First Study of Molecular detection of Ovine Herpesvirus 2 DNA using heminested PCR in Pakistan

Aayesha Riaz (✉ aayeshariaz@uaar.edu.pk)

University of Arid Agriculture <https://orcid.org/0000-0001-6411-5753>

Inga Dry

The University of Edinburgh The Roslin Institute

Robert G. Dalziel

The University of Edinburgh The Roslin Institute

Saif Ur Rehman

University of Arid Agriculture

Muhammad Ali Abdullah Shah

University of Arid Agriculture

Naeem Akhtar

University of Arid Agriculture

Arfan Yousaf

University of Arid Agriculture

Ruqia Mehmood Baig

University of Arid Agriculture

Research

Keywords: Malignant Catarrhal Fever, OvHV-2, Heminested PCR, First Report, Pakistan

Posted Date: June 5th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-30077/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

Malignant catarrhal fever (MCF) is a highly fatal lymphoproliferative disease of cattle, deer, bison, water buffalo and pigs caused by the gamma herpesviruses; alcelaphine herpesvirus 1 (AIHV-1) and ovine herpesvirus 2 (OvHV-2). AIHV-1 is known to cause wildebeest-associated MCF whereas OvHV-2 is known to cause sheep-associated MCF. AIHV-1 and OvHV-2 cause inapparent infection in their reservoir hosts (wildebeest for AIHV-1 and sheep for OvHV-2) but fatal lymphoproliferative disease in susceptible species.

Methods

This study was aimed to find prevalence of OvHV-2 in sheep, goat, cattle and buffalo in Rawalpindi and Islamabad cities of Pakistan, using molecular and phylogenetic methods. Blood samples were collected consisting of sheep (n = 54), goat (n = 50), cattle (n = 46) and buffaloes (n = 50) from slaughter houses and farms. The samples were subjected to hemi-nested PCR, sequencing, and phylogenetic analysis of OvHV-2 POL gene and OvHV-2 tegument protein gene.

Results

The highest percentage of positive samples was found to be in sheep (13%) whereas positive samples from goat, cattle and buffaloes had percentage of 11%, 9% and 6.5% respectively. Four positive PCR products from sheep samples were also sequenced. The sequences obtained were submitted to NCBI GenBank database under the accession numbers of MK852173 for POL gene, and MK840962, MK852171 and MK852172 for ORF75 tegument protein gene. Phylogenetic analysis revealed close identities with some sequences of this virus from worldwide including neighbouring countries including China, India and Iran.

Conclusion

This study is the first study in Pakistan on the prevalence of OvHV-2 in carrier and susceptible species. This report provides important data about the presence of OvHV-2 in apparently healthy cattle and buffaloes, which might transmitted to them from OvHV-2 positive sheep or goats. It is concluded that a countrywide, large scale OvHV-2 prevalence study should be conducted to avoid economic losses due to any outbreak of MCF.

Introduction

The Pakistani livestock sector contributes approximately 11% to Pakistans's annual gross domestic product and provides employment to approximately 62% of the population in rural areas when subsidiary industries are taken into account (1). The national herd of Pakistan incorporates 29.6 million cattle, 27.3 million buffalo, 53.8 million goats and 26.5 million sheep (Habib et al., 2016). Infectious diseases that affect animal that constitute the national herd impact both the security of the food chain and the wider economy.

Malignant catarrhal fever (MCF) is an acute, systemic, usually lethal lymphoproliferative disease of cattle and even-toed ungulates including pigs, deer, bison and water buffalo (2, 3, 4, 5). MCF may be caused by any of the gamma herpesviruses, identified so far, that comprise the *macavirus* classification group. The two most well studied of these *macaviruses*, Ovine Herpesvirus-2 (OvHV-2) and Alcelaphine Herpesvirus-1 (AIHV-1), are maintained asymptotically in sheep and wildebeest reservoir populations respectively (6, 7). Reactivation of these viruses from latency in asymptomatic reservoir populations and subsequent transmission of infectious virus, to susceptible *Ariodactyla* species may result in the development of MCF. Though, OvHV-2 transmission to susceptible animals, via aerosol, has been reported to occur up to 5 km from where reservoir animals are located (8, 9) the most common route of transmission is most likely direct contact of susceptible species with nasal secretions of reservoir animals, containing infectious virus, on mixed grazing areas.

Clinically, MCF is characterized by fever, excessive salivation, nasal and ocular discharge. Lesions are usually present on buccal cavity and muzzle. Enlarged lymph nodes is also a characteristic sign of MCF. Post mortem lesions include presence of petechial haemorrhages on the mucosa of buccal cavity, in the gastrointestinal and respiratory tracts (9, 10). Symptoms of MCF in susceptible

species are similar to those of other diseases, such as vesicular stomatitis virus, foot and mouth disease and rinderpest (6, 12). Clinically affected susceptible hosts do not shed infectious virus and are considered as dead end hosts (13, 14).

MCF cases have been documented in both India (15) and Iran (16, 17), which border Pakistan. In Pakistan in most rural areas, livestock farms, animal hospitals and research centres, it is a common practice to keep MCF reservoir and susceptible animals in close proximity (18, 19). It is likely, therefore that the lack of documented cases of MCF in Pakistan, is a result of underreporting due to a lack of testing and awareness amongst veterinarians. In this study, for the first time in Pakistan, PCR testing of blood samples derived from abattoirs and farms was used to investigate the prevalence of OvHV-2 in cattle, buffalo, sheep and goats in Rawalpindi District and Islamabad city.

Materials And Methods

Samples collection

Two hundred blood samples consisting of sheep (n = 54), goat (n = 50), cattle (n = 46) and buffaloes (n = 50) samples were randomly collected from slaughter house (Sihala slaughter house, Rawalpindi) and farms of Rawalpindi and Islamabad, Pakistan. Blood samples were collected in ethylene diamine tetra acetic acid (EDTA) containing tubes and transported to the Virology laboratory of Department of Parasitology and Microbiology, FV&AS, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi for further processing. On arrival at the labs the blood samples were refrigerated at 4° C.

.Processing of Samples and DNA isolation

The buffy coat layer was removed carefully for DNA extraction and stored in separate tubes at -20°C. DNA isolation was performed using 100–120 µl from the buffy coat layer was performed using the PureLink® Genomic DNA Kit (Invitrogen). Concentration of extracted DNA in the samples was measured using a Nano-Drop spectrophotometer. DNA samples were stored at -20 °C.

Construction Of The OvHV-2 Positive Control

A section of the polymerase gene was amplified from DNA, extracted from BJ1035 cells (20), using Qiagen's DNA blood and tissue kit (Qiagen, UK). Regions of the polymerase gene (POL gene) were amplified in accordance with the primers specified by (21). The amplified product was purified, using Qiagen's PCR purification kit (Qiagen,UK) in accordance, and cloned into Topo vector pcr2.1+ (Life Technologies, UK). The purified DNA was used as a positive control in all documented OvHV-2 POL gene PCRs.

Heminested PCR to amplify OvHV-2 POL and tegument gene

Heminested PCRs were performed to amplify fragments of OvHV-2 POL gene using the extracted DNA samples. The following primer sets were used (22)

Primer POL1: 5'-GGC(CT)CA(CT)AA(CT)CTATG CTACTC CAC-3'

Primer POL2: 5'-ATT(AG)TCCACAACTGTTTTGT-3

Primer OHVPOL: 5'-AAAAACTCAGGGCCATTCTG-3'

DNA samples along with a positive and a negative control were selected for PCR. Primer POL1 and Primer POL2 were used in the first step for primary amplification. To perform PCR, *Taq* 2X Master Mix (NEB, UK) was used. Approximately 1 µg of the extracted DNA was used in a total volume of 50 µl reaction mixture for PCR. Thermal cycling conditions were carried out with one cycle of 95 °C for 15 minutes; followed by 25 cycles of 94 °C for 60 seconds, 60 °C for 60 seconds and 72 °C for 60 seconds; with a final extension at 72 °C for 10 minutes. For negative control nuclease free water was used. For Secondary amplification primer OHVPOL and POL2 were used. Samples of 4 µl of each primary amplification product were placed in PCR tubes and 46 µl of master mix was added to each tube. Cycling conditions for the secondary PCR were the same as for the primary PCR, except that 30 cycles of amplification were used. After amplification 10 µl of each second PCR reaction was run on a 1.8% agarose gel.

Heminested PCRs to amplify a fragment of the OvHV-2 ORF75 (tegument protein gene) were also performed on the DNA samples positive for the OvHV-2 POL gene. The following primer sets were used (21).

Primer 556: 5'-AGTCTGGGTATATGAATCCAGATGGCTCTC-3'

Primer 555: 5'TTCTGGGGTAGTGGCGAGCGAAGGCTTC-3'

Primer 755: 5'-AAGATAAGCACCAAGTTATGCATCTGATAAA-3'

The primer 556 and 755 amplifying fragment of 422 bp for the primary amplification step of the PCR. Primer 556 and Primer 555 were used in the secondary amplification step to amplify a fragment of 238 bp product. In both amplifications steps the PCR and electrophoresis conditions were same as that of used for heminested PCR for POL gene.

Sequence and Phylogenetic Analysis

Four positive PCR products from DNA samples taken from different sheep, one from POL gene PCR and three from tegument protein gene PCRs from the second amplification step were sent for gene sequencing to Macrogen® Korea. Primer OHVPOL and POL2 were used to sequence POL gene PCR product and primer 556 and 555 were used to sequence tegument protein gene PCR product. Sequences derived from this study and those obtained from the GenBank database were aligned by the CLUSTAL_W method in the software Seaview®. The distances were computed mean-wise and overall using MEGA7®. The gene sequences were translated using Seaview®. Sequences were subsequently analyzed with neighbor joining to construct the phylogenetic tree (19). The statistical significance of the relationships obtained was determined by bootstrap re-sampling analysis with 1000 repetitions.

The sequences were deposited to GenBank database. Accession numbers MK852173 for OvHV-2 POL gene and MK840962, MK852171 and MK852172 for the OvHV-2 tegument protein genes, were assigned by NCBI for the nucleotide sequences. The NCBI accession numbers for the amino acid sequences for the POL gene was QDG03185 and for the tegument protein were QDC27829, QDG03183 and QDG03184 respectively.

Results

Two hundred blood samples were taken randomly from different species included 46 cattle, 50 buffalo, 50 goats and 54 sheep from different farms, small holdings at household level and slaughter house of Rawalpindi and Islamabad Pakistan. All the animals were apparently healthy at the time of sampling. DNA was isolated from buffy coat of each of the blood sample and the extracted DNA was subjected to heminested PCR, using primers recommended by the OIE for the detection of OvHV-2. Initial screens of the samples were carried out using primers targeted towards the virus POL gene. A plasmid containing the region of the OvHV-2 POL gene, targeted by the POL primers, derived from the OvHV-2 immortalised cell line BJ1035 was utilised as a positive control. Samples registered as positive for OvHV-2 had 172 bp amplicon and no amplicon was observed in negative control samples tested at the same time (Fig. 1a). The positive DNA samples were further PCR tested for amplification of a region of tegument protein gene (Fig. 1b). Out of 200 samples, 79 were determined to be positive for both OvHV-2 POL and tegument genes using hemi-nested PCR (Table 1). The highest percentage of positive samples was found to be in sheep (13%) whereas positive samples from goat, cattle and buffaloes had percentage of 11%, 9% and 6.5% respectively (Table 1).

Table 1
Species wise breakdown of positive sample (%) calculated from total number of samples.

S. No.	Species	No. of Samples	Positive Samples	% out of Total (n = 200)
1	Cattle	46	18	9
2	Buffalo	50	13	6.5
3	Goat	50	22	11
4	Sheep	54	26	13

Molecular phylogenetic analysis of regions of POL gene and tegument protein gene of OvHV-2

The nucleotide sequences of the POL gene region and tegument protein gene region of four OvHV-2 positive samples were compared with seven sequences and ten sequences respectively, reported by other researchers from around the world. Phylogenetic trees were

constructed on the basis of aligned and retrieved sequences from NCBI showing 99 to 100% homology with our sequences.

The phylogenetic analysis demonstrated that the sequence of the POL gene obtained in the present study (Accession number MK852173) clustered most closely to OvHV-2 isolates from Germany (HM216472 and AF327831) (Fig. 2a). The isolated sequence showed a more distant relationship to isolates of caprine herpesvirus 2 (KJ867526) and Rupicapra pyrenaica gammaherpesvirus 1 (KP260923).

The phylogenetic analysis for the three sequences of tegument protein gene obtained in the present study (MK840962, MK852171 and MK852172) showed close similarities (99 to 100%) with the other reported sequences. Two branches of the tree are observed with high bootstrap value. MK840962, MK852171 and MK852172 clustered with OvHV-2 sequences isolated from India (MF685297), Japan (LC203437), Egypt (KP015737), Turkey (JN084009), Italy (KJ420947) and Brazil (KJ658293). A second complex branch with isolates from Brazil (HQ223415) India (MK059980 and KR092147) and South Africa (EU851178) were found (Fig. 2b), with high bootstrap value, to be more distant from the sequences obtained from the Rawalpindi and Islamabad study area.

Discussion

In 1981 a case of MCF was reported on the basis of clinical signs and symptoms (see Additional File 1) (18). The disease was reported in a buffalo heifer in a village near Faisalabad. The animal showed all the signs and symptoms of MCF and died after an illness of 19 days. No postmortem examination of the carcass was performed (18). In neighbouring countries like India (15, 24), China (23) and Iran (16, 17) documented reports of MCF and the prevalence of OvHV-2, have been published from time to time. However, since the report from Ashfaq and Mohammad in 1981, no reports on cases of MCF in Pakistan have been published. This present study is therefore the first confirmed report of the prevalence of OvHV-2 amongst the national herd of Pakistan.

It is a well-established fact that sheep act as asymptomatic carriers of OvHV-2 and in Pakistan it is a common practice to keep sheep, goats, cattle and buffaloes in close proximities to each other, often with shared common feeding areas² (8, 18). In the present study, hemi-nested PCR assays were used to find the presence of OvHV-2 DNA in blood of apparently healthy animals. The hemi-nested PCR used to detect OvHV-2 DNA is highly sensitive, specific and is the diagnostic tool for SA-MCF recommended by the OIE (9, 25). DNA Sequence analysis confirmed the similarity of our Sequences with other OvHV-2 tegument protein gene and POL gene. Our results revealed an overall prevalence of OvHV-2 of 40% in the Rawalpindi District and Islamabad study area. Of the samples taken from apparently healthy animals, 48% of the sheep, 52% of the goats, 39% of the cattle, and 26% of the buffalo tested were found to be positive for OvHV-2 using PCR (Table 1).

Previous studies into the prevalence of OvHV-2 from around the world have indicated that goats, believed to be the natural hosts of Caprine Herpesvirus 2 (CpHV-2), are also susceptible to infection by OvHV-2 (16, 24–29). Studies on wild ruminants in Iran, indicated an OvHV-2 prevalence in goats of 10% (16). The prevalence of OvHV-2 amongst the goats in our study area is higher, however it is consistent with that reported by sampling goats tested in the Kashmir valley (61%) where mixed grazing of goats and sheep is practiced (25, 28). Whilst, goats have been shown to transmit CpHV-2, to susceptible species (5, 29) it has not yet been demonstrated that goats can transmit OvHV-2 to naïve animals (30). Though the majority of cases of OvHV-2 infection of goats appear to be subclinical, reports have tied OvHV-2 infection with clinical symptoms in goats including corneal opacity and pyrexia with neurological signs (31, 32). Further research is required to understand both the impact of OvHV-2 on the health and productivity of goats and whether goats can act as a reservoir animal, and source of infection for susceptible animals for OvHV-2.

Results of our study also indicated high levels of samples taken from apparent healthy cattle (40%) and buffalos (26%) to be positive for OvHV-2. This may suggest that under natural exposure conditions sub-clinical OvHV-2 infections regularly occur in cattle and buffaloes. This observation is consistent with experimental studies, using OvHV-2, that have demonstrated infection can occur in cattle without concurrent development of clinical MCF (33, 34). Furthermore, experimental evidence has indicated that whether an animal develops clinical signs of MCF may depend on the infectious dose of OvHV-2 that the animal receives (35).

Several studies in Pakistan focused on diseases in cattle and buffaloes such as Rinderpest, peste des petits ruminants (PPR), foot and mouth disease (FMD), theileriosis and babesiosis (18, 36), which share clinical symptoms with MCF, have reported large percentages of symptomatic animals that tested negative for the presence of the pathogens under investigation. Veterinarians in Pakistan do not test for MCF routinely and so its impact on the national herd and economy may be underestimated. Movements of animals between cities for trade purposes during religious and social festivals caused mixing of different animal species which provides opportunity for transmission of infections like MCF. In Pakistan and neighbouring countries free movement of goats and sheep across the borders make

MCF a transboundary disease (15). Phylogenetic analysis has shown that the OvHV-2 detected in Pakistan, showed no geographic separation from isolates from around the world. Diagnostic tests, using nested PCR, should therefore be able to identify all cases of MCF, caused by OvHV-2 whether they have resulted from within the national herd or from transboundary cases. Furthermore, the development of improved laboratory diagnostic capability through availability of sensitive and specific molecular tools like PCR, which enables detection of viral DNA, provides a reliable test to distinguish MCF cases from other diseases that induce similar clinical symptoms in affected species.

MCF causes significant economic losses worldwide in major ruminant species. MCF also poses threat to other susceptible species which are housed in close proximity to infected species (15). Whilst transmission of OvHV-2 over distances of five kilometres between lambs and bison without any physical contact has been reported (8, 37), close proximity of susceptible species with infected sheep, and potentially goats, on mixed pasture provides a greater risk of transmission of virus and development of MCF. Since there are no vaccines available for the disease so far and on control measures is the best strategy (15), such as segregating animals by 5 km or housing lambs separate from susceptible species, to reduce the incidence (38).

Continued investigation of the epidemiology and patho-physiology of OvHV-2, particularly in regards to cellular tropism, virus replication, and viral protein expression, is necessary for a more complete understanding of the virus and its association with MCF in cattle and buffaloes. Development of penside testing for MCF and other related with similar clinical symptoms diseases (like FMD and PPR) could help in the early diagnosis of the diseases and lead to better husbandry/ planning during potential outbreaks for veterinarians and government officials.

Conclusion

MCF is a fetal lymphoproliferative disease of susceptible ruminants and causes enormous economic losses to livestock farmers worldwide. In Pakistan the research on MCF is negligible. Our study demonstrated the prevalence of OvHV-2 in carrier species as well as in apparently healthy susceptible species (cattle and buffaloes). Present study is the first report to the best of our knowledge, on the prevalence of OvHV-2 in Pakistan. A large scale detailed study is required to achieve a better understanding of prevalence and pathogenesis of the virus in natural and susceptible hosts. Factors affecting the virulence of OvHV-2 in susceptible species are also needed to be investigated as the present study has shown the presence of virus in apparently healthy susceptible animals.

Abbreviations

MCF
Malignant catarrhal fever
AIHV-1
Alcelaphine herpesvirus 1
OvHV-2
Ovine herpesvirus 2
WA-MCF
Wildebeest-associated MCF
SA-MCF
Sheep-associated MCF
POL gene
Polymerase gene
Tegument protein gene
ORF75

Declarations

Ethics Approval

All the sampling was done under strict ethical conditions, with the permission of the owners. The animals were handled and samples were taken by Pakistan Veterinary Medical council certified professional veterinary clinicians/practitioners (Doctor of Veterinary Medicine). This work was approved by Ethics Committee for use of Animal & Human Subjects, Arid Agriculture University and Higher

education commission of Pakistan (SRGP-620). All the methods used including check up and taking samples were done without giving any harm to the animals. Also no anaesthesia, euthanasia, or any kind of animal sacrifice was the part of the study.

Consent to publication

Not Applicable

Availability of data and material

Not Applicable

Competing interests statement:

The authors declare that they have no financial and non-financial competing interests.

Funding:

AR was funded by Higher Education Commission Pakistan; grant number SRGP 620. ID and RGD were funded by the BBSRC programs: BBS/E/D/20241864 and BBS/E/D/20002173

Authors' contributions

AR, ID and RGD Conceived and designed the experiments. AR, ID, NA performed the experiments, AR, ID, SUR, MAAS, AY, RMB Contributed reagents/materials/analysis tools. AR and ID were the major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We are thankful to Aqsa Zulfiqar, Sehrish Sardar and Muzammil Tariq for their help in the study.

References

1. Khan IAKM. Developing Sustainable Agriculture in Pakistan: CRC press; 2018. Available from: <https://www.routledge.com/Developing-Sustainable-Agriculture-in-Pakistan-1st-Edition/Khan-Khan/p/book/9780815366539>.
2. Buxton D, Reid HW, Finlayson J, Pow I. Pathogenesis of Sheep-Associated Malignant Catarrhal Fever in Rabbits. *Res Vet Sci*. 1984;36(2):205–11.
3. Schultheiss PC, Collins JK, Spraker TR, DeMartini JC. Epizootic malignant catarrhal fever in three bison herds: differences from cattle and association with ovine herpesvirus-2. *J Vet Diagn Invest*. 2000;12(6):497–502.
4. Teankam K, Tantilertcharoen R, Boonserm T, Suadsong S, Banlunara W. Malignant catarrhal fever in swamp buffaloes (*Bubalus bubalis*): A retrospective pathological study of outbreaks in Thailand. *The Thai Journal of Veterinary Medicine*. 2006;36(1):19–30.
5. Stahel ABJ, Baggenstos R, Engels M, Friess M, Ackermann M. Two Different Macaviruses, ovine herpesvirus-2 and caprine herpesvirus-2, Behave Differently in Water Buffaloes than in Cattle or in Their Respective Reservoir Species. *PloS one*. 2013;8(12).
6. Russell GC, Stewart JP, Haig DM. Malignant catarrhal fever: a review. *Veterinary journal*. 2009;179(3):324–35.
7. Kumati OB. Virus life cycle and the parthenogenesis of malignant catarrhal fever (Doctoral dissertation, University of Nottingham).
8. Li H, Cunha CW, Taus NS, Knowles DP. Malignant catarrhal fever: inching toward understanding. *Annu Rev Anim Biosci*. 2014;2:209–33.
9. Li H, Karney G, O'Toole D, Crawford TB. Long distance spread of malignant catarrhal fever virus from feedlot lambs to ranch bison. *Can Vet J*. 2008;49(2):183–5.
10. OIE
OIE. Malignant. catarrhal fever.
www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/MALIGNANT_CATHARRAL_FEVERpdf2013.
11. The Center for Food Security and Public Health. Malignant Catarrhal Fever Factsheet.
www.cfsph.iastate.edu/Factsheets/pdfs/malignant_catarrhal_feverpdf2012.

12. Cunha CW, Gailbreath KL, O'Toole D, Knowles DP, Schneider DA, White SN, et al. Ovine herpesvirus 2 infection in American bison: virus and host dynamics in the development of sheep-associated malignant catarrhal fever. *Veterinary microbiology*. 2012;159(3–4):307–19.
13. O'Toole D, Li H. The pathology of malignant catarrhal fever, with an emphasis on ovine herpesvirus 2. *Veterinary pathology*. 2014;51(2):437–52.
14. Berezowski JA, Appleyard GD, Crawford TB, Haigh J, Li H, Middleton DM, et al. An outbreak of sheep-associated malignant catarrhal fever in bison (*Bison bison*) after exposure to sheep at a public auction sale. *J Vet Diagn Invest*. 2005;17(1):55–8.
15. Sood R, Khandia R, Bhatia S, Hemadri D, Kumar M, Patil SS, et al. Detection and molecular characterization of naturally transmitted sheep associated malignant catarrhal fever in cattle in India. *Trop Anim Health Pro*. 2014;46(6):1037–43.
16. Momatz HHF, Keyvanfar H, Abbasian B. PCR for detection of Ovine Herpesvirus-2 in cow and sheep of Iran. *Research Journal of Biological Sciences*. 2009;4(6):558–61.
17. Hemmatzadeh F, Boardman W, Alinejad A, Hematzade A, Moghadam MK. Molecular and Serological Survey of Selected Viruses in Free-Ranging Wild Ruminants in Iran. *PloS one*. 2016;11(12).
18. Ashfaq M, Muhammad K. Malignant catarrhal fever in a buffalo. *Pakistan Veterinary Journal*. 1981;1(4):179–80.
19. Chaudry RA, Akhtar AS. Rinderpest-like diseases in Pakistan. Central Treaty Organization,; 1973.
20. Schock A, Collins RA, Reid HW. Phenotype, growth regulation and cytokine transcription in Ovine Herpesvirus-2 (OHV-2)-infected bovine T-cell lines. *Veterinary immunology and immunopathology*. 1998 Nov 6;66(1):67–81.
21. Baxter SIF, Pow I, Bridgen A, Reid HW. Pcr Detection of the Sheep-Associated Agent of Malignant Catarrhal Fever. *Archives of virology*. 1993;132(1–2):145–59.
22. Flach EJ, Reid H, Pow I, Klemm A. Gamma herpesvirus carrier status of captive artiodactyls. *Research in veterinary science*. 2002 Aug 1;73(1):93 – 9.
23. Ochirkhuu N, Konnai S, Odbileg R, Murata S, Ohashi K. Molecular epidemiological survey and genetic characterization of ovine gammaherpesvirus-2 in Mongolian livestock. *J Vet Med Sci*. 2017;79(12):2040–2.
24. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol*. 2016;33(7):1870–4.
25. Wani SA, Bhat MA, Samanta I, Buchoo BA, Ishaq SM, Pandit F, et al. Clinical, serological and molecular evidence of sheep-associated malignant catarrhal fever in India. *Vet Rec*. 2004;155(8):242–4.
26. Wiyono A. The detection of ovine herpesvirus-2 in reservoir host of malignant catarrhal fever in Indonesia by means of polymerase chain reaction. *Jurnal Ilmu Ternak dan Veteriner*. 1999;4:121–7.
27. Li H, Keller J, Knowles DP, Crawford TB. Recognition of another member of the malignant catarrhal fever virus group: an endemic gammaherpesvirus in domestic goats. *J Gen Virol*. 2001;82:227–32.
28. Wani SA, Samanta I, Pandit F, Buchoo BA, Peer F, Bhat MA. Molecular epidemiology of ovine herpesvirus type 2 infection in Kashmir, India. *Vet Rec*. 2006;159(18):587–90.
29. Vikoren T, Li H, Lillehaug A, Jonassen CM, Bockerman I, Handeland K. Malignant catarrhal fever in free-ranging cervids associated with OVHV-2 and CPHV-2 DNA. *J Wildlife Dis*. 2006;42(4):797–807.
30. Li H, Keller J, Knowles DP, Taus NS, Oaks JL, Crawford TB. Transmission of caprine herpesvirus 2 in domestic goats. *Veterinary microbiology*. 2005;107(1–2):23–9.
31. Foster AP, Twomey DF, Monie OR, Kiupel M, Hoffmann I, Willoughby K. Diagnostic Exercise: Generalized Alopecia and Mural Folliculitis in a Goat. *Veterinary pathology*. 2010;47(4):760–3.
32. Jacobsen B, Thies K, von Altröck A, Forster C, König M, Baumgartner W. Malignant catarrhal fever-like lesions associated with ovine herpesvirus-2 infection in three goats. *Veterinary microbiology*. 2007;124(3–4):353–7.
33. Cook E, Russell G, Grant D, Mutisya C, Omoto L, Dobson E, et al. A randomised vaccine field trial in Kenya demonstrates protection against wildebeest-associated malignant catarrhal fever in cattle. *Vaccine*. 2019;37(40):5946–53.
34. Kojouri GA, Mahmoodi P, Momtaz H. Identification of SA-MCFV DNA in blood, lymph node, and spleen of adult sheep, healthy cattle, and MCF cattle by PCR. *Comp Clin Pathol*. 2009;18(2):113–8.
35. Powers JG, VanMetre DC, Collins JK, Dinsmore RP, Carman J, Patterson G, et al. Evaluation of ovine herpesvirus type 2 infections, as detected by competitive inhibition ELISA and polymerase reaction assay in dairy cattle without clinical signs of malignant catarrhal fever. *Javma-J Am Vet Med A*. 2005;227(4):606–11.

36. Taus NS, Oaks JL, Gailbreath K, Traul DL, O'Toole D, Li H. Experimental aerosol infection of cattle (*Bos taurus*) with ovine herpesvirus 2 using nasal secretions from infected sheep. *Veterinary microbiology*. 2006;116(1–3):29–36.

37. Zulfiqar S, Shahnawaz S, Ali M, Bhutta AM, Iqbal S, Hayat S, Qadir S, Latif M, Kiran N, Saeed A, Iqbal F. Detection of *Babesia bovis* in blood samples and its effect on the hematological and serum biochemical profile in large ruminants from Southern Punjab. *Asian Pacific journal of tropical biomedicine*. 2012;2(2):104–8.

38. Moore DA, Kohrs P, Baszler T, Faux C, Sathre P, Wenz JR, et al. Outbreak of malignant catarrhal fever among cattle associated with a state livestock exhibition. *Javma-J Am Vet Med A*. 2010;237(1):87–92.

Figures

Figure 1

a: Nested PCR results showing approximately 172bp bands of POL gene. The primers POL1 and POL2 target a segment of the DNA polymerase gene which is conserved in both OvHV-2 and AIHV-1, amplifying a fragment of 386bp. OHVPol is specific primer for OvHV-2 which amplify 172bp products.L1 and L2: Samples showing positive results, P: Positive control, M: 100bp Ladder, N: Negative control. b: Nested PCR results showing approximately 238bp bands of tegument protein gene. L1, L4-L7: Samples showing positive results M: 100bp Ladder, N: Negative control

Figure 2

Page 9/10

a: The phylogenetic analysis of the segment of POL gene sequence and their closely related reference sequence obtained from GenBank constructed using Maximum Likelihood method in MEGA 7 software. b: The phylogenetic analysis of the segment of tegument protein gene sequence and their closely related reference sequence obtained from GenBank constructed using maximum likelihood method in MEGA 7 software.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.png](#)