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Prevalence and PCR based molecular characterization of goat pox virus from field Outbreaks of Multan and Bahawalnagar, Pakistan

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Abstract

This study was designed to check the prevalence and PCR base molecular characterization of goat pox virus (GTPV) in the Multan and Bahwalnagar regions of Punjab, Pakistan. Capripox virus (CPPV) is the cause of goat pox (GTP) and sheep pox (SPP) disease; it highly affects the morbidity and mortality rate of goats and sheep. In this study the 80 tissue and blood samples of goats were collected on age basis from the goat farms, slaughter houses, tanneries and domestic animals. The epidemiological data was also collected. The collected samples were processed for DNA extraction and characterized the goat pox virus (GTPV) with specific reported and designed primers of P32 gene by PCR. Then each amplified product was analyzed by Gel Electrophoresis visualized by fluorescence in UV light. This study showed that Infants of goats in Multan showed 25% while adult goat in Multan showed 14.2% positive results. In Bahawlnagar, the effected infants of goats found were 31.25% while adult goats with positive signs were 11.1%. Both primers were equally effective for the characterization of unknown samples. The most effected goats were adult female and infants. The data was highly significant to $p < 0.05$.

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Introduction

Domesticated animal's generation influences a stimulating commitment to Pakistan agriculture that contributes around 56% of development in agribusiness and around 11% to the GDP. Animals are dominant supporter, representing around 11.8% bid to GDP. Animals incorporate the cows, wild oxen, sheep and goat (Rehman *et al.,* 2017). The flexibility of goats to various conditions and flawless association results to extend their world, under which those animals create and spare themselves. Pakistan is third most prominent country with relevancy glut of goats after China and India having 66.6 million heads. While Pakistan is at fourth position in goat's drain and sheep growing international locations with each year generation of 822 thousand tons of drain and 657 thousand tons of meat (Aziz, 2010).

Disease predominance is affected by organic framework, physiographic, soil sorts, precipitation, temperature and relative moistness (Bhanuprakash *et al.*, 2006). Chance additives of the ailment, intercourse, breed, along with age and physiological fame of animal (Selvaraju, 2014). SPP and GTP infections might be inactivated at 65°C within 30 minutes or 56°C within 2h. They can make position at a pH in range of 8.6 and 6.6. These contaminations are inclined to particularly acidic or basic pH; 2% HCl can crush these infections within 15 min (Yeruham *et al.,* 2007; Yune and Abdela, 2017).

Animals are helpless all through all age associations, and it has an exceptionally vital part in agrarian monetary framework. From the field of announced ailment flare-ups, it was learnt that the ailment could not be seen in immunized populaces however saw in unvaccinated creature (Roy *et al.,* 2018). GTP infection transmitted by contact with infected animals, their vaporizers, nasal release, salivation or dried scabs. The infections are also promptly transported on garments and rigging. Creepy crawlies e.g. the stable fly (*Stomoxys calcitrans*) act occasionally as mechanical vectors. Epidemiology of the ailment is likewise essential in diagnoses of SPP and GTP. In the kind shape, more typical in grown-ups, just skin injuries arise, particularly under the tail, and there may be no fundamental reaction and animals recover in 3-4 weeks. Developing life clearing and auxiliary pneumonia are also viewed on the complexities (Yune and Abdela, 2017; Juneja and Ganguly, 2017). Movements of infected animals act as principle source of spreading infections. SPPV and GTPV may make critical harm fleece and covers up, diminished lamb and drain creation (Babiuk *et al.,* 2008). The death rate of sheep and goat will currently and again be significantly high, among sheep and grown-ups (Tuppurainen *et al.,* 2017). CPPV cause high mortality and morbidity rate in dairy cattle, goats and sheep (Sajid *et al.,* 2013). The disease is basic both in stormy and winter season; however, the death rate is highest in the stormy season due to the rapid transmission of virus. It was also likewise watched that 33.33% disease was in roaming groups and 10% in settled crowds (Massoud *et al.,* 2016). As of late landowners of Tharparkar and its encompassing zones have endured immense financial misfortunes as death of thousands of sheep because of this disease (Anonymous).

Unstable and specific nuclear procedures are used for acknowledgment of CPPV concentrating on the RPO30, P32 and GPCR characteristics (Zhou *et al.*, 2012). It is understood that heterogonous diagnostic reagents tend to be less productive than homologous reagents for corroborative finding (Yune and Abdela, 2017). Prognosis of SPP is often supported extraordinarily function clinical symptoms ELISA check virus neutralization test, virus isolation (Tian *et al.*, 2010) and PCR measures (Balinsky *et al.,* 2008; Adedeji *et al*., 2019).

The Objective of this study is to check the prevalence of Goat pox disease in Multan and Bahwalnagar regions and molecular characterization of goat pox virus by PCR. The PCR test has enormous potential for the discovery of pathogens and for the most part utilized as the touchy and corroborative determination of the personality of the illness. The utilization of PCR strategy for SPP and GTP infection distinguishing proof thought to be a basic, quick and indicative technique.

Materials and methods

Ethical Approval

As per the committee for control and supervision of experiments on animal's guidelines, studies involving the collection of field clinical samples do not require any approval from the Institute's Ethics Committee.

Study areas and sample collection

The present study was designed to collect the samples of infected goats based on age and characterize them by PCR from Multan and Bahawalnagar regions of Punjab, Pakistan.

The samples were collected from different hides, slaughter houses and goat markets from Multan and Bahawalnagar regions. Multan is 30°15' N latitude, 71°36' E longitudes, located in Punjab, Pakistan. Bahawalnagar is 30° 00 N latitude, 73° 15E longitudes, located in Punjab, Pakistan. The distance between Multan and Bahawalnagar is directly 173 km. The Bahwalnagar district is spread over 8878sq. km area with hot and dry climate with minimum and the maximum temperature is recorded as 110°C and 500°C. It comprises five administrative units, Minchan Abad, Chishtian, Fort Abbas, Haroon Abad, and Bahawalnagar.

Multan is the major financial focal point and social of southern Punjab, situated on banks of Chenab River. Multan District is encompassed by the Khanewal towards the North and North East, the Vehari towards the East and Lodhran to the South. The district of Multan is spread over a zone of 3,721 square kilometers. Multan is known for having a portion of the most humid climate in Pakistan. The most elevated temperature is around 52° and the least recorded temperature is approximately –1°C.

Blood samples, hair samples, and skin tissues were collected from the animals showing typical signs and symptoms i.e. dullness, lack of apatite, pustules, scabs, and depression of goat pox disease. About 80 samples were collected from infected goats present in different herds. Blood samples were collected in EDTA tubes and tissue samples were collected in SDS solution without EDTA tubes. By following Biosafety procedures all the samples were stored in the icebox and brought to GCUF Zoology Research Lab through proper transportation and packaging. The collected samples were centrifuged on 10000rpm for just one minute so, the debris was settled down and a clear solution was used for the further process. Viral DNA was isolated from collected samples by following the procedure of (Sambrock *et al.,* 1989).

The DNA Extraction Method

Fifty µl from each collected sample was taken in Eppendorf tubes and homogenization was carried out by using 400µl TAE buffer. Then added 400µl of 20% SDS and 100µl of 20mg/ml proteinase K. After Incubation, for 4 hours at 55°C these tubes were vortexed for 15-30 sec. The supernatant was taken after 10 min centrifugation and transferred to another tube. DNA was precipitated by adding 300-400µl of isopropanol or super cold 100% ethanol and kept at -4°C for 60 minutes. Centrifuged again for 10 mins. Pellet was washed with 70% ethanol and was dried for 10-15 mins. DNA was permitted to suspend in 25µl of sterile water (d_3 H_2 O) for further processing. Nanodrop was used for the DNA quantification (Laimen *et al.*, 2011)

Primers

A conserved region of P32 gene was used for the detection of GTPV. The following table shows the sequence and nucleotide position of the reported primer used in this study (Bowden *et al.*, 2008) (see Table 1).

Table 1. Reported and designed primers of Goat pox virus

Gene	Primer Type	Primer Name	Primer Sequence	Size
P32	Reported	P1 Forward	5´CTAAAATTAGAGACTA TACTTCTT3´	969 bp
P32	Reported	P2 Reverse	5´CGATTTCCATAAACTAA AGTG3´	
P32	Designed	S1 Forward	5´-ATGGAAATCGTA TGCCGAT-3´	530 bp
P32	Designed	S2 Reverse	5´-AAACCAATGGA TGGGATACAT-3´	

PCR conditions and Reactions

For PCR of random amplified Polymorphic DNA analysis, the concentration of genomic DNA 10 X PCR buffer with $(NH_4)_2$ $SO_{4.mg}Cl_2$, dNTPs, 10 –mer random

primer and DNA Taq Polymerase were optimized. The 10 base oligonucleotide primers used for amplification of genomic DNA were obtained from Gene link Company. PCR was conducted in 50µl PCR tubes containing the 25µl master mixture. PCR reaction was done in Persona Autorisieter Master cycler of the Eppendorf, Germany. The PCR for goat pox virus were performed by using 10µL of template placed in 50µL of the ultimate extend of a 10 X reaction mixture containing 50 mM KCL, 10 mM Tris-HCl, 1.5mMmgCl2, 200mM of dNTP and 100pmol of primer sets for the gene encoding for the viral attachment protein as described by (Ireland and Binepal, 1998) and 2U Taq-DNA polymerase. Amplification consisted of an initial denaturation step at 94°C for 4 min, which will be followed by 35 cycles at 47°C for 1 min, 72°C for 1 min, 95°C for 45 sec and, finally, extension at 72°C for 10 min in a thermal cycler. PCR products were visualized in 1% agarose gel containing ethidium bromide. PCR products were evaluated on 1.5% agarose gel prepared in TAE buffer. DNA samples were then loaded with DNA loading buffer. The gel was run out at 120 volts for about 30-40 mins. The gel has been examined under ultraviolet transilluminator and photographed using a gel documentation system (WEALTEC, Dolphin-Doc) (Chopade *et al.,* 2013).

Results

The clinical signs of GTP were found in the form of pustules, papules, nodules, and scabs mostly on the hairless areas such as groin and perineum in addition to nose, eyes and lips making the feed intake painful. It was found that overall prevalence of CPPV was more in Bahawalnagar, as we found the affected animals which shows the typical signs have a prevalence of 24% and in Multan 20%. This above percentage showed the combined effect of the CPPV on sheep and goat. While the separate effect shows some differences regarding the percentage. Infants of goats in Multan showed 25% positive results while adult goat in Multan showed 14.2% positive results. In Bahawalnagar, the affected infants of goats found are 31.25% while adult goats with positive signs are 11.1% (See Table 2).

Table 2. PCR based detection of virus from suspected samples collected from different herds and Slaughter Houses.

Species	Multan				Bahawalnagar			
	Samples			% positive	Samples			%positive
		Infected	Healthy			Infected	Healthy	
Infant Goats	16	4	12	25%	32	10	22	31.25%
Adult Goat	14	2	12	14.2%	18	2	16	11.1%
Total		30		20%		50		24%

The PCR measure was observed delicate and the exactness and unwavering quality was affirmed by sequencing the relating amplicons of GTPV field disengage accessible for this examination (see Fig. 3 and 4). The flow thinks about recommended that the two preliminaries of P32 and other quality can be utilized for atomic recognizable proof of GTP malady. The accessibility of a simple to-utilize sub-atomic technique is required for the distinguishing proof of CPPV. The distinguishing proof of appropriate focus in the viral genome of P32 to separate antibody strains from GTPV field disengages incredibly decreases the expenses, by permitting the sequencing of little genome pieces. The present test is expected to be utilized by every veterinary research facility, incorporating those with constrained assets. It can also be utilized as a lead instrument for the immediate screening of neurotic examples gathered in CPPV flare-ups, particularly those happening in already immunized little ruminant populaces. (See Fig. 1 and 2)

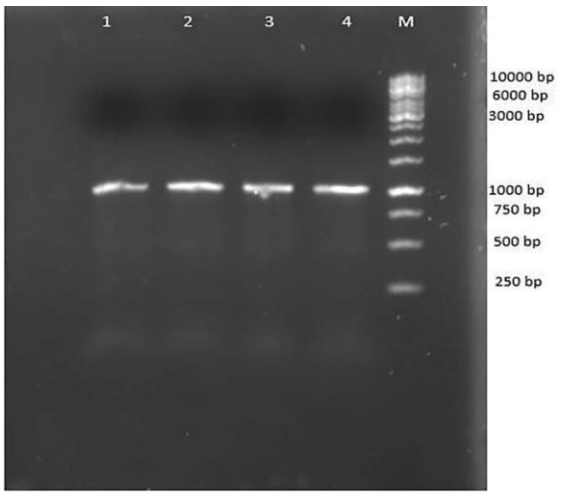

Fig. 1. Agarose gel showing 969bp PCR product of p32 gene of Capri pox virus using P1 and P2 primers (Lane 1- 4: positive samples, Lane M: 1Kb Ladder).

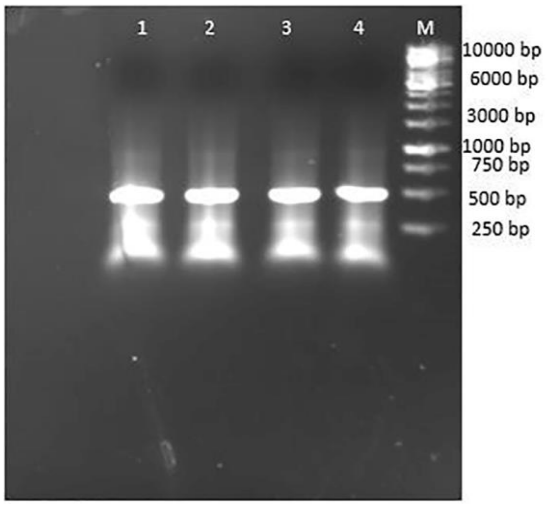

Fig. 2. Agarose gel showing 530 bp PCR product of p32 gene of Capri pox virus using S1 and S2 primers (Lane 1- 4: positive samples, Lane M: 1Kb Ladder).

Discussion

Goat Farming is incredible and popular business model in Pakistan and about 66.6 million goat population recorded per economic survey of 2016. CPPV contamination of sheep and goat effects in most important monetary losses and it also hinders the worldwide trade (Anonymous). In Pakistan, the disease is primary in arid regions accompanied by southern and northern regions of Punjab Pakistan (Sajid *et al.,* 2012).

The current study of CPPV based on the prevalence and characterization of SPPV and GTPV in the areas of Pakistan. SPPV and GTPV are endemic in some countries. These viruses can easily be diagnosed clinically as SPPV and GTPV, but it cannot differentiate at field level. Although, there are many reports of CPPV outbreak and prevalence in goats and sheep and their causative agents. But there is a need to identify causative agents properly in outbreaks of CPPV. There are some game animals play role in maintaining and cause an outbreak of CPPV for the identification and differentiation of GTPV, SPPV and LSDV, the phylogenetic analysis used in many studies. CPPV, RPO30, PCR used for the fast differentiation of GTPV and SPPV without using the technique of gene sequencing. Mahmoud *et al.,* 2016 used the species-specific primer, CPPV RPO30 PCR method to detect and identify the infection. Their results declared that the sequencing RPO30 gene-based PCR assay give a good picture of molecular epidemiology of CPPV infection.

Bora *et al.,* 2018 used the P32 primer for the detection of GTPV by PCR method. Shehbaz and Hussain in 2017 used P32 primer for differentiation of GTPV, SPPV, and LSDV by PCR method. Zhou *et al.,* 2012 found that the PCR assay developed a specific and sensitive method for differentiation and detection of infection in SPPV and GTPV. Abd-Elfatah *et al.,* 2018 and Adedeji *et al.,* 2019 find out the PCR and EM are a rapid and sensitive method for differentiation and characterization or identification of SPPV and GTPV. Roy *et al.,* 2018 studied the molecular epidemiology of GTPV by using the technique of PCR. As many other studies concluded that the P32 primer and PCR methods are the best parameters used ever for the phylogenetic analysis and use of RPO30 gene-based PCR assay used for molecular epidemiology. (Karapinar *et al.,* 2017; Mahmoud *et al.,* 2016; Zhao *et al.,* 2017, 2014; Venkatesan *et al.,* 2016, 2014, 2010; Santhamani *et al.,* 2013; Yan *et al.,* 2012).

It was found that the clinical signs of GTP may be variable. Skin lesions are seen and characterized by papules, nodules, pustules, and scabs. Overall prevalence of CPPV was more in Bahawalnagar as we found the affected animals which have a prevalence of 24% and in Multan 20%. This above percentage showed the combined effect of CPPV on adult goat and infant goat. While the separate effect shows some differences regarding the percentage. Infant Goats in Multan showed 25% positive results while adult goats in Multan showed 14.2% positive results. In Bahawalnagar, the affected infant goats found are 31.25% while adult goat with positive signs are 11.1%. The samples were characterized first by reported primers. P1 and P2 primer pair yield a PCR product of 969 bp representing the P32 gene. Then newly designed primer was also checked and found that these primers are also giving good results, while new designed S1and S2 primer yielded a PCR product of 530 bp.

Disease caused by CPPV is important causes of economic loss in goat and sheep farming. It was determined that 33.33% disease was in nomadic herds and 10% in settled herds (Massoud *et al.,* 2016). Farmers of Tharparkar and its surrounding areas have suffered huge economic losses in the form of death of thousands of sheep due to this disease (Anonymous).

Based on the results of the present study following conclusion are drawn

P32 gene is an important gene used for characterization of GTPV. Specific primers are used which gives 969bp product of PCR. Also, the newly designed primer has given 530bp product of PCR. Both reported, and newly designed primers showed positive results for identification of GTPV and negative as normal skin samples. The seasonal calendar of GTPV was outlined by informant groups and it was claimed to occur during the long and short rainy seasons of a year. Furthermore, GTP was listed to be one of the most common five goat diseases in the area by the farmers. Generally, the disease and associated morbidity and mortality were less commonly seen in adult age groups as compared to young age groups. P32 is the major immune dominant gene having amplicon size of 1024bp and 1027bp in GTPV and SPPV respectively. Also, P32 is gene sequencing data widely used for differentiating SPPV and GTPV and phylogenetic analysis of CPPV (Zhou *et al.,* 2012). Several researchers used the *P32* gene to detect SPV and GPV because it contains a most significant antigenic determinant present in all species of CPPV genus (Tian *et al.,* 2010).

Based on p32 gene analysis, it used to be determined the GTPV sequences are all most conserved. From the field of stated disorder outbreaks, it was once learned that the disease may want to no longer be determined in vaccinated populations but observed in unvaccinated animals (Roy *et al.,* 2018).

Conclusion

The results of this study showed that disease mostly prevailed in Bahawalnagar (24%) than Multan (20%). We declare this % age by using gene sequencing of P32. The animals were declared positive based on the PCR test. The normal skin samples were also preceded for negative control, but the virus was not detected, and vaccination of blood samples was taken as positive control. PCR was optimized for detection of Capri pox virus using specific primers. The samples were characterized first by reported primers. P1 and P2 primer pair yield a PCR product of 969bp representing the P32 gene. Then newly designed primer was also checked and found that these primers are also giving good results, while new designed S1and S2 primer yielded a PCR product of 530bp.

After conducting the study, we reach a point that the major factor in the spread of the disease was a movement of nomadic peoples along with their animals in different areas. It can be controlled by the carpet vaccination at the entry point and damping down vaccination in the endemic areas. Similarly, a bivalent vaccine of Capripox can give good results.

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Conflict of interest

Authors affirm no conflict of interest regarding article publication.

References

Abd-Elfatah EB, El-Mekkawi MF, Bastawecy IM, Fawzi EM. 2018. Identification and phylogentic analysis of sheep pox during an outbreak of sheep in Sharkia Governorate, Egypt.

Adedeji AJ, Moller J, Meseko CA, Adole JA, Tekki IS, Shamaki D, Hoffmann B. 2019. Molecular characterization of Capripox viruses obtained from field outbreaks in Nigeria between 2000 and 2016. Transboundary and emerging diseases.

Aziz MA. 2010. Present status of the world goat populations and their productivity. World **861(1078.2),** 1.

Babiuk S, Bowden TR, Boyle DB, Wallace DB, Kitching RP. 2008. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. Transboundary and Emerging Diseases **55(7),** 263-272.

Balinsky CA, Delhon G, Smoliga G, Prarat M, French RA, Geary SJ, Rodriguez LL. 2008. Rapid preclinical detection of sheeppox virus by a real-time PCR assay. Journal of clinical microbiology **46(2),** 438-442.

Bhanuprakas, V, Indrani BK, Hosamani M, Singh RK. 2006. The current status of sheep pox disease. Comparative Immunology, Microbiology and Infectious Diseases **29(1),** 27-60.

Bora DP, Venkatesan G, Neher S, Mech P, Barman NN, Ralte E, Das SK. 2018. Goatpox outbreak at a high altitude goat farm of Mizoram: possibility of wild life spill over to domestic goat population. Virus Disease **29(4),** 560-564.

Bowden TR, Babiuk SL, Parkyn GR, Copps JS, Boyle DB. 2008. Capripoxvirus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. Virology **371(2),** 380-393.

Chopade NA, Kaore MP, Chavan CA, Rautmare SS, Tembhurne PA, Bhandarkar AG, Kurkure NV. 2013. Detection of Capripoxvirus from Field Outbreak of Sheep Pox in Maharashtra State by Polymerase Chain Reaction. Indian Vet. J **90(3),** 110-111.

Ireland DC, Binepal YS. 1998. Improved detection of capripoxvirus in biopsy samples by PCR. Journal of Virological Methods **74,** 1-7.

Juneja R, Ganguly S. 2017. Sheep Pox and Goat Pox: The Animal Diseases of Importance for Transboundary Control. International Journal **3(1),** 30.

Karapinar Z, Ilhan F, Dincer E, Yildirim S. 2017. Pathology and Phylogenetic Analysis of Capripoxvirus in Naturally Infected Sheep Sheeppox Virus. Pakistan Veterinary Journal **37(1).**

Lamien CE, Lelenta M, Goger W, Silber R, Tuppurainen E, Matijevic M, Diallo A. 2011. Real time PCR method for simultaneous detection, quantitation and differentiation of capripoxviruses. Journal of virological methods **171(1),** 134-140.

Mahmoud MA, Khafagi MH. 2016. Detection, identification, and differentiation of sheep pox virus and goat pox virus from clinical cases in Giza Governorate, Egypt. Veterinary World **9(12),** 1445.

Massoud AH, Charbonnier LM, Lopez D, Pellegrini M, Phipatanakul W, Chatila TA. 2016. An asthma-associated IL4R variant exacerbates airway inflammation by promoting conversion of regulatory T cells to T H 17-like cells. Nature medicine **22(9),** 1013.

Rehman A, Jingdong L, Chandio AA, Hussain I. 2017. Livestock production and population census in Pakistan: Determining their relationship with agricultural GDP using econometric analysis. Information Processing in Agriculture **4(2),** 168-177.

Roy P, Jaisree S, Balakrishnan S, Senthilkumar K, Mahaprabhu R, Mishra A, Karmakar AP. 2018. Molecular epidemiology of goat pox viruses. Transboundary and emerging diseases **65(1),** 32-36.

Sajid A, Chaudhary ZI, Maqbol A, Anjum AA, Sadique U, Hassan ZU, Shahid M. 2013. Comparative sensitivity of PCR and cell culture technique for the identification of goat pox virus. J. Anim. Plant Sci **23(1 Supplement),** 31-34.

Sajid A, Chaudhary ZI, Sadique U, Maqbol A, Anjum AA, Qureshi MS, Shahid M. 2012. Prevalence of goatpox disease in Punjab province of Pakistan. J. Anim. Plant Sci **22(Sup 2),** 28-32.

Sambrock J. 1989. In vitro amplification of DNA by the polymerase chain reaction. Molecular Cloning, A Laboratory Manual **2,** 1418-1419.

Santhamani R, Yogisharadhya R, Venkatesan G, Shivachandra SB, Pandey AB, Ramakrishnan MA. 2013. Detection and differentiation of sheeppox virus and goatpox virus from clinical samples using 30 kDa RNA polymerase subunit (RPO30) gene based PCR. Veterinary World **6(11),** 923-925.

Selvaraju G. 2014. Epidemiological measures of disease frequency against sheep pox. International Journal of Scientific Research vol **8 (2).**

Shehbaz HA, Hassan IQ. 2017. Phylogenetic analysis of sheep pox virus isolates based on P32 gene in Iraq. Journal of Entomology and Zoology Studies **5(6),** 704-708.

Tian H, Chen Y, Wu J, Shang Y, Liu X. 2010. Serodiagnosis of sheeppox and goatpox using an indirect ELISA based on synthetic peptide targeting for the major antigen P32. Virology journal **7(1),** 245.

Tuppurainen ESM, Venter EH, Shisler JL, Gari G, Mekonnen GA, Juleff N, Babiuk S. 2017. Capripoxvirus diseases: current status and opportunities for control. Transboundary and Emerging Diseases **64(3),** 729-745.

Venkatesan G, Balamurugan V, Bhanuprakash V. 2014. Multiplex PCR for simultaneous detection and differentiation of sheep pox, goat pox and orf viruses from clinical samples of sheep and goats. Journal of Virological Methods **195,** 1-8.

Venkatesan G, Balamurugan V, Bhanuprakash V, Singh RK, Pandey AB. 2016. Loop-mediated isothermal amplification assay for rapid and sensitive detection of sheep pox and goat pox viruses in clinical samples. Molecular and cellular probes **30(3),** 174-177.

Venkatesan G, Balamurugan V, Singh RK, Bhanuprakash V. 2010. Goat pox virus isolated from an outbreak at Akola, Maharashtra (India) phylogenetically related to Chinese strain. Tropical Animal Health and Production **42(6),** 1053-1056.

Yan XM, Chu YF, Wu GH, Zhao ZX, Li J, Zhu HX, Zhang Q. 2012. An outbreak of sheep pox associated with goat poxvirus in Gansu province of China. Veterinary microbiology **156(3-4),** 425-428.

Yeruham I, Yadin H, Van Ham M, Bumbarov V, Soham A, Perl S. 2007. Economic and epidemiological aspects of an outbreak of sheeppox in a dairy sheep flock 236-237.

Yune N, Abdela N. 2017. Epidemiology and Economic Importance of Sheep and Goat Pox: A Review on Past and Current Aspects. J Vet Sci Technol **8(430),** 2.

Zangana IK, Abdullah MA. 2013. Epidemiological, clinical and histopathological studies of lamb and kid pox in Duhok, Iraq. Bulgarian Journal of Veterinary Medicine **16(2),** 133-138.

Zhao Z, Fan B, Wu G, Yan X, Li Y, Zhou X, Li J. 2014. Development of loop-mediated isothermal amplification assay for specific and rapid detection of differential goat pox virus and sheep pox virus. BMC microbiology **14(1),** 10.

Zhao Z, Wu G, Yan X, Zhu X, Li J, Zhu H, Zhang Q. 2017. Development of duplex PCR for differential detection of goatpox and sheeppox viruses. BMC veterinary research **13(1),** 278.

Zhou T, Jia H, Chen G, He X, Fang Y, Wang X, Jing Z. 2012. Phylogenetic analysis of Chinese sheeppox and goatpox virus isolates. Virology Journal **9(1),** 25.