruminant movements occurred exclusively between northern and eastern regions before movements were dominated by the western region until the last quarter of 2017, with 200 out-bound movements per month being the highest in 2018. The highest frequency of out-bound small ruminant movements (over 350) was observed in the northern region of Uganda, around 2019 (**Fig. 3a**).

Similar trends were observed in the frequency of in-bound small ruminant movements across the study period. Three distinct peaks of high frequency of in-bound monthly small ruminant movements of around 120, 150 and over 200 were observed in 2014, 2018 and 2019 respectively. Frequency of in-bound small ruminant movements was generally dominated by the eastern and northern regions of Uganda with the western and central region dominating only around the last quarter of 2017 (**Fig. 3b**).

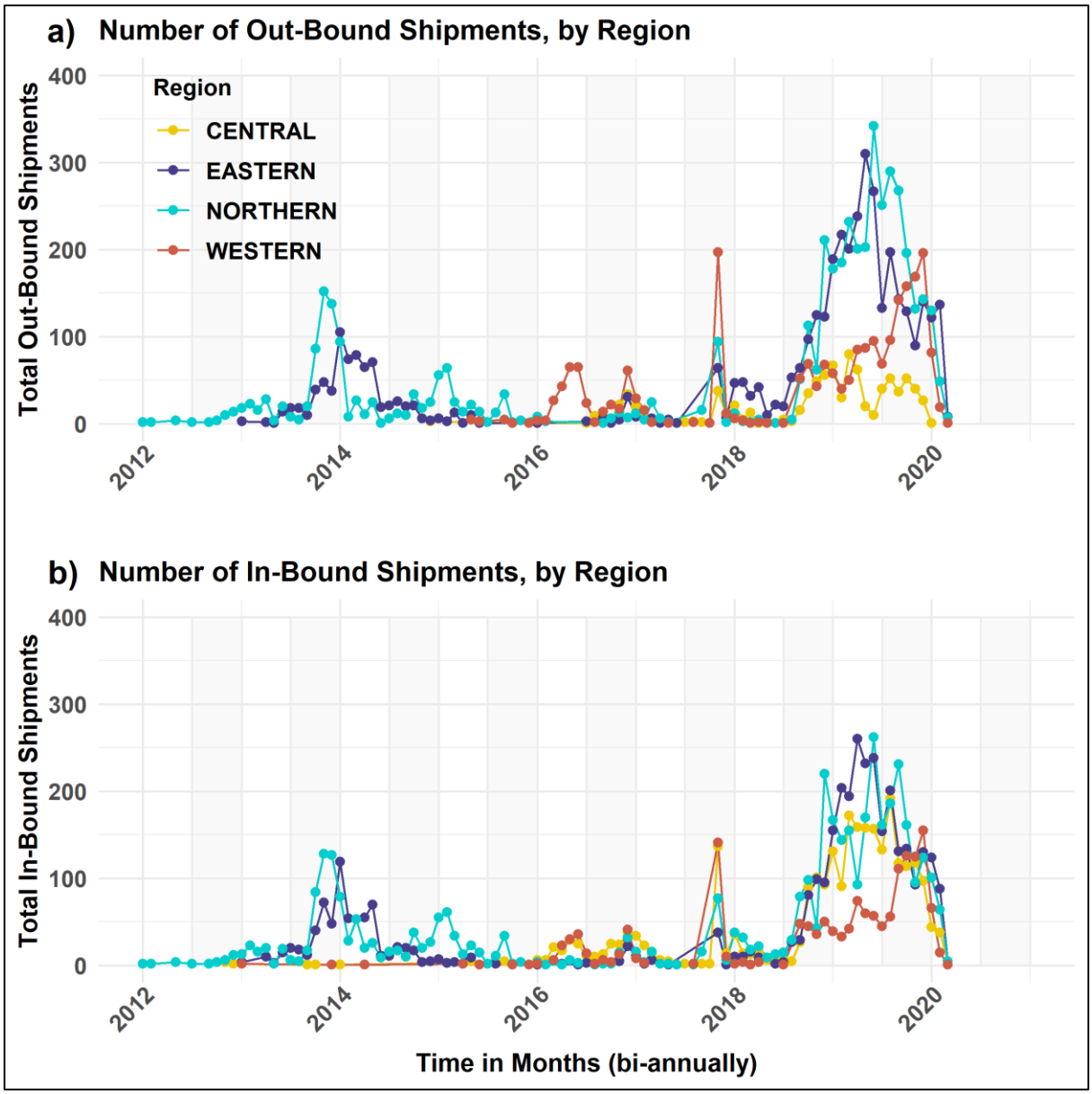

Fig. 3 The total frequency of small ruminant shipments for all 127 districts for a period of 95 months. Panel a) shows the frequency of monthly in-bound small ruminant movements between districts across the four regions of Uganda. Panel b) shows the total frequency of out-bound small ruminant shipments. The movements were aggregated by a triplicate-variable containing origin—destination—month temporal windows and plotted on a bi-annual scale.

Small ruminants, and indeed other livestock, are relocated for purposes including slaughter, breeding, social functions (e.g., dowries), or research purposes, among others. Different means of transport, for example trekking (movements by hoof), truck, boat, motorcycle, bicycle and/or ferry were also observed depending on the convenience, distance and number of animals involved. The two most common reasons for moving small ruminants were for slaughter and breeding, which accounted for 73.8% (8588/11631) and 26.1% (3037/11631) of all small ruminant movement transactions, respectively. Social functions, like dowry, and research purposes contributed the least to the purpose of small ruminants' movement. Movements by trucks and trekking dominated the modes of transportation accounting for 72.7% (8,457/11631) and 20.6% (2,392/11631) of all transactions, respectively (**Table 2**).

Table 2: Total number of small ruminants moved across districts as summarized by "purpose" and "mode of transportation" for the entire study period (January 2012 to January 2020).

Mode of transportation	**Purpose and total number of small ruminants moved**				
	Breeding (%)	Slaughter (%)	Dowry (%)	Research (%)	Total (%)
Truck	1,475 (48.6)	6,979 (81.3)	2 (40)	1 (100)	8,457 (72.7)
Trekking	1,272 (41.9)	1,118 (13.0)	2 (40)	0 (0)	2,392 (20.6)
Motorcycle	191 (6.3)	439 (5.1)	1 (20)	0 (0)	631 (5.4)
Bicycle	99 (3.3)	28 (0.3)	0 (0)	0 (0)	127 (1.1)
Boat	0 (0)	23 (0.3)	0 (0)	0 (0)	23 (0.2)
Ferry	0 (0)	1 (0.0)	0 (0)	0 (0)	1 (0.0)
Overall	**3,037 (26.1)**	**8,588 (73.8)**	**5 (0.0)**	**1 (0.0)**	**11,631 (10)**

During the period 2012-2020, more than 200,000 small ruminants were moved across Uganda districts. Over 90% of the animals were moved in trucks, whereas the rest were either trekked or carried on motorcycles (**Fig. 4a**). Similarly, over 90% (>150,000 animals) of all small ruminants were destined for slaughter while the rest were moved for other purposes including breeding, dowry and research (**Fig. 4b**).

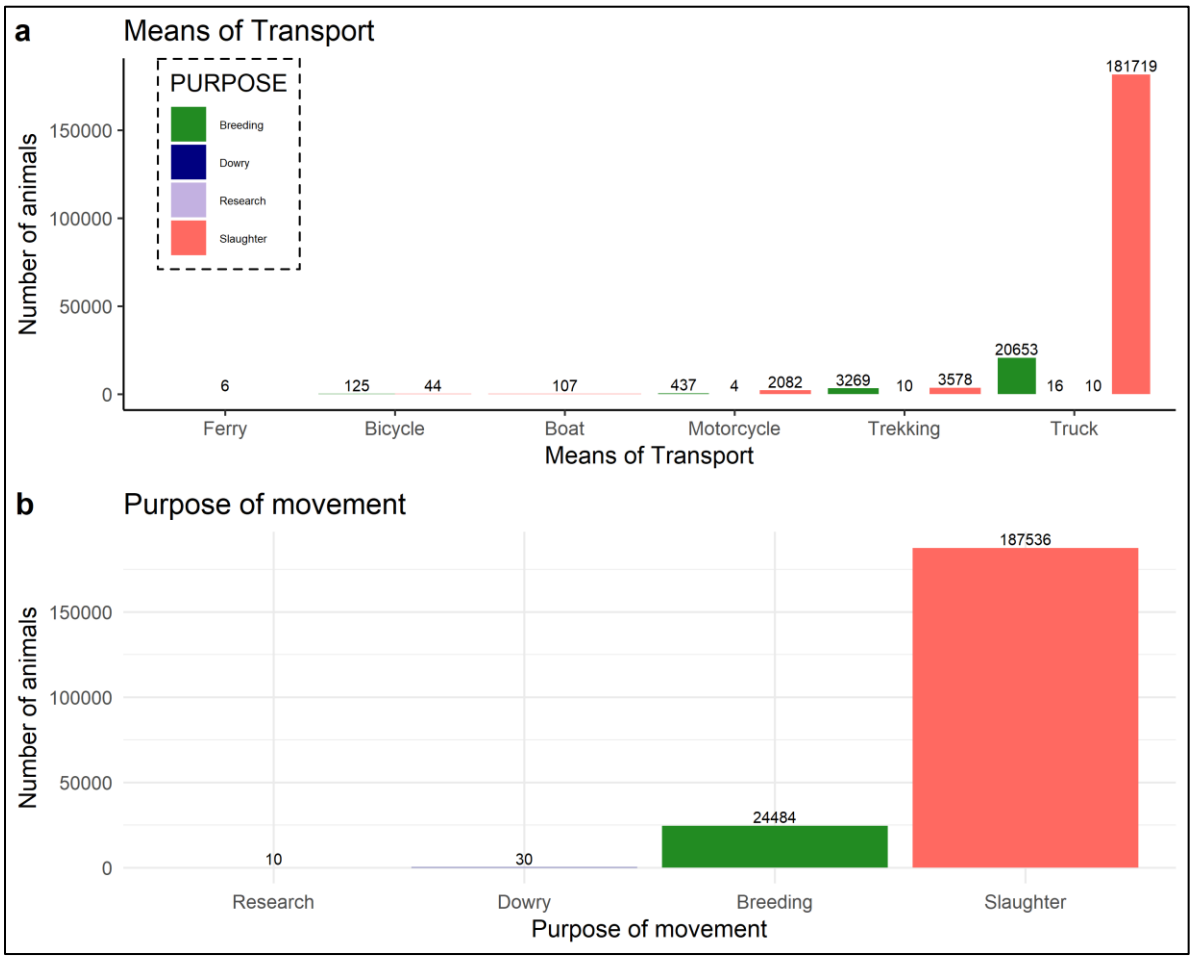

Fig. 4: A summary of mode of transportation and purpose of small ruminant movement over time. Panel **a**) shows a summary of the different *means of transport* available for movement of small ruminants in Uganda whereas panel **b**) shows the summary of all the purposes motivating these movements. The numbers on top of each bar indicate the total number of small ruminants moved.

Network-level metrics were computed to evaluate the importance of individual districts or groups of districts in the small ruminant movement network. The network size was 127 districts with a diameter of 9 districts and an edge density of 0.165 over the entire study period. We observed a moderate to high tendency for districts to ship more animals between districts from the *same* region than outside the region (nominal assortativity: 0.495).

The monthly small ruminant movements between districts gradually increased from time to time. In the first year 2012, the small ruminant movement network was dominated by only districts from northern Uganda. Small ruminant movements to other regions were observed by the second year (2013) with western and northern regions dominating receipt of animals from within and between regions. The network was generally highly fragmented in the first few years with no strong tendencies of consistent partnering between districts. By the third year (2014), AMP records showed that the central region of Uganda joined the network and

dominated the interactions as movement networks continued to grow as time went on and by 2020, small ruminants had been shared across all regions in Uganda. The communities formed across temporal cross-sections of the dynamic network of the small ruminant movement network from the earliest onset (first month) to the end of the study (last month) (**Fig. 5**).

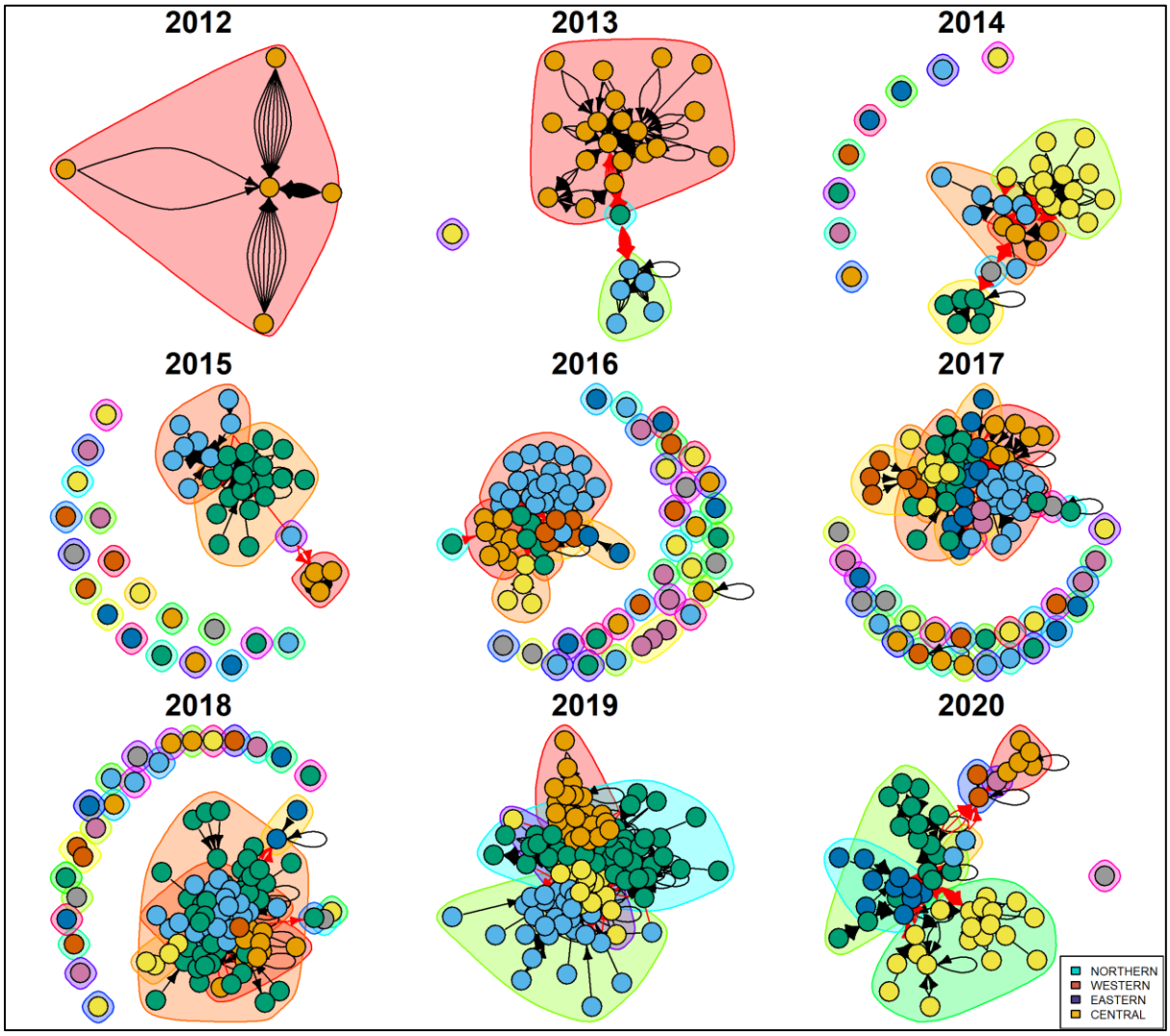

Fig.5: Small ruminant network communities over time (2012-2020). The dots represent individual districts, and their colors indicate the region to which the districts belong.

Different districts in Uganda formed three communities over the course of the aggregated 95-month time window (study period). Initially, the network was very fragmented over time (Figure 5); however, by the end of the study period, districts appeared to gravitate towards the formation of three tightly knit communities in which movement transactions occurred (**Fig. 6a**). The communities consisted of districts that were generally spatially close to each other with a wide connection to spatially distant neighbors (**Fig. 6b**).

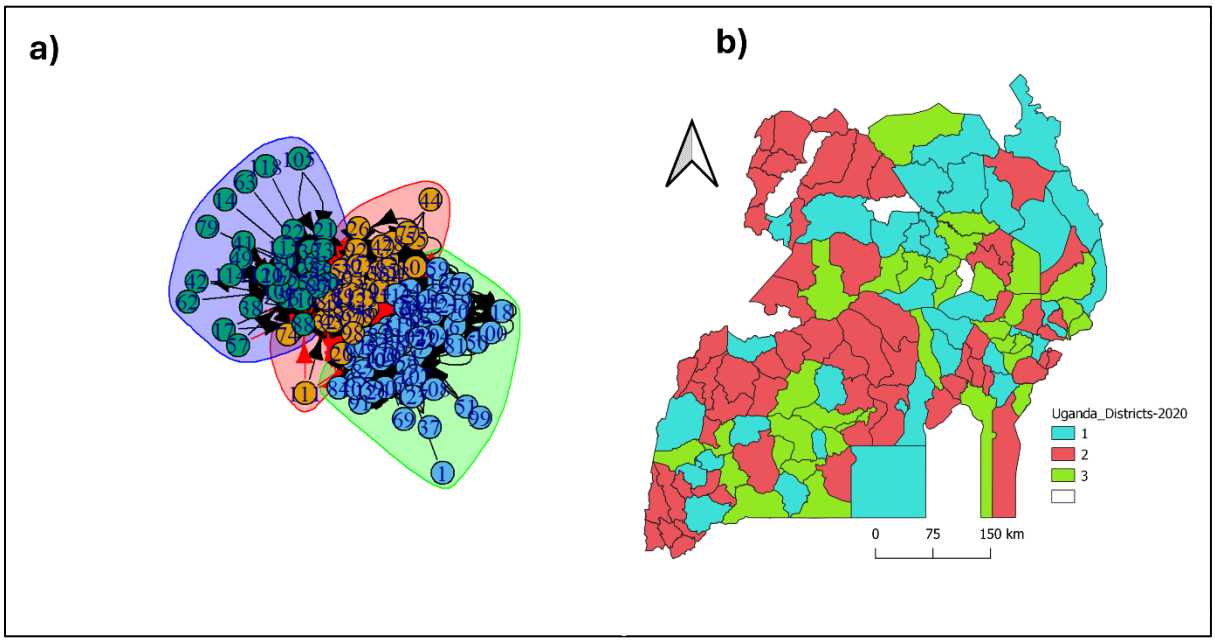

Fig. 6: Community formation for the entire study period (2012-2020) (a). The different districts were colored by the community they belonged to (b). The map was drawn using open-access datasets from Uganda Burau of Statistics and open source QGIS software.

Generally, districts participated more in receiving than sending out animals as revealed by a higher average in-degree than average out-degree. The mean in-degree for the entire network was about 42, suggesting that, on average, each district received animals from 42 unique districts. Districts from the central region had the highest average in-degree (in-degree: 10) followed by the eastern region with an average in-degree of 6, whereas the northern and western regions had the least in-degree averages of 5.3 and 4.3, respectively, suggesting lower levels of engagement. All districts with the highest tendency to receive animals (i.e., highest in-degree) were from the central region, including the highly populated Kampala, Wakiso and Mukono districts (**Fig. 7**).

The average out-degree across all districts, on the other hand, was 6. The biggest sources of small ruminants to other districts (as defined by their out-degree) were Lira, Kaberamaido, Kiruhura and Mukono for the northern, eastern, western and central regions, respectively (**Fig. 7**). The dominant small ruminant destinations in Uganda were the central (Kampala and Wakiso districts) and southwestern subregions (Mbarara and Kiruhura districts) whereas the eastern and northern regions were more of origins of small ruminants to other parts of the country (**Fig. 7**).

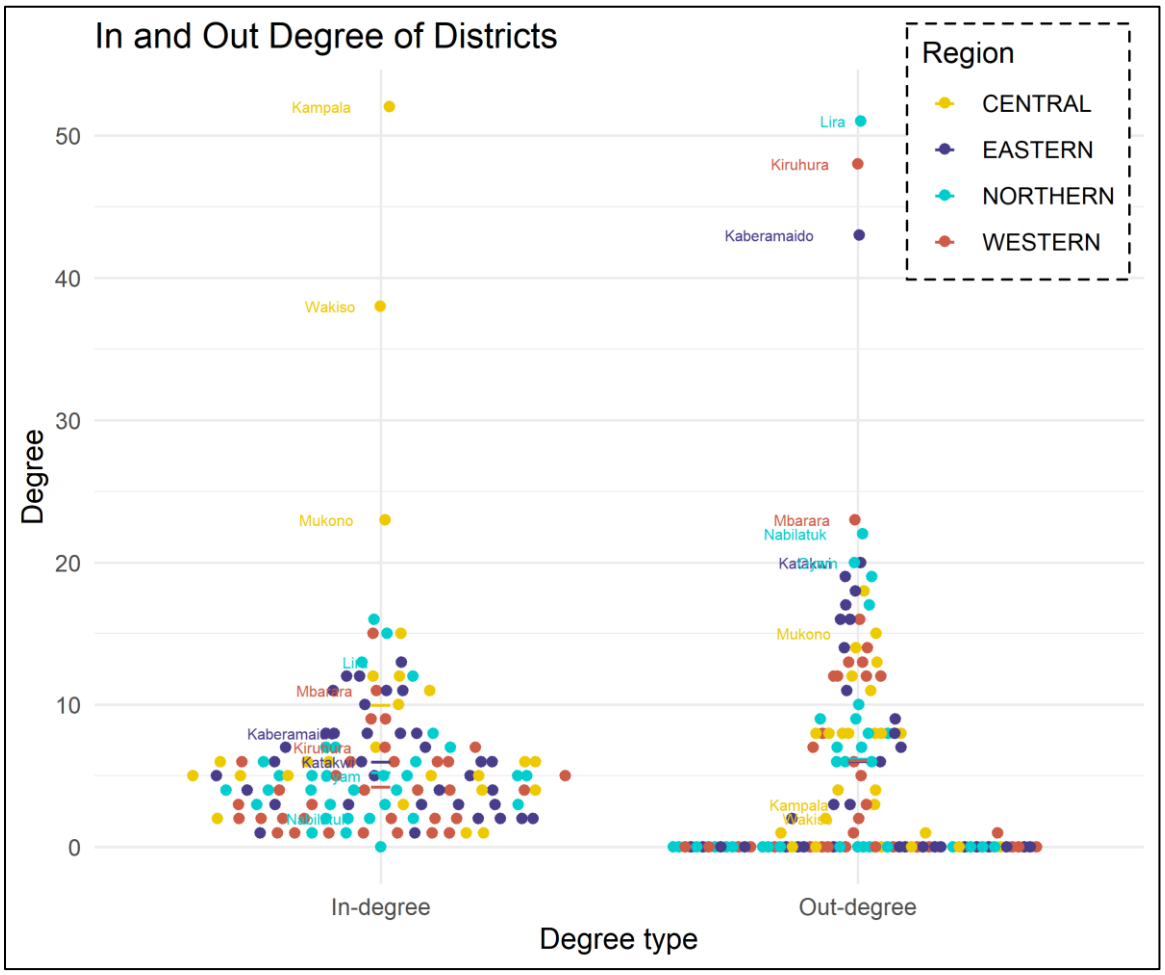

Fig. 7: The dot plots show the in- and out-degree of different districts across regions of Uganda. The in-degree and out-degrees ranged from 0-52. The color of the dots corresponds to the region in which a district is from. The cross bar along the central axis of each dot plot represents the average degrees for each region.

Interestingly, Kampala and Wakiso districts in central Uganda had the highest hub scores of 100 and 63 respectively. These were followed by Amuru (Acholi subregion) and Tororo (Elgon subregion) districts with hub scores of 40 and 35, respectively. Mbarara, Kasese, Lira and Mbale districts were additionally important districts where most small ruminant shipments would be headed (**Fig. 8**).

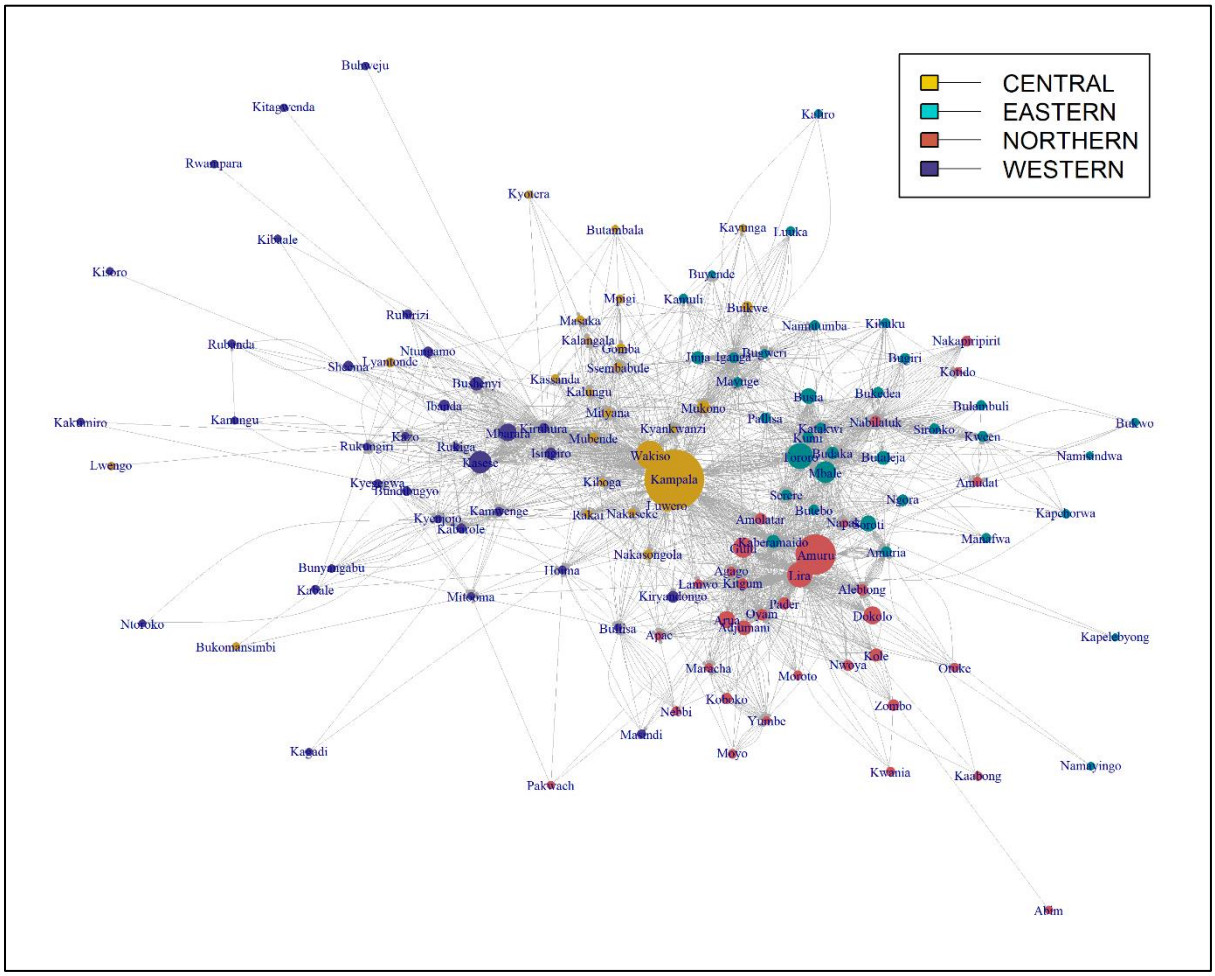

Fig. 8: Static network for small ruminant movements in 127 districts of Uganda. Here, the size of the node is scaled to represent its Hub Score.

Generally, districts received more animal shipments than they shipped out animals as the in-degree and backward reachability scores were higher than the downstream reachability across the study period (**Fig. 9b**).

In-degree, forward reachability, closeness centrality and hub score values varied significantly ($p < 0.05$) across subregions of Uganda. Districts from central Uganda and those that lie at international borders received disproportionately more small ruminants than other districts in Uganda (Fig. 9a). Small ruminants from central and western Uganda districts reached more districts in Uganda than any other subregion. Closeness centrality varied significantly across Ugandan sub region (Fig. 9b). Closeness centrality measures an individual node's average farness (inverse distance) to all other nodes in the network sucl h that the higher the closeness values, the closest the node is to every other node. Closer nodes distribute information more rapidly and more efficiently. Districts from different regions had a statistically significant closeness centrality measure. (**Fig. 9c**). On average, districts from central Uganda including Kampala were closer to all other districts than those from the other regions and acted as the

most important link for animal movements to trickle into other regions (**Fig. 9c**). Hub score values significantly differed across subregions. On average, Kampala was the most significant hub for receiving small ruminants from equally important locations in Uganda. This was followed by Acholi subregion whereas the least hub score values were observed in the Karamoja subregion (**Fig.9d**).

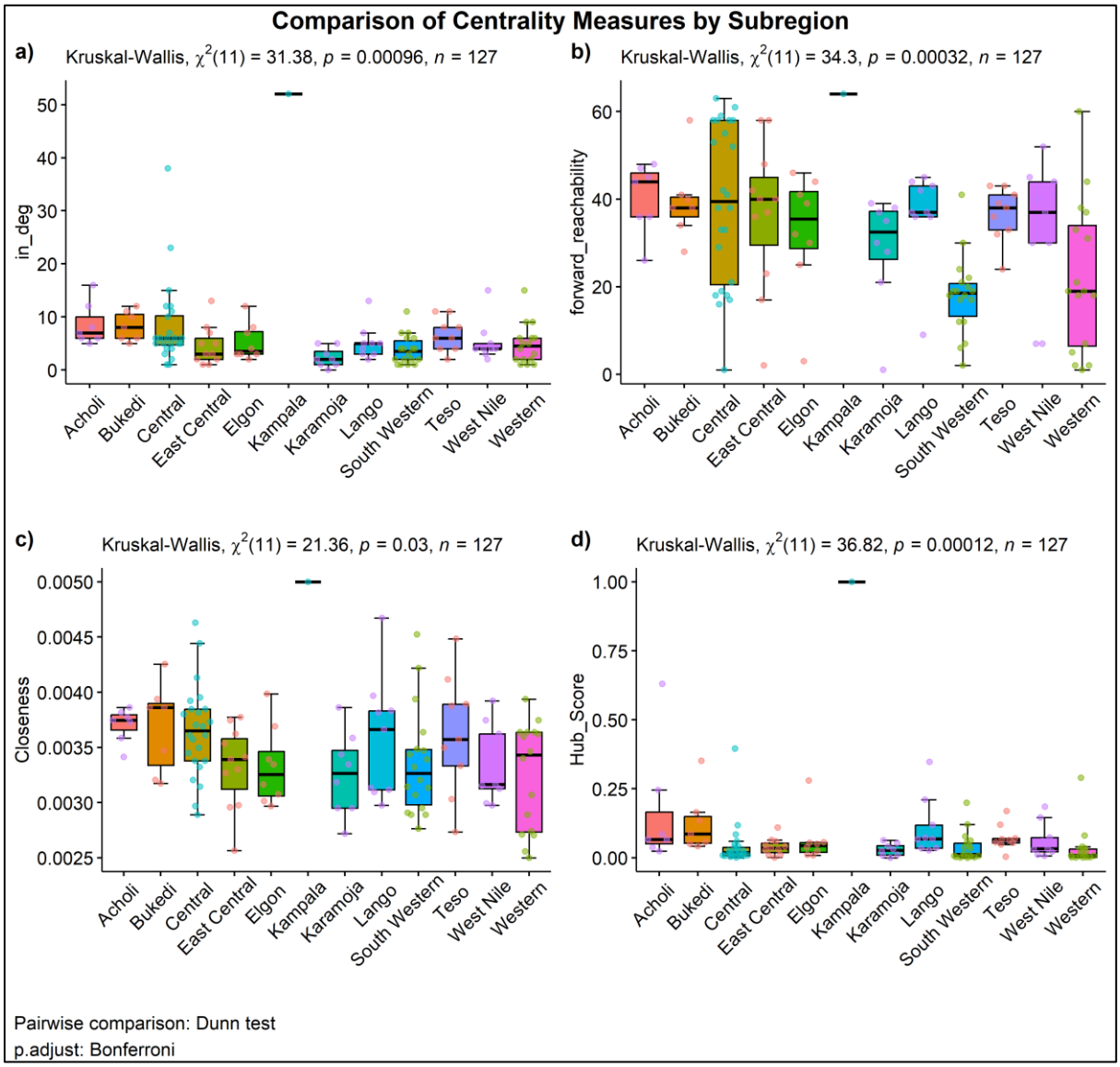

Fig. 9: Box plots show the pairwise comparison of statistically significant measures by subregion. In-degree (a), forward reachability (b), Closeness centrality (c), and Hub score (d). The dots represent individual districts whereas dot colors represent the sub-regions from which a district is drawn.

Six measures including out-degree, authority score, betweenness, eigenvector centrality, backward reachability, were also compared across subregions, and were not statistically significant. However, some of the metrics revealed individual districts with extreme values that are worth noting (**Figure 10**).

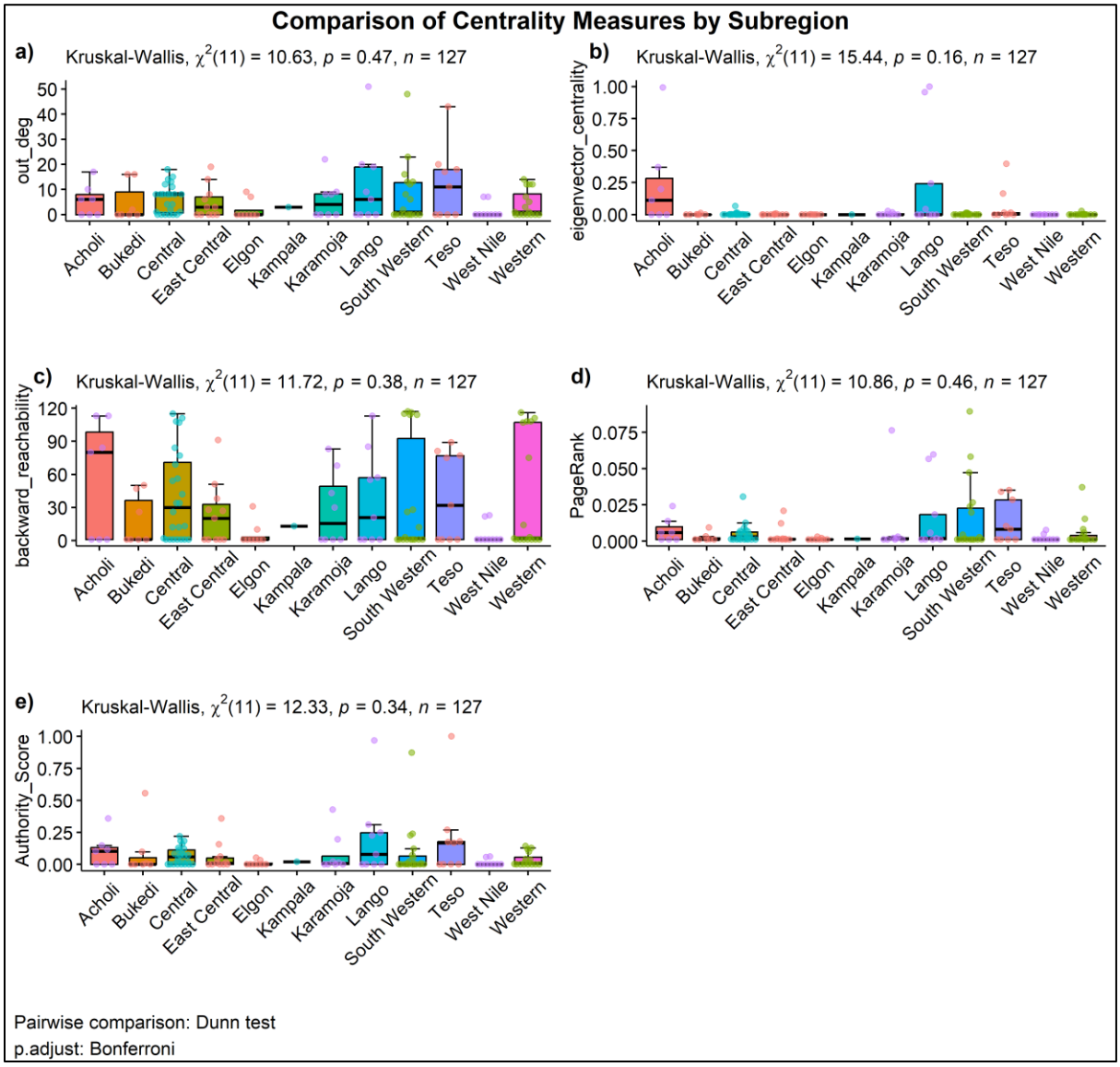

Fig. 10: Box plots show the pairwise comparison of centrality measures by subregion- that were not statistically significant. Out-degree (a), Eigenvector centrality (b), backward reachability (c), PageRank (d). and authority score (e). The dots represent individual districts whereas dot colors represent the regions from which a district is drawn.

Discussion

This study set out to identify the districts with the highest connectivity in the small ruminant movement network for the period 2012—2020 using the social network analysis approach. The available livestock mobility literature in Uganda is based on Animal Movement Permit (AMPs) data from only 2019 (González-Gordon et al., 2023) and shortly after between 2019-2021 (Hasahya et al., 2023). Both studies acknowledge a potential limitation that was posed by the COVID-19 related lockdown periods that negatively impacted livestock trade that saw a sharp decline in demand for livestock and livestock products leading to majority of key players diversifying into other economic activities (Ilukor et al., 2022). This study builds on already existing literature to expound more on small ruminant movement networks across all districts of Uganda for a longer period (2012 – 2020).

Influential districts in the animal movement network can be a basis for targeted control interventions such as vaccination, quarantine, and biosecurity measures. The most important districts and activities in the small ruminant as well as all livestock movements in Uganda were those with the highest levels of connectivity through network centralization measures, namely degree, betweenness, closeness, PageRank among others. On average, for every centrality measure tested in this study, there were notable outlier districts. Such outliers have previously been described as super-spreaders of infections as these are the individuals onto which the entire network is anchored (Zhang et al., 2019). There were more districts with outgoing animal shipments than incoming shipments, an observation similar to what has been reported by another study on cattle movements in Uganda (Hasahya et al., 2023).

The districts with exceptionally higher levels of incoming animals imply that they are at increased risk of receiving infected animals and thus could be very important for surveillance activities depending on the purpose of movement (VanderWaal et al., 2016). For example, most small ruminants moved during the period 2012—2020 were moved for slaughter purposes and thus abattoirs could act as important sentinels for disease surveillance, including zoonotic diseases. Likewise, the districts that have very high tendencies to send out animals to other districts are likely to spread infections in case they have infectious animals and could therefore be targeted for interventions such as biosecurity measures and vaccination against priority diseases to reduce the likelihood of transmission to other areas.

There were districts such as Kampala, Wakiso Lira and Kaberamaido with exceptionally high degree centrality and betweenness centrality. Such districts are very likely to act as spillways that enable the rapid flow of infectious disease agents to other districts that would have otherwise been poorly connected. Once such districts are carefully identified, they could be targeted with interventions to increase chances of disrupting the flow of potentially infectious animals and thus reduce the impact and extent of disease outbreaks (VanderWaal et al.,

2016), for instance quarantine facilities.

The central region was the most favorite destination of animals largely because of the urban and peri-urban nature of the central region in Uganda which is associated with high demand for livestock and livestock products. This is similar to an observation made by a study on cattle movement networks in Uganda (González-Gordon et al., 2023). Most of the small ruminants were shipped for purposes of slaughter followed by breeding. As has been reported elsewhere, most small ruminants reared in Uganda are the indigenous type and therefore, the motivation for their rearing is for sale, social functions and family consumption to provide animal-source proteins. Moreover, small ruminant keepers in Uganda do not frequently re-stock animals as they often maintain and multiply their own stocks as previously reported (Nkamwesiga et al., 2023).

The northern region of Uganda was identified as a general source of animals for slaughter in the rest of the country. Districts such as Nabilatuk (Karamoja sub-region), Lira and Kaberamaido were among the most important districts in the dissemination of animals to other regions especially central and western regions. Lira and Kaberamaido districts have previously been identified as important districts in the cattle movement networks in Uganda (Hasahya et al., 2023). Because of this, it is not surprising that the northern region in Uganda, especially the Karamoja sub-region, has previously been blamed for being the source of small ruminant diseases such as Peste des petits ruminants (PPR) to other regions especially the central and southwestern regions (Nkamwesiga et al., 2022). Animal movements provide a golden opportunity to spread diseases over long distances in the shortest time possible especially if no biosecurity measures are available to minimise this risk as previously reported (Wolff et al., 2017).

The small ruminant movement networks were more fragmented in the earlier years (2012-2016), however, the networks became strongly connected thereafter, an observation that is generally consistent with previous studies in Uganda (González-Gordon et al., 2023; Hasahya et al., 2023). Despite no obvious temporal trends in the number of movement transactions over time, there were three distinct periods of high volume of small ruminants moved that coincided with the monthly shipment frequency (Fig. 2 and Fig. 3). The observed peaks of high small ruminant movements coincide with months with festivities, for example between March and April (Easter holiday) and around December (Christmas holiday). Hypothetically, if an infectious disease was introduced into one of the districts with the highest connectivity, the fastest spread of an outbreak would have been observed in this period 2017 and 2018. Indeed, this observation coincides with the shift in PPR focus from the Karamoja sub-region to central and southwestern Uganda resulting in the first major outbreak reported in western Uganda in the same period (Nkamwesiga et al., 2022). The rapid increase in the frequency and number

and of animals moved between regions in the periods 2018 and 2020 also coincided with the highest number of outbreaks of PPR reported in multiple districts in Uganda (Nkamwesiga et al 2024 (Manuscript Under development). The progressive increase in the number and frequency of small ruminants, especially into urban districts follows the progressive increase in both human and livestock populations over time (UBOS & MAAIF, 2024).

Interestingly, similar to what has been previously reported by Hasahya et al. (2023), districts at international border points and those that make up cities and urban centres in Uganda were identified as the most important players in the small ruminant movement network. For example, districts such as Amuru (border Uganda-South Sudan), Tororo (border Uganda-Kenya), Kasese (border Uganda-Democratic Republic of Congo) and Isingiro (border Uganda-Tanzania border) were among the districts that were influential in the network. Urban centres and cities in Uganda have a relatively higher human population which in turn drives the demand for small ruminant meat to feed the urban dwellers. Moreover, there is a lot of human activity at international borders which facilitates trade of animals and some of the animals, maybe headed for export through illegal or legal means especially around the porous Ugandan borders (Mugezi et al., 2020). For the case of transboundary animal diseases such as PPR and FMD, the observed influence of some districts at the border points indicates a potential risk of spread of diseases from Uganda to other countries and vice versa. The trucks moving animals to markets and the traders themselves can facilitate dissemination of infectious diseases such as PPR and FMD (Mugezi et al., 2020). This observation of increased flow of small ruminants to districts along the international borders calls for more strict regulation of livestock movement by measures such as establishing quarantine stations to minimize the potential likelihood of disease introduction into Uganda or vice-versa.

Conclusions and recommendations

The districts that were identified as influential in the small ruminant networks can be good starting points to correctly institute animal disease control measures especially quarantine, vaccination and enhanced biosecurity. Such influential districts in networks have previously been linked with the likelihood of driving the spread of infectious diseases in a very short time because of how quickly animals from them can potentially reach many districts in the country. The districts such as Kampala, Wakiso, Lira and Kaberamaido that demonstrated high levels of connectivity, especially by the different centrality measures should be prioritized for surveillance and control activities to increase the impact and effectiveness of such activities. Districts with high degree centrality and betweenness would increase the accuracy and sensitivity of active surveillance efforts other than blindly implementing such activities. This would in turn improve timely detection of disease outbreaks and reduce the spatial extent and impact thus improving the profitability of small ruminant production venture.

Data Availability

All the data used in this study is available on reasonable request from the Uganda Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and the corresponding authors.

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Author contributions statement

JN: study design, data collection and analysis, writing, and reviewing; KRG: data analysis, visualisation, writing and reviewing; PL: data collection, data curation and reviewing; KR: grant acquisition, writing and reviewing, AP, HK and DM: study design, writing, and reviewing; all authors have read and approved this manuscript for publication.

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Competing interests

The authors declare no competing interests.

Chapter 7: General Discussion

This thesis is based on four specific objectives which have been prepared as stand-alone publications and submitted to relevant reputable peer-reviewed open-access journals. Two of the chapters have been accepted and published online while the last two chapters are currently under peer review. The four chapters aimed to paint a complete epidemiological picture of the Peste des petits ruminants (PPR) situation in Uganda through advanced epidemiology, molecular biology, and social network analysis approaches.

We observed that PPR outbreaks tended to be more frequent in districts (or geographical locations) with relatively high small ruminant density, extended road networks and that lie in the dry belt and thus receive relatively low rainfall per year. The districts or geographical locations with high small ruminant density coupled with extended road networks are more likely to participate in small ruminant movement into and outside the district for different purposes including trade, breed improvement social functions among others (Hardstaff et al., 2015; Okello et al., 2021). Areas that receive relatively low amounts of rainfall also tend to move animals over short and long distances (sometimes across international borders) in search for fresh pasture and water for their animals especially during the drought periods. Drought-facilitated long distance animal trekking has been linked with reduction in livestock immunity and thus making them more susceptible to disease in addition to increasing chances of contact between susceptible and infected animals resulting in increased disease transmission and size of outbreaks (Fèvre et al., 2006; Macpherson, 1995).

The observed annual patterns of PPR outbreaks enabled us identify geographical locations (individual districts or groups of districts) in Uganda with a much more reduced tendency for near-future PPR outbreaks to occur, increased likelihood and those with no obvious tends of outbreaks We identified the Karamoja sub-region, a region characterised by large transhumant small ruminant flocks in a community that lies at across the international border between Uganda, Kenya and South Sudan. The Karamoja region was the first area to report a confirmed PPR outbreak in Uganda and persisted for over 10 years before it was confirmed in other regions (Luka et al., 2012). This persistence attracted a lot of control interventions especially vaccination from both government of Uganda and development partners such as FAO, USAID which contributed to the observed down trend. However, the observed down trend could also be as a result of massive disease suppression by mass vaccination which has reduced the disease impact and ability to recognize outbreaks and report them as the outbreak no longer involves large number of animals (as a significant number of them are/were vaccinated). We predicted an uptrend of PPR outbreaks in central and southwestern Uganda districts, areas that lie at the along the cattle corridor and have relatively large flock sizes.

Moreover, more than 90% of all reported outbreaks in the subsequent years (2021—2023) occurred along the predicted uptrend belt of Uganda (Nkamwesiga et al.,2024 Unpublished). The districts in central and southwestern regions of Uganda are one of the leading participants in long distance movement of animals for purposes of trade, slaughter, breed improvement and social functions, activities that have previously been associated with driving disease transmission (Mugezi et al., 2020).

Guided by the observed and predicted trends of PPR outbreaks in Uganda, we also set out to determine the levels of exposure to the PPR virus across the three main small ruminant production systems to paint the picture of the extent disease spread. In addition, we identified individual animal and production system-level risk factors of this exposure. The prevalence of PPRV specific antibodies was the highest in the pastoral production system, moderate in the agropastoral and lowest in the mixed-crop livestock production systems of Uganda. In Uganda, the pastoral production system involves large flocks of small ruminants (> 50 heads per household) that freely browse on a large expanse of land. This phenomenon is associated with increased within and between herd commingling which in turn increase the likelihood of infectious disease transmission. One infected animal introduced in one of the flocks can potentially infect many other animals during communal grazing as there is little or no restriction of flocks mixing or movement in this system of production. More than 90% of the available land is used for livestock production and contributes to more than 50% of the household livelihood. However, in the agropastoral production system, the land is shared between crop agriculture and livestock keeping which necessitates moderate restriction of animals from encroaching on crop gardens. As a result, the agropastoral production system is characterised by many fenced farms and crop gardens with only between 10% and 50% of household income derived from livestock production. Fencing reduces the chances of nose-to-nose contact between animals from different flocks thus reducing the likelihood of disease transmission (Ochwo et al., 2019).

In the mixed crop-livestock production system, less than 10% of household income and livelihood is derived from livestock keeping with the majority of households involved in crop agriculture. The households keep relatively small flock sizes (5 to 10 animals) which majorly managed by tethering on ropes to prevent them from encroaching on nearby crop gardens. In this production system, small sized small ruminant flocks that are heavily restricted in movement limits livestock disease transmission through diminished capacity to sustain outbreaks due to lack of enough susceptible individuals and limited opportunities for contact respectively. We also observed that female animals that were older than 1 year were more likely to be exposed to PPR virus as compared to male and younger animals. Farmers tend to

keep more female animals than male especially those interested in multiplying flocks and breeding. Consequently, female animals are kept in flocks for a longer period (some times > 4 years), a phenomenon that increases their chances of being exposed to PPR virus and or vaccination. Moreover, older animals were also more likely to be seropositive as compared to animals younger than 1 year, because age is a pre-disposing factor to infections (Thrusfield, 2010). Contrary to a previous observation that goats are more likely to be PPRV antibody seropositive as compared to sheep, we did not find a statistically significant difference between seroprevalence in sheep and goats (Akwongo et al., 2022; Dubie et al., 2022). This is likely true because many of such studies tended to over sample goats and thus made conclusions without necessarily granting each small ruminant species an equal chance to be included in the sample certainly because farmers keep more goats than sheep (or vice-versa) in sub-Saharan Africa and Asia respectively (Gilbert et al., 2018; Robinson et al., 2014).

Upon assessing the levels of exposure to PPRV across different production system, we went head to identify important districts or groups of districts that likely facilitate transmission of animals from one corner of the country to another. The transmission of infectious diseases such as PPR with no known reservoirs and do not persist in the environment, certainly requires direct contact between susceptible and infected animals, a scenario made possible through livestock movement (Fèvre et al., 2006; Pirtle & Beran, 1991). We observed that there were two periods in which the highest frequency and quantity of small ruminant movement were recorded. The first and second peaks were observed in periods between 2017—2018 and 2019—2020 respectively. Both these periods coincided with the first and highest number of confirmed PPR outbreak reported in central and southwestern Uganda, regions outside the Karamoja subregion where PPR was first reported and persisted for over a decade (Mulindwa et al., 2011). Moreover, both peaks of the highest frequency and volume of small ruminant movement transactions were dominated by small ruminant movement shipments between districts in the northern and western regions of Uganda. Livestock movement have preciously been associated with both short and long-distance transmission of infectious diseases such as FMD and PPR (EFSA AHAW Panel, 2015; Ekwem et al., 2021).

The districts with exceptionally higher levels of incoming animals imply that they are at increased risk of receiving infected animals and thus could be very important for surveillance activities depending on the purpose of movement (VanderWaal et al., 2016). For example, the majority of small ruminants moved in the study period, were moved for slaughter purposes and thus abattoirs could act as important sentinels for disease surveillance. Likewise, the districts that have very high tendencies to send out animals to other districts are likely to spread infection in case they have infectious animals and could therefore be targeted for

interventions such as biosecurity measures and vaccination against priority diseases to reduce the likelihood of their transmission to other areas.

There were districts such as Kampala, Wakiso Lira and Kaberamaido with exceptionally high degree centrality and betweenness centrality. Such districts are very likely to act as spillways that enable the rapid flow of infectious disease agents to other districts that would have otherwise been poorly connected. Once such districts are carefully identified, they could be targeted with interventions such as quarantine to increase chances of disrupting the flow of potentially infectious animals and thus reduce the impact and extent of disease outbreaks (VanderWaal et al., 2016).

As the infectious diseases such as PPR spread from one area to another, there is increasing need to track the virus evolution over time. Continuous molecular characterisation of viruses enables us to keep track of the antigenic variation in order to support adequate development of diagnostics in addition to tracking the direction of spread of the virus to guide identification of potential sources of the infection and better planning of counter measures (Artika et al., 2020; WHO, 1981). Furthermore, PPR global eradication campaign was crafted with other core objectives of effective control of PPR and other important small ruminant diseases (FAO & WOAH, 2016). Interestingly, there is a number of small ruminant infectious diseases whose clinical presentation can potentially be confused with that of PPR. Contagious ecthyma (Orf, *Parapoxvirus ovis*), Sheep and goat pox (GTP, Goat pox virus), Bluetongue (BT, Bluetongue virus), Foot and mouth disease (FMD, Foot and mouth disease virus), Contagious caprine pleuropneumonia (CCPP, *Mycoplasma capricolum* subspecies *capripneumoniae*) and pasteurellosis (*Pasteurella multocida, Mannheimia haemolytica and others*) are among the small ruminant diseases with comparable symptoms for the differential diagnosis of PPR (Balamurugan et al., 2014b; Diallo, 1995). Co-infection of PRV and other small ruminant diseases complicates diagnosis, prognosis and treatment outcomes of the affected animals thus increasing the burden of such a disease on the livelihood of the farmers and the entire small ruminant value chain.

All the PPR positive samples sequenced from this study belonged to PPRV lineage III. Moreover, for the last 15 years, only PPRV lineage III has been reported from outbreaks in Uganda (Muniraju, Munir, Banyard, et al., 2014; Nkamwesiga et al., 2019). It is reasonable to conclude that PPRV lineage IV has since disappeared, or its earlier description was indeed a laboratory contamination as has previously been suggested (Dundon et al., 2020). Phylogenetic analysis of the nucleotide sequences from this study revealed close relatedness among the virus isolates from majority of the districts around Uganda to those reported in neighboring countries such as Burundi and Tanzania (Kinimi et al., 2021; Niyokwishimira et al., 2019). This strongly suggests interconnectedness of the districts through animal movements for different purposes such as trade, social exchanges and breeding as previously suggested (Dundon et al., 2017). Most of the sequences from this study were closely related with the recent isolates from Burundi, a result that resonates well with a recent report of trade of animals for breeding purposes from Bushenyi district in Western Uganda to Burundi that coincided with the first PPR outbreak in Burundi (Dundon et al., 2020). The PPRV lineage III isolates from Kazo and Kasese districts in southwestern Uganda shared a very close relationship with each other, but most interestingly, they shared a close relationship with isolates from Ngorongoro, northern Tanzania. This relationship strongly affirms the importance of nucleotide sequencing in tracking the transboundary movement of diseases through human socioeconomic activities that must be incorporated in endemic countries' disease control programs to be able to reduce transmission and spread of PPR.

The commonest co-infection detected was PPRV and ORFV. The Parapoxvirus ovis, sometimes referred to as; contagious ecthyma, sore mouth, or scabby mouth, is a DNA virus that belongs to the Poxvirus family and parapoxvirus genus, very common in most areas where small ruminants are kept. Even though it can infect humans and usually causes a self-limiting skin infection, this virus mainly affects sheep and goats. Although the disease is largely believed to cause a self-limiting disease, it causes serious mortalities in naïve kids and labs (Bala et al., 2018). Coupled with other coinfections such as PPR that are associated with more serious pathologies to the animals, Orf can exacerbate the already constrained small ruminant production venture. Detection of PPRV and ORFV coinfection in this study contributes to the limited body of literature that has previously reported PPRV and ORFV coinfection in PPR endemic countries (Saravanan et al., 2007).

Although it is the first time to report co-infection of PPRV and GTPV in Uganda, it has previously been reported in Nigeria under relatable field conditions (Akanbi et al., 2020). In most PPR endemic countries, clinical diagnosis and indeed PPR disease prognosis are often complicated by coinfections such as CCPP that we detected in this study. Comparable percentage positivity of the animals that tested positive for CCPP in this study were previously reported in the neighboring Tanzania (Kgotlele et al., 2019). CCPP causes significant global economic losses of more than USD 500 million annually although studies documenting the extent of spread and risk factors are still limited across the globe (Ahaduzzaman, 2021; Iqbal Yatoo et al., 2019). Our goal in this study was to determine the lineages of the PPRV and other co-infections based on reports of potential PPR outbreaks in Uganda. Only 26.7% (4/15) of the outbreaks were linked to diseases other than PPR, while over 73.3% (11/15) of the districts that reported PPR-like sickness in this study had at least one animal testing positive for PPR.

Summary

Epidemiology of Peste des petits ruminants in Uganda

Peste des petits ruminants (PPR) is a viral disease that affects domestic small ruminants (goats and sheep) and some wild small ruminants. By 2015, more than 70 countries in Africa and Asia were confirmed as affected although the threat to Europe through Turkey and Bulgaria was quickly neutralised. In Uganda, PPR was first reported in 2007 the Karamoja subregion of northeastern Uganda in 2007, a region comprised of 9 different districts. PPR persisted in this region. Upon eradication of Rinderpest in 2011, PPR was identified as the next target for eradication because of how closely related their aetiologies and epidemiological situations were. Indeed, in 2016, the Food and Agriculture Organisation of the United Nations (FAO) and the World Organisation for Animal Health (WOAH) launched a four-stage global PPR control and eradication (PPR-GCEP) by 2030. The control plan was set up to leverage on the momentum gained from PPR control activities to tackle other small ruminant diseases identified by small ruminant keepers through joint activities such as concurrent vaccination against multiple diseases. The PPR-GCEP demands that every PPR affected country complies with the program and implements respective activities to warrant progression from one stage to another until PPR is eradicated following a self-administered PPR Monitoring and Assessment Tool (PMAT). After all steps are achieved, countries will apply for declaration of freedom from disease and continue with activities aimed at maintenance of disease-free status.

This thesis was designed to update the PPR epidemiological situation in Uganda, by generating data to fit into PPR-GCEP stage 1 (assessment stage) as well as identify areas for targeting of interventions – which is the cornerstone for the PPR-GCEP stage III (control stage), activities that directly contribute the PPR-GCEP. To achieve the set objectives, this study employed a range of advanced epidemiology, and social network analysis techniques to analyse archived PPR outbreak data and small ruminants movement data respectively. Additionally, the study employed molecular biology and molecular epidemiology techniques to identify the circulating PPR virus and other relevant coinfections in all cases of PPR-like disease reported in Uganda during the study period (202-2022).

Despite the disease persistence in this region for over a decade before it spread to other districts of Uganda, this study identified that the Karamoja subregion was now a diminishing hotspot whereas two new foci of transmission had come up in the central and southwestern regions of Uganda. Concentration of control interventions by government of Uganda and development partners in the Karamoja subregion coupled with rampant shipment of animals

between districts from the northern, central, and western regions could have resulted in this shift in the disease focus. However, the districts in the pastoral production system (where the Karamoja subregion falls), had the highest levels of exposure to PPRV as compared to agropastoral and mixed cop-livestock production systems because of the stark difference in the small ruminant management practices amongst these systems. Small ruminants in the pastoral production system are allowed to roam freely on a large expanse of land whereas the those in the other systems are somewhat restricted in movement by fenced farms and or tethering by ropes which greatly protects limits their chances of contact with other flocks with potentially infected animals.

This study confirmed PPRV, CCPP, ORFV and GTPV coinfection among animals from 15 different reports of PPR-like disease that were investigated in this study. This observation further complicates field clinical diagnosis of PPR especially in countries without extensive laboratory diagnostics capacity such as Uganda. In conclusion, there is need to incorporate the detected coinfections in the panel of molecular diagnostics in Uganda to be able to achieve the target of effective PPR control of PPR and other small ruminant diseases. Furthermore, this study reports for the first-time coinfection of other important small ruminant diseases together with PPRV, an observation we believe will improve preparedness for proper disease management options such as chemotherapeutic treatment and vaccination to simultaneously target different disease pathogens. Animal movement control especially at international borders needs to be strengthened to reduce the likelihood of importing or exporting PPR infected small ruminants.

Findings of this study provide a basis for more robust timing and prioritization of control interventions such as vaccination to contribute to the global goal of control and eradication by 2030. For instance, these findings can be used to test a risk based PPR vaccination program by prioritising vaccination of small ruminants in PPR Up Trend districts. The districts that were identified as influential in the small ruminant networks can be good starting points to correctly institute animal disease control measures especially quarantine, vaccination and enhanced biosecurity. Such influential districts have previously been linked with the likelihood of driving the spread of infectious diseases in a very short time because of how quickly animals from them can potentially reach many districts in the country. The districts such as Kaberamaido, Lira, Nabilatuk that demonstrated high levels of connectivity especially by the different centrality measures should be prioritized for surveillance and control activities to increase the impact and effectiveness of such activities.

Districts such as Kampala, Mukono, Wakiso and Lira with high degree centrality and betweenness would increase the accuracy and sensitivity of active surveillance efforts other than blindly implementing such activities. This would in turn improve timely detection of disease outbreaks and reduce the spatial extent and impact thus improving the profitability of small ruminant production venture.

Prioritization of interventions in terms of both space and time and for example districts with uptrend, drought-prone and those with high density of small ruminants and the time of the year when the amount of rainfall is low. Targeting PPR control interventions (vaccination and livestock movement control) to and from pastoral and agro-pastoral small ruminant production systems that are prone to PPR incursions is recommended to prevent PPRV spread to low-risk smallholder small ruminant production systems.

Zusammenfassung

Epidemiologie der Peste des petits ruminants in Uganda

Die Peste des petits ruminants (PPR) ist eine Viruserkrankung, die kleine Hauswiederkäuer (Ziegen und Schafe) und einige wilde kleine Wiederkäuer befällt. Im Jahr 2015 wurden mehr als 70 Länder in Afrika und Asien als betroffen bestätigt, obwohl die Bedrohung für Europa durch die Türkei und Bulgarien schnell neutralisiert wurde. In Uganda wurde die PPR erstmals 2007 in der Subregion Karamoja im Nordosten Ugandas gemeldet, einer Region, die aus 9 verschiedenen Distrikten besteht. Die PPR blieb in dieser Region bestehen. Nach der Ausrottung der Rinderpest im Jahr 2011 wurde die PPR als nächstes Ziel für die Ausrottung identifiziert, da die Ätiologie und die epidemiologische Situation der beiden Krankheiten eng miteinander verbunden sind. Tatsächlich haben die Ernährungs- und Landwirtschaftsorganisation der Vereinten Nationen (FAO) und die Weltorganisation für Tiergesundheit (WOAH) 2016 einen vierstufigen globalen Plan zur Bekämpfung und Ausrottung der PPR (PPR-GCEP) bis 2030 ins Leben gerufen. Der Bekämpfungsplan wurde aufgestellt, um die durch die PPR-Bekämpfung gewonnene Dynamik zu nutzen und andere von den Tierhaltern identifizierte Krankheiten kleiner Wiederkäuer durch gemeinsame Aktivitäten wie gleichzeitige Impfungen gegen mehrere Krankheiten zu bekämpfen. Das PPR-GCEP verlangt, dass jedes von der PPR betroffene Land das Programm einhält und entsprechende Maßnahmen durchführt, um den Übergang von einer Stufe zur nächsten zu gewährleisten, bis die PPR nach einem selbst verwalteten PPR-Überwachungs- und Bewertungsinstrument (PMAT) getilgt ist. Wenn alle Schritte erreicht sind, beantragen die Länder die Erklärung der Seuchenfreiheit und setzen die Maßnahmen zur Erhaltung des seuchenfreien Status fort.

Ziel dieser Arbeit war es, die epidemiologische Situation der PPR in Uganda zu aktualisieren, indem Daten für die PPR-GCEP-Phase 1 (Bewertungsphase) erhoben und Bereiche für gezielte Maßnahmen identifiziert wurden, die den Grundstein für die PPR-GCEP-Phase III (Kontrollphase) bilden, d. h. für Aktivitäten, die direkt zum PPR-GCEP beitragen. Um die gesetzten Ziele zu erreichen, wurden in dieser Studie eine Reihe fortschrittlicher epidemiologischer und sozialer Netzwerkanalysetechniken eingesetzt, um archivierte PPR-Ausbruchsdaten bzw. Daten über die Verbringung kleiner Wiederkäuer zu analysieren. Darüber hinaus wurden im Rahmen der Studie molekularbiologische und molekularepidemiologische Verfahren eingesetzt, um das zirkulierende PPR-Virus und andere relevante Koinfektionen in allen Fällen von PPR-ähnlichen Erkrankungen zu identifizieren, die während des Studienzeitraums (202-2022) in Uganda gemeldet wurden.

Obwohl die Krankheit in dieser Region über ein Jahrzehnt lang persistierte, bevor sie sich auf

andere Bezirke Ugandas ausbreitete, wurde in dieser Studie festgestellt, dass die Unterregion Karamoja nun ein abnehmender Hotspot ist, während zwei neue Übertragungsherde in der zentralen und südwestlichen Region Ugandas entstanden sind. Die Konzentration der Bekämpfungsmaßnahmen der ugandischen Regierung und der Entwicklungspartner auf die Subregion Karamoja in Verbindung mit dem regen Tiertransport zwischen den Distrikten der nördlichen, zentralen und westlichen Regionen könnte zu dieser Verlagerung des Krankheitsschwerpunkts geführt haben. In den Bezirken des pastoralen Produktionssystems (zu dem die Subregion Karamoja gehört) war die PPRV-Belastung jedoch am höchsten im Vergleich zu den agropastoralen und gemischten Produktionssystemen mit Viehhaltung, da die Praktiken der Kleinwiederkäuerhaltung in diesen Systemen sehr unterschiedlich sind. Die kleinen Wiederkäuer in den pastoralen Produktionssystemen dürfen sich auf einem großen Stück Land frei bewegen, während die Tiere in den anderen Systemen durch eingezäunte Farmen oder durch Anbinden mit Seilen in ihrer Bewegungsfreiheit eingeschränkt sind, was die Möglichkeit des Kontakts mit anderen Herden mit potenziell infizierten Tieren stark einschränkt.

In dieser Studie wurde eine Koinfektion mit PPRV, CCPP, ORFV und GTPV bei Tieren aus 15 verschiedenen Berichten über PPR-ähnliche Erkrankungen, die in dieser Studie untersucht wurden, bestätigt. Diese Beobachtung erschwert die klinische Felddiagnose von PPR weiter, insbesondere in Ländern ohne umfangreiche Labordiagnosekapazitäten wie Uganda. Zusammenfassend lässt sich sagen, dass die festgestellten Koinfektionen in das Panel der Molekulardiagnostik in Uganda aufgenommen werden müssen, um das Ziel einer wirksamen Bekämpfung von PPR und anderen Krankheiten kleiner Wiederkäuer zu erreichen. Darüber hinaus wird in dieser Studie zum ersten Mal über die Koinfektion anderer wichtiger Krankheiten kleiner Wiederkäuer mit PPRV berichtet, eine Beobachtung, von der wir glauben, dass sie die Vorbereitungen für geeignete Optionen zur Krankheitsbekämpfung, wie z. B. chemotherapeutische Behandlung und Impfung, die gleichzeitig auf verschiedene Krankheitserreger abzielen, verbessern wird. Die Kontrolle von Tierverbringungen, insbesondere an internationalen Grenzen, muss verstärkt werden, um die Wahrscheinlichkeit der Einfuhr oder Ausfuhr von mit PPR infizierten kleinen Wiederkäuern zu verringern.

Die Ergebnisse dieser Studie bilden eine Grundlage für eine solidere Zeitplanung und Priorisierung von Bekämpfungsmaßnahmen wie Impfungen, um zum globalen Ziel der Bekämpfung und Ausrottung bis 2030 beizutragen. So können diese Ergebnisse beispielsweise genutzt werden, um ein risikobasiertes PPR-Impfprogramm zu testen, bei dem die Impfung von kleinen Wiederkäuern in PPR-Up-Trend-Distrikten Priorität hat. Die Bezirke, die als einflussreich in den Netzwerken für kleine Wiederkäuer identifiziert wurden, können gute Ausgangspunkte für die korrekte Einführung von Maßnahmen zur

Tierseuchenbekämpfung sein, insbesondere Quarantäne, Impfung und verbesserte Biosicherheit. Solche einflussreichen Bezirke wurden schon früher mit der Wahrscheinlichkeit in Verbindung gebracht, dass sie die Ausbreitung von Infektionskrankheiten in kürzester Zeit vorantreiben, da die Tiere von dort aus potenziell viele Bezirke im Land erreichen können. Distrikte wie Kaberamaido, Lira und Nabilatuk, die insbesondere durch die verschiedenen Zentralitätsmaße ein hohes Maß an Konnektivität aufweisen, sollten bei Überwachungs- und Kontrollmaßnahmen Vorrang haben, um die Wirkung und Effektivität dieser Maßnahmen zu erhöhen. Bezirke wie Kampala, Mukono, Wakiso und Lira mit einem hohen Grad an Zentralität und Verflechtung würden die Genauigkeit und Sensibilität aktiver Überwachungsmaßnahmen erhöhen, anstatt diese blindlings durchzuführen. Dies würde wiederum die rechtzeitige Erkennung von Krankheitsausbrüchen verbessern, die räumliche Ausdehnung und die Auswirkungen verringern und damit die Rentabilität der Produktion von kleinen Wiederkäuern verbessern.

Räumliche und zeitliche Priorisierung der Maßnahmen, z. B. in Bezirken mit Aufwärtstrend, in dürregefährdeten Bezirken, in Bezirken mit hoher Dichte an kleinen Wiederkäuern und in Bezirken, in denen es zu dieser Jahreszeit wenig regnet. Es wird empfohlen, die Maßnahmen zur PPR-Bekämpfung (Impfung und Kontrolle der Verbringung von Tieren) auf pastorale und agro-pastorale Produktionssysteme für kleine Wiederkäuer auszurichten, die anfällig für PPR-Einschleppungen sind, um die Ausbreitung von PPRV auf kleinbäuerliche Produktionssysteme mit geringem Risiko zu verhindern.

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List of Publications

Oral presentations

i. 16th International Symposium of Veterinary Epidemiology and Economics, August 7 — 2, 2022 | Halifax Marriott Harbourfront Hotel, Halifax, Canada

ii. German Veterinary Medical Society (DVG-Vet-Congress) — Conference on Tropical Veterinary Medicine & International Animal Health, October 14 - 15, 2022 | Estrel Congress Center Berlin, Germany

iii. 6th Peste des Petits Ruminants Global Research and Expertise Network (PPR—GREN) Meeting, 28 Nov 2023—30 Nov 2023 | Clarion Bangalore Hotel, Bengaluru, India

iv. Peste des Petits Ruminants (PPR) Special Prize 2022 https://www.world-food-forum.org/news/detail/winners-announced-for-2022-transformative-research-challenge-peste-des-petits-ruminants-(ppr)-special-prize/en

v. Seminar at BfR: In the framework of the "Buliding Uganda's Investment in Livestock Development" (BUILD) project, 2023-04-06, 1:00 pm to 3:15 pm | BfR Conference Room

Poster presentations

i. 5th Peste des Petits Ruminants Global Research and Expertise Network (PPR-GREN) Meeting, 07 Dec 2022 —09 Dec 2022 | Faculty of Science, University of Montpellier, Montpellier, France

ii. Science fare at the international Livestock Research Institute, Nairobi Kenya May 2022 https://www.ilri.org/news/ppr-epidemiology-inform-risk-based-control-uganda | ILRI fellows science seminar serries

Journal Articles in line with the PhD thesis

1. **Nkamwesiga, J**., Korennoy, F., Lumu, P., Nsamba, P., Mwiine, F. N., Roesel, K., Wieland, B., Perez, A., Kiara, H., & Muhanguzi, D. (2022). Spatio-temporal cluster analysis and transmission drivers for Peste des Petits Ruminants in Uganda. *Transboundary and Emerging Diseases*, *69*(5), e1642–e1658. https://doi.org/https://doi.org/10.1111/tbed.14499

2. **Nkamwesiga, J**., Lumu, P., Nalumenya, D. P., Korennoy, F., Roesel, K., Wieland, B., Perez, A., Kiara, H., & Muhanguzi, D. (2023). Seroprevalence and risk factors of Peste des petits ruminants in different production systems in Uganda. *Preventive Veterinary Medicine*, *221*, 106051. https://doi.org/10.1016/j.prevetmed.2023.106051

Other journal article contributions during the PhD period (2021—2024)

1. Yessenbayev, K., Mukhanbetkaliyev, Y., Yessembekova, G., Kadyrov, A., Sultanov, A., Bainiyazov, A., Bakishev, T., **Nkamwesiga, J**., Korennoy, F., & Abdrakhmanov, S. (2023). Simulating the Spread of Peste des Petits Ruminants in Kazakhstan Using the North American Animal Disease Spread Model. *Transboundary and Emerging Diseases*, *2023*, 1–9. https://doi.org/10.1155/2023/7052175
2. Coffin-Schmitt, J. L., Moore, E. V, McKune, S. L., Mo, R., **Nkamwesiga, J**., Isingoma, E., Nantima, N., Adiba, R., Mwiine, F. N., Nsamba, P., Hendrickx, S., & Mariner, J. C. (2021). Measurement and sampling error in mixed-methods research for the control of Peste des Petits Ruminants in the Karamoja subregion of Northeastern Uganda: A cautionary tale. *Preventive Veterinary Medicine*, *196*, 105464. https://doi.org/https://doi.org/10.1016/j.prevetmed.2021.105464
3. Tweyongyere, R., **Nkamwesiga, J**., Etiang, P., Mugezi, I., Wamala, H., Wasswa, A. T., Kamusiime, M., Ainebyoona, S., Abizera, H., Mwiine, F. N., & Muhanguzi, D. (2024). Seroprevalence of contagious bovine pleuropneumonia (CBPP) in cattle from Karamoja region, North-eastern Uganda. *BMC Veterinary Research*, *20*(1), 97. https://doi.org/10.1186/s12917-024-03938-8
4. Etiang, P., Atim, S. A., **Nkamwesiga, J**., Nalumenya, D., Byaruhanga, C., Odongo, S., Vudriko, P., Ademun, A. R., Biryomumaisho, S., Erume, J., Masembe, C., Thomson, E. C., Muhanguzi, D., & Tweyongyere, R. (2024). Identification and distribution of Rhipicephalus microplus in selected high-cattle density districts in Uganda: signaling future demand for novel tick control approaches. *BMC Veterinary Research*, *20*(1), 119. https://doi.org/10.1186/s12917-024-03979-z
5. Mugasa, C. M., Mirembe, B. B., Ochwo, S., **Nkamwesiga, J**., Ndekezi, C., Tusabe, T., Musoba, A., & Kankya, C. (2023). Molecular Detection of Cryptosporidium Species in Wildlife and Humans at the Wildlife-Human Interface around Queen Elizabeth National Park, Uganda. *Parasitologia*, *3*(2), 181–193. https://doi.org/10.3390/parasitologia3020019
6. Muhanguzi, D., Ndekezi, C., **Nkamwesiga, J**., Kalayou, S., Ochwo, S., Vuyani, M., & Kimuda, M. P. (2022). *Anti-Tick Vaccines: Current Advances and Future Prospects BT - Vaccine Design: Methods and Protocols, Volume 2. Vaccines for Veterinary Diseases* (S. Thomas, Ed.; pp. 253–267). Springer US. https://doi.org/10.1007/978-1-0716-1888-2_15
7. Okella, H., Georrge, J. J., Ochwo, S., Ndekezi, C., Koffi, K. T., Aber, J., Ajayi, C. O., Fofana, F. G., Ikiriza, H., Mtewa, A. G., **Nkamwesiga, J.,** Bassogog, C. B. B., Kato, C. D., & Ogwang, P. E. (2020). New Putative Antimicrobial Candidates: In silico Design of Fish-Derived Antibacterial Peptide-Motifs. *Frontiers in Bioengineering and Biotechnology*, *8*(December), 1–10. https://doi.org/10.3389/fbioe.2020.604041
8. Okella, H., Ikiriza, H., Ochwo, S., Ajayi, C. O., Ndekezi, C., **Nkamwesiga, J**., Kaggwa, B., Aber, J., Mtewa, A. G., Koffi, T. K., Odongo, S., Vertommen, D., Kato, C. D., & Ogwang, P. E. (2021). Identification of Antimicrobial Peptides Isolated From the Skin Mucus of African Catfish, Clarias gariepinus (Burchell, 1822). *Frontiers in Microbiology*, *12*, 794631. https://doi.org/10.3389/fmicb.2021.794631
9. Okella, H., Okello, E., Mtewa, A. G., Ikiriza, H., Kaggwa, B., Aber, J., Ndekezi, C., **Nkamwesiga, J**., Ajayi, C. O., Mugeni, I. M., Ssentamu, G., Ochwo, S., Odongo, S., Tolo, C. U., Kato, C. D., & Engeu, P. O. (2022). ADMET profiling and molecular docking of potential antimicrobial peptides previously isolated from African catfish, Clarias gariepinus. *Frontiers in Molecular Biosciences*, *9*, 1039286. https://doi.org/10.3389/fmolb.2022.1039286

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Competing interests

In the context of this work, there are no conflicts of interest due to contributions from third parties.

Declaration of independence

I hereby confirm that I have written this thesis independently. I certify that I have used only the sources and aids indicated.

Berlin, 18.10.2024

Joseph Nkamwesiga

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