An Overview of Morbilliviruses and the Wildlife Spillover Risk of Peste des Petits Ruminants from Bharal (*Pseudois nayaur*) to Human Hotspots and Vulnerable Species in the Tibetan Plateau and Adjacent Himalayan-Sichuan-Yunnan Region
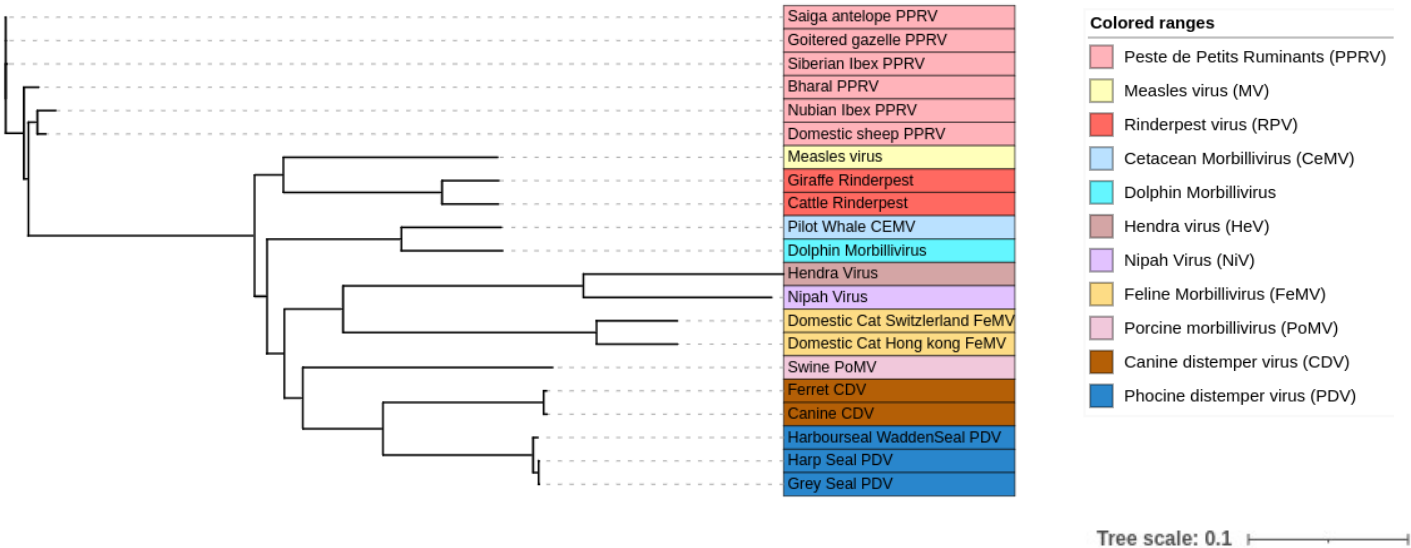
Introduction and aim:

The genus Morbillivirus belongs to the viral family paramyxoviridae which are enveloped viruses with non-segmented negative RNA genomes, comprised of N,P,M,F, H and L proteins (Sourimant and Plemper, 2016)(De Vries et al, 2015) that have an estimated size of 15-16 kb (Bailey et al, 2005) . Genetic and structural analyses indicate that morbilliviruses are able to exploit receptor variability across species and are thus able to adapt to – and emerge in new hosts (Ohishi et al,. 2010). These airborne viruses are highly infectious and cause severe clinical manifestation in human and animal hosts such as respiratory, neurological and gastrointestinal symptoms aswell as immunosuppression leading to fatal infection in many affected hosts. As such, Morbilliviruses have been associated with mass die-offs in and have led to large-scale epidemics in both terrestrial and marine mammal populations. There are believed to be several genera of morbilliviruses, of which the main ones include: Measles Virus (MeV), Canine Distemper Virus (CDV), Rinderpest Virus (RPV) which previously infected cattle but was declared eradicated in 2011, Peste de petits ruminants (PPRV) affecting ruminants such as sheep and Goats, Phocine distemper virus (PDV) affecting pinnipeds, Dolphin morbillivirus (DMV) and Cetacean Morbillivirus (CeMV). Novel strains however appear in various host species frequently such as Feline morbillivirus (FeMV) and Bat morbillivirus..

Thus, the objectives of the following paper are to construct a phylogenetic tree of morbilliviruses using RNA polymerase sequences and compare genetic similarity, and based on the results, select a virus and identify focal mammalian host species and related vulnerable species at risk of spillover events. Then, assess spatial overlap between the focal host and selected species in R (version 4.3.3) using IUCN shape data (IUCN, 2024). Areas of high human population density will be analysed to identify potential hotspots for zoonotic spillover. Monitoring and mitigation strategies to minimize the risk of viral spillover events will be proposed based on the findings.

Methods and Results:

To compare the genetic similarity between *Morbillivirus* strains, 20 viral strains were identified from the NCBI database from various hosts and locations, and their L genes were isolated using ORF Finder. The sequences were then aligned using Clustal Omega, and poorly aligned regions were removed using Jalview to focus on conserved sections. The L genes were specifically chosen for comparison because they are highly conserved over time and represent the longest section of the viral RNA genome (approx 6kb) , providing robust data for alignment analysis. Additionally, Hendra and Nipah virus strains were included as outgroups to help distinguish *Morbillivirus* strains from each other. -For alignment results, **see appendix**--.

Colored ranges
- Peste de Petits Ruminants (PPRV)
- Measles virus (MV)
- Rinderpest virus (RPV)
- Cetacean Morbillivirus (CeMV)
- Dolphin Morbillivirus
- Hendra virus (HeV)
- Nipah Virus (NiV)
- Feline Morbillivirus (FeMV)
- Porcine morbillivirus (PoMV)
- Canine distemper virus (CDV)
- Phocine distemper virus (PDV)

Tree scale: 0.1

The alignment results reveal the sequence relationships between morbillivirus strains, with branch lengths indicating genetic divergence. Clusters of genetically similar viruses are visible, such as the PDV and CDV groups. This genetic similarity, but overall distinction, has also been confirmed through studies involving genetic analysis (Kennedy et al., 2019). Similarly, the PPRV viral strains show high levels of genetic similarity to one another. In contrast, significant divergence is observed in viruses such as FeMV and the outgroup viruses HeV and NiV. This aligns with the literature, as Hendra and Nipah viruses belong to the Henipavirus genus within the Paramyxoviridae family. While FeMV is classified as a morbillivirus, genetic analyses suggests genetic distance within the genus (De Vries et al., 2015).

Based on these alignment results, a decision was made to analyse the Peste de petits ruminants (PPRV) Virus, due to its high sequence homology despite isolation in various genetically distant small ruminants and wild ungulates, indicating high transmission risk across species. The Bharal – Blue sheep – (*Pseudois nayaur*) was then selected as a focal host species to assess potential zoonotic spill over risk, due to the virus having been isolated in it previously, and due to the large range it covers, spanning a large span of the Tibetan plateau, and areas of inner China (Harris R.B, 2014).

5 additional species that were rated as Vulnerable or above by IUCN with overlapping ranges and were then selected based on different criteria via the Onezoom Tree of life tool (Onezoom, 2024) the overlap ranges were also quantified. The Takin, Red Serrow and red goral were selected due genetic similarities, sharing recent common ancestry. Additionally these options were justified by the fact that PPRV strains had previously been isolated in Saiga antelopes and Goitered gazelles, which have a more distant ancestry to Bharal than those selected.

It was also then decided to include Yak and Wild Bactrian camels in the overlap analysis. Previous Studies have observed natural and experimental infection of PPRV in to Bovid and Camilidae species with clinical manifestation (Schulz et al, 2019) (Rahman et al, 2020). With a recent study involving dromedaries having observed further transmission to sheep and goats (Saeed et al, 2022). Potential concern of pathogenic crossover from Bharal to members of these families was therefore justified.
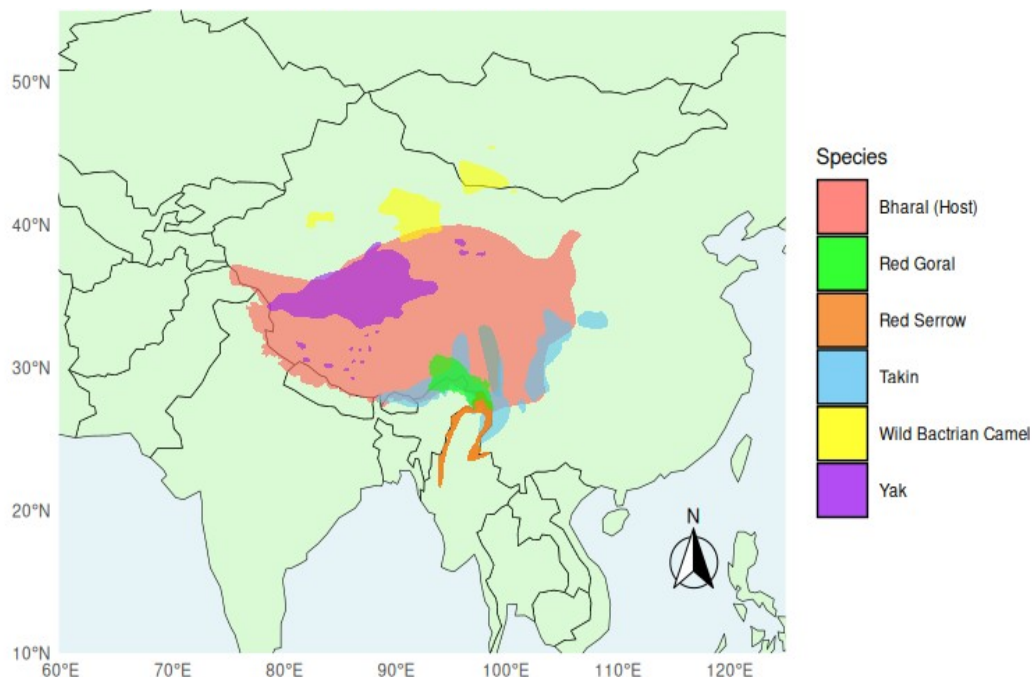
Figure: *A map of Central Asia with overlapping species range data. It highlights potential areas of significant species overlap and increased risk of interspecies transmission.*

Species of concern	IUCN Redlist Status (2024)	Proportion of overlap with host species	Overlap Range (Km²)
Bharal (*Pseudois nayaur*) – Host species	Least Concern	1	2858577.5
Takin (*Budorcas taxicolor*)	Vulnerable	0.66599514	293331.62
Burmese Red Serrow (*Capricornis rubidus*)	Vulnerable	0.11184868	9661.52
Red Goral (*Naemorhedus Baileyi*)	Vulnerable	0.88241258	109902.2
Yak (*Bos mutus*)	Vulnerable	0.98317031	462044.43
Wild Camel (*Camelus ferus*)	Critically Endangered	0.08179438	16475.85

Table: *Conservation status of species of concern, aswell as proportion of and overlap range with host species per species compared.*

The shape data indicates a geographic region of concern with increased cross-species transmission risk where multiple species ranges overlap on the Burmese border and Yunnan and Sichuan Chinese provinces. Highlighting a particular area of concern in regards to Interspecies transmission risk.

The map was then further modified using NASA 2020 population density data (NASA, 2020). To indicate how species ranges overlap with areas of high human population, and indicate areas of high potential zoonotic spillover risk.
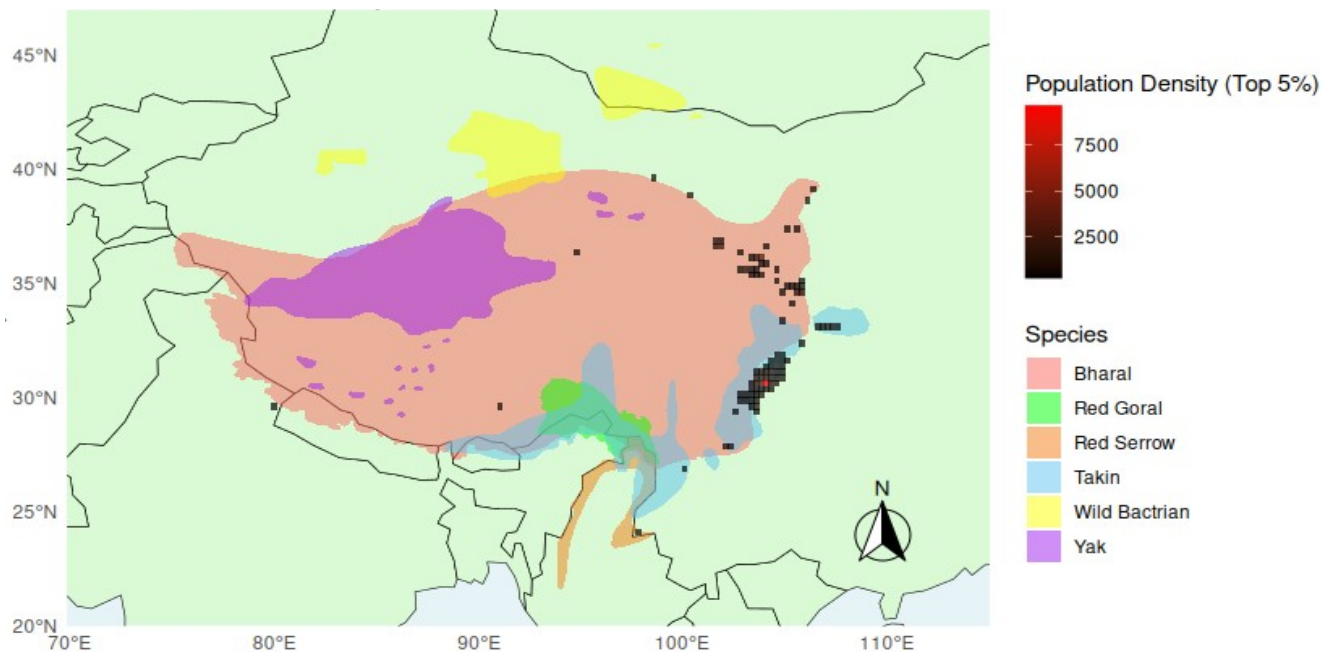
Figure: *A map displaying human population data and species ranges was created, where raster data representing the top 95% of population densities per square kilometer were selected to identify human hotspots. A gradient was applied to these rasters to highlight areas with the highest human populations within these hotspots.*

Species	Number of Zoonotic Spillover Hotspots in range (km^2)	Minimum Population density in range (per Km^2)	Maximum Population density in range (per Km^2)	Median Population Density in range (Per Km^2)
Bharal (*Pseudois nayaur*) – Host species	55	0	3171.3	1.67
Takin (*Budorcas taxicolor*)	56	0.019	9660.5	1.181
Burmese Red Serrow (*Capricornis rubidus*)	1	0.372	281.32	1.656
Red Goral (*Naemorhedus Baileyi*)	0	0.019	69.98	2.848
Yak (*Bos mutus*)	0	0	6.853	0
Wild Camel (*Camelus ferus*)	0	0	38.59	2.546

Table: The above table describes the number of population hotspots that fall within species ranges, aswell as the min, max and median human population densities across all species.

Discussion of Results, an assessment of risk and proposed mitigation strategies:

The above data displays potential risk areas for not only transmission from the host species to overlapping vulnerable species, but also from potential terrestrial hosts to human populations. Particular areas of risk include the Sichuan, and Yunnan Provinces aswell as areas in north Myanmar. Amongst these an area that aligns with the location of Chengdu city, provides an area of particular zoonotic spillover risk with a maximum population density of 9660.5 per Km$^{2.}$. Multiple population hotspots can also be found in the Ningxia and Qinghai regions of China, further representing risk areas.

Across the entire host range however, human population density appears to be very low. With a median value of 1.67 per km^2. Areas with lower zoonotic spillover risks appear to be across the Tibetan Plateau, which also coincides with the ranges Yak and Bactrian Camels, where both have a max population of 6.853 and 38.59 respectively across their entire ranges. Additionally Given the large ranges Yaks and Bactrian Camels occupy despite their low population numbers (both under 10000 individuals) (IUCN, 2024), contact and inoculation with PPRV from Bharals is unlikely. However, if establishment occurs, risk is heightened due to the group behavior exhibited by both yaks and Bactrian camels, which form herds of up to 20 individuals (Xue et al., 2015; Liu et al., 2019), facilitating spread of infection throughout the entire groups.

Risk of interspecies contact and establishment is higher in regions where Bharal, Takin and Red Goral and Serrow ranges overlap. All species inhabit mountainous areas above 2000 meters with forested or grassy terrains (Chen et al, 2019)(Hua and Jinchu, 2001)(Abedin et al, 2024)(Mishra and Jonsingh, 1996). However transmission and establishment risk in populations is highest between Takin and Bharal specifically. As both display herd behaviour unlike gorals and serrows, forming groups of 6-20 individuals with both preferring alpine meadows. Transmission risk of PPRV increased by either direct contact of bodily secretions or aerosol droplets, or by innoculation with the virus from contaminated environments like shared food sources (Libeau, 2014).

Given these results. Various mitigation strategies might be implemented to reduce both zoonotic transmission risk and wildlife spillover risk. With main target areas including the Adjacent Himalayan-Sichuan-Yunnan Region and north of there due to higher species overlap and population density. These could include:

- Vaccinations are available for PPRV. Including live attenuated and thermostable vaccines. The live attenuated vaccine remains the gold standard for effectivity and provide life-long immunity but

require refrigeration. However, effective thermostable vaccines have been developed which can be shipped to resource-poor areas. Facilitating potentially large scale vaccination campaigns to bharal (Sen et al, 2010) (Mariner et al, 2017). Additionally DIVA vaccines could also be implemented potentially aid in serosurveillance as they would allow one to distinguish between animals that obtained immunity from vaccinations or natural immunity (Jia et al, 2020).

- Active serosurveillance of Bharals could effectively complement vaccination campaigns by identifying regions herds have had prior exposure to PPRV, thereby informing epidemiologists about high-risk areas for viral transmission. Coupled with ongoing serosurveillance of ruminant livestock, could provide a more comprehensive understanding of disease spread and help target control efforts to areas with the highest outbreak risk.

- Information Campaigns and Wildlife-Livestock Interface management would also provide a strong front in controlling the spread of PPRV but minimizing the risk of contact between wild and domestic populations, therefore reducing the risk of disease spillover, which has been observed before in PPRV affected livestock hosts.

Mitigation strategies such as these have successfully been implemented in China (Legnardi et al., 2022) and could serve as an effective approach to combat the threat of interspecies and zoonotic spillover from Bharal within its range.

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Alignment Results:

See the viral seqence aligments here:

 https://drive.google.com/file/d/1B48rOa-ZMAoTmbdIafd5qFFdd6phwyDE/view?usp=sharing

CLUSTAL O(1.2.4) multiple sequence alignment

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Hendra                          TTGGCGATATGAATCCATGGCTATTTTTGCAGAAAGACTTGACGAAATTTATGGCTTACC60
Nipah_Virus                     TTGGAGATACGAATCAATGGCTATATTTGCTGAACGTCTGGATGAGATATACGGTTTACC        60
FelisCatus_Hongkong_FeMV        CTGGCGATATGAATCAAGTAGTATGTTTGCAGAGAGACTTAATGAAATTTATGGACTGCC
                                60
FelisCatus_Switzerland_FeMV     TTGGCGATATGAGTCAAGTAGTGTATTTGCAGAGAGACTCAATGAAATTTACGGATTGCC
                                60
Porcine_morbillivirus_Swine_Mexico    TTGGCGTTATGAGACAATAAGCATCTATGCCCAGAGGTTAAATGAGATTTATGGACTCCC
                                60
Dolphin                         TTGGAGGTATGAGACAATCAGCATATTTGCCCAGAGACTAAATGAGATTTATGGGTTGCC        60
Pilot_Whale_CEMV                TTGGAGGTATGAGACAATCAGCATATTTGCACAGAGATTAAATGAGATCTATGGCTTGCC        60
Canine_CDV                      TTGGCGTTATGAGACCATCAGTATTTTTGCTCAGAGATTAAATGAAATCTATGGTCTCCC        60
Ferret_Australia_CDV            TTGGCGTTATGAGACCATCAGTATTTTTGCTCAGAGATTAAATGAAATCTATGGTCTCCC        60
                          Harbourseal_Netherlands_WaddenSeal_PDV
          TTGGAGATACGAGACAATCAGCATATTCGCTCAAAGGTTAAATGAAATCTATGGGCTTCC60
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