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Clinical signs, molecular detection and phylogenetic analysis of sheep and goat pox viruses in sheep in Mosul city, Iraq

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Abstract

The genus capripox viruses, including sheep and goat pox viruses, represent one earnest pox infection with remarkable economic importance in animal farms. The study aimed to describe the clinical sheep pox disease, molecular disclosure of sheep and goat pox virus in clinically exposed sheep against the P32 gene of capripoxvirus, and for the initial record of phylogenetic analysis for these viruses in sheep in Mosul city, Iraq. Clinical findings revealed a fever of 40-41°C, diarrhea, and death of infected sheep. The skin lesions characteristic of erythema, papules, pustules, and dry black scabs disseminated on top of the skin frame. The percentage of infected sheep and fatal rate ranged between 10-40% and 2-6%, respectively. Moreover, ten local sequences of the sheep pox virus were obtained, and then individual sequence analysis was performed using BLASTn. No goat pox virus was detected in infected sheep. Two sequences were recorded under the accession numbers (PP992317.1 and PP992318.1) in the GenBank. The outcomes indicate that the study sequence was analogous to those of Iraq, India, Germany (99.89% identity) and Russia (99.79% identity). Thus, it is recommended that the farmers be aware of the occurrence of disease in susceptible animals, restrict new entry animals, apply vaccination, and eliminate clinically infected sheep, which are of priority to prohibit more losses.

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Introduction

The capripoxvirus genus is in the family of Poxviridae; it includes lumpy skin disease, goats' and sheep's pox virus, which affects cattle, goats, and sheep and induces remarkable financial losses (1,2). The virus causes subversive ailments with great publicity in some regions (3). The disease clinically shows fever, papules, and/or nodules; in some cases, the lesions can involve internal organs and death (4). The causative virus is enveloped, dual ss deoxyribonucleic acid, with genome lengths of approximately 150 bp., and open reading frames with 96% and 97% homology with the other members of pox virus, respectively (5,6). The transmission usually occurs directly or indirectly from contamination with lesions and secretions containing high viral concentration, fomite, respiratory droplets, and oronasal discharged from acutely infected sheep. The virus can survive several months on wool or dried scabs. Also, the disease can be distributed mechanically by arthropod vectors (3,7,8). The disease is chronic in various areas: the Middle East, North Africa, Asia, Turkey, Iraq, Europe and wherever sheep production is intensive; also, recent outbreaks have been documented in Greece, Bulgaria and Russia (9). Despite numerous efforts to eradicate it, especially in endemic areas, sheep pox still seriously threatens animal health and productivity (10,11). Small ruminants are considered of great value for the production of wool or hair and meat, and the pox virus is a notifiable illness that affects the productivity of these animals because of serious clinical pictures and high mortality rates, as well as its significant impact on the marketing of sheep (12,13). Clinically, outcomes of pox disease are manifested by rising

temperature and the development of various distinguishable lesions in the form of papules, pustules and scabs on the skin parts, including nostrils, medial surface of the thigh, around the tail, cheeks, lips, which concurrently cured within a few weeks (14). Boshra *et al.* (15) revealed moderate to acute fever, erythema, vesicles, and scabs on different skin parts in infected animals. Moreover, the injury could also be as well extended to internal organs, displaying respiratory disturbance, diarrhea, anorexia, body loss, superficial enlarged lymph nodes, nasal discharges and increased rate of abortion. Young ages are more susceptive than older ones, and mortality rates could be between 50% and 70% related to pneumonia (8,16). Several factors associated with the link between sheep and goat pox have been recorded, such as sex, age, breed, immunology, and health status of animals (17). The mortality rate may reach 50% in a quietly susceptible farm (18). The incident is furthermore influenced by ecosystems, regions, rain, and humidity (19). Although the sheep pox virus is host-specific, a few strains are ineffective against heterologous hosts. Furthermore, a recent study revealed that some genes are related to host priority (15). Although there are several laboratory aids for diagnosis of the condition, including electron microscopy, ELISA, isolation of the virus, and neutralization test (20,21), based on the fact of the relative antigenic, virulence linkage and engaged over 96% sequence homology between members of this genus, neither clinically nor the traditional serological assays could capable to differentiate between sheep and goat pox virus, as so molecular techniques targeting Capripox viruses specific genes such as P32 and RPO30 genes are required (19-23). The PCR technique is a rapid précised for detecting and differentiating sheep and goat pox (24-26).

In Mosul, Iraq, sheep are considered to be of great economic value for producing meat, wool, and trade, and diseases like sheeppox affect their productivity. This work aimed to consider clinical, molecular, and phenotype analysis of sheep and goat pox viruses in sheep in the study area.

Materials and Methods

Ethical approval

The (UM.VET.2023.114) was granted ethical permission by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Mosul, on October 18, 2023**.**

Animals' area and samples collection

For this work, 250 local male sheep aged 7 months to 1 year, for fattening purposes, represented 14 flocks (at least 250 sheep per flock) from clinically suspected sheep pox disease were examined during an outbreak from October to December 2023 in different unvaccinated sheep farms of Mosul city. 42 scab samples collected and conserved at -20ºC for the molecular analysis. Mortality rate, morbidity rate and clinical signs which are initially suspected of pox disease have been recorded.

Molecular analysis

Following the manufacturer's instructions, viral DNA of the pox virus was extracted from a skin specimen utilizing an Add Bio (Korea) extraction kit. The molecular technique was carried out with specific primers for the P32 gene (27): P32-F: CTAAAATTAGAGAGCTATACTTCTT and P32-R: CGATTTCCATAAACTAAA GTG (Macrogen Co, Korea). The PCR reaction mixtures and thermal cycler (BioRad, USA) were done according to Hamouda *et al.* (28) with some modifications (Table 1).

Table 1: Cycling conditions of PCR for amplification of pox virus

Step	Temperature (ºC)	Time
Activation	95	10 min
Denaturation	95	45 sec
Annealing	55	45 sec
Extension	72	1 min
Final extension	72	5 min

Finally, gel electrophoresis utilizing agarose gel 1.5% (AddBio, Korea) combined with GelRed dye 3 µl (AddBio, Korea). A 7 µl of each PCR product was loaded into each well of the agarose gel in the electrophoresis tank that contained TBE buffer 1X. Subsequently, the electrophoresis was run for one hour at 300 MP, and 80 V. Standard molecular weight marker 5 µl was utilized, which is 100 DNA marker. In order to document and identify the expected bands, the gel was looked at under a UV light source using a gel system (28). The purified amplicons were sent to Macrogen Company in Korea to perform the DNA sequence. The coding sequencing of 2 strains undergo to GenBank for documentation, and for building a Phylogenetic tree, the neighbour-joining method using MEGA version 11 software was performed (29).

Statistical analysis

All data from the current study are described in Excel 2010 for Windows 10.

Results

In the current investigation, the recorded clinical signs with different frequencies in affected sheep included fever, depression, loss of appetite, diarrhea, recumbency, and death. The characteristics of skin lesions (papules, erythema, pustules, and dry black scabs) are disseminated over the face, neck, subaxillary, inner thigh, and fatty tail. The morbidity rates ranged between the farms from 10% to 40%, and the mortality rate was between 2% and 6 %. The present work was revealed by utilizing a PCR reaction for 82 skin samples

from sheep targeting the P32 gene with a band of approximately 390 bp (Figure 1). In this study, and from determining 42 scab samples by PCR, 10 obtained sequences of the sheep pox virus were detected in Mosul City using the individual sequence analysis (BLASTn). No goat pox virus was detected. Moreover, 2 accession numbers (PP992317.1 and PP992318.1) were recorded in Genbank (Table 2). The study also revealed by equivalent the local sequence (PP992317.1and PP992318.1) of the P32 gene and the analysis of the phylogenetic tree using MEGA11 software with the attainable database in GenBank, outcomes indicate that the study sequence was neatly analogous with those data of Iraq, India and Germany (99.89% identity), and with Russia (99.79% identity) (Table 3 and Figure 2).

Table 2: Sheep pox sequences and accession numbers in sheep

Accession numbers	Sequencing 5'..... '3
PP992317.1	ATTCTTAGTTGTAAAATTTATACAATGAAAAATTGTACAAAATTTCTTTTATACTTGGTCT CTTACTGGTATGCGAAGTACATTTTTCTACCGTATATTTAATTAAATGCGGTGTTTCAGG AAGTTTTAACTGTCCATTTTTATTAATGATTAAATCATATATTTCTTTCGTTGTTAGATTT TTAAATGGTATTTTTCCTGAAAATATTTCCCACAAAACAACGCCAAAACTATAGATATCA TCATCTATAGTGCGTTCATTAAACACATTTGATAACATTTTATGAGATTGATAAACTATA GCGTTTATATTTTTAAAAGGTGGATTGGATAGTATTTTTTCTAAACCATGACATATAATC TTTACTTTATAATTTTCCTCTACTAAAAAACTAACGCTTGAAATACATCCATATGGTTTAT TATTTGTGTATTTATAAACGTTATATAAACCAACACTGCAATCTATTGCCATATCTAATTT TGTTTTAAAAGTTAAATCTCTTTCTTTTCTTATTACATTTTTTAAATATCCTCTTTTACAAT ATTCTAATACCAACGAAAGCCTTGGAAGGCCGTCACACATTTCTATATAAAATGCGTAA ATTTTTAATATATTATTACTGTCTATCCTTCTTAAATTTTTTATTTCGTTATTTGAAATATC CATTAGTATTTTGTGACTCGTGTGAGATTTTTTAAAAGTTCTAACGATAACTTCTTTATTA TTAAAAATACATTTATGAATGTAATTTTGATCATTTTCTTTTATAACAACACTTGTATAAG ATTGATAAACATCATCTGATTTTATACATCTTATTGGATTAATATAGCTTTGGTTTATTAT ATTTTGTGAATTTTCTGTAGTTATTTTTTTTTACTAAATTCCAAAATGAGTTTCTTTCGTCC AT
PP992318.1	TGGTTGTCATATTAAGCTATACAAATCATTGTATTTTTTTTTTTACCTTTTCATTTTCTATTT CATCATTTTCACATATTTGAAAATTAAACCACGCATAAACGAAAGCTCTAATTTTATTTC TATGATAACATTTTTTTATTATTTTAATATTATTTATTTCGTTAAAGTTTATATATATATAC GTTTTTTCTATATTAAAGGATGGGTATATATGCGTTAAAATTTTTTTTAGTGGTGCATAAT TATCAAATATGAAATAGTTAATCGATGACCTTAAAATCAAATTTTTAAATATATCTGTGT TGTTTTTTATTACAAATGGATTTATTAAAAAGATTTCATATGAACTATTTTTCATTTCTGG GCAGTTGACAAATAATAATCTCTTCCATATTTTTTTTTTCAAAATAAGTAACATCTTGTATT ATTATTTTATCGTAATCAGTGATATTATCATTTTTTTGAATAATTTCATTACACCATTCTT TTTGGCATCCTGACAAAATAAATTCTCTTGTTGTAACATATCTATCTTTAATAATGTGAG AAGACTTTACATTTTTTAATTCTAACACACCATCGTTGTCAGAACTTACTTCATATTGAG AAAAAAAATATTTATATCTATTAATTTTTTCTAAAACATTTTCAAAAAAACCTTTATGTTT TCCCAATAGAAAAAATGTTACGTCATGCAAATCAATTTTTGGATATTCGTTATTTTTTATT ATACAAATATTCAT

Table 3: Homology of sheep pox according to BLASTn in GenBank of NCBI

Deposit No.	Query %	Identity %	NCBI Accession No.	Region
PP992317.1 PP992318.1	100	99.89	MN072631.1	Iraq
	100	99.89	MN072630.1	Iraq
	100	99.89	MN072626.1	Iraq
	100	99.89	MN072628.1	Iraq
	100	99.89	MG000157.1	India
	100	99.89	MT137384.1	India
	100	99.89	MW020571.1	Germany
	100	99.79	OQ434235.1	Russia
	100	99.79	ON961655.1	Russia

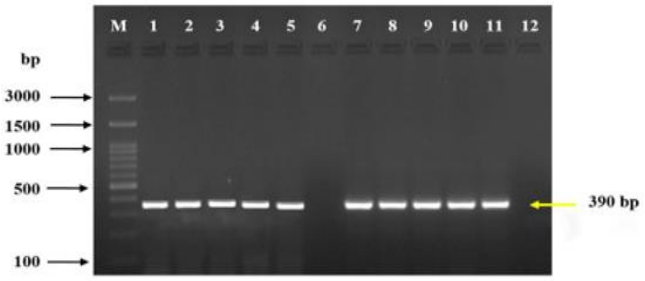

Figure 1: Electrophoresis form: lane M) Exact 100-3000bp DNA ladder; Lane 1-5,7, -11) c- PCR identified the P32 gene of the sheep pox virus with a band size around 390bp; Lane 12) negative control.

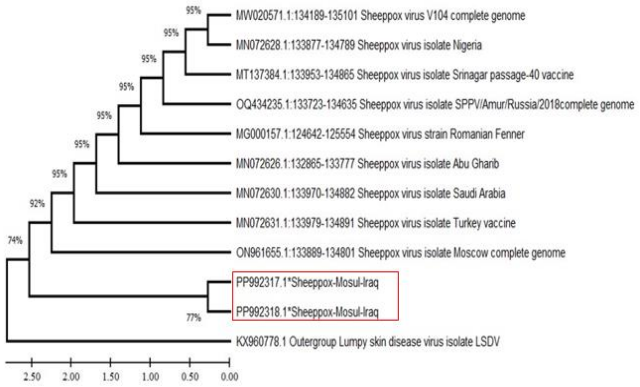

Figure 2: Phylogenic tree of sheep pox virus from Mosul/Iraq. bootstrap analysis with 1000 re-samplings.

Discussion

This is the first phylogenetic analysis of sheep pox virus in sheep in Mosul city, Iraq. The observed clinical signs in the current work in sheep positive for pox virus revealed body temperature of 40-41°C, depression, inappetence, diarrhea, recumbency and death (1,4). Characteristic skin lesions (papules, erythema, pustules and dry black scabs) disseminated with different frequencies over the face, neck, subaxillary, inner thigh and fatty tail. The morbidity rates vary between the farms and range from 10 to 40%, and the mortality rate ranges between 2-6%. Our results are the same as the former literature (28,30). It has been mentioned that the genus capripoxvirus, including sheep pox virus, mostly targets sheep and is manifested by intense skin lesions, elevation of body temperature, mild to severe diarrhea, respiratory participation and death and subsequent marked impacts to sheep producers.

Further, individual and multiple cases with no response to treatment and control efforts propose that the sheeppox virus may succumb to mutations that can cope with host defence by blocking or inhibiting host immunity proteins (31,32). Shehbaz and Hassan (33) revealed a 36% sheep pox virus rate based on the P32 gene in sheep in Al-Diwaniya, Iraq. In Anbar, Dayla, Baghdad, and Najaf provinces, the sheep pox virus was 75% (34). Differences in the rates between studies may related to the area, population volume, management and vaccination program. These reasons were recorded by Zangana and Abdullah (7), Hassan *et al.* (35), and Shihab and Hassan (36).

The present study confirms the detection of the sheep pox virus by utilizing a PCR reaction from skin samples targeting the P32 gene at 390 bp, signaling the sensitivity and reality of molecular analysis of the P32 gene to detect the capripoxvirus genus. This information is parallel to previous research (10,28,33). The sheep pox virus was detected in Mosul City using the individual sequence analysis (BLASTn), and no goat pox virus was detected. Moreover, 2 sequences were allocated with numbers (PP992317.1 and PP992318.1). The present finding showed that PCR was a more helpful and excellent tool for discriminating the pox virus. Several investigations indicate that the PCR technique is fast, critical and accurate for detecting and recognizing pox virus (26,37). In contrast, Shehbaz and Hassan (33) revealed the detection of Sheep (MF289491.1) and Goat (KJ026556.1) pox virus from cutaneous lesions based on the P32 gene in sheep in Al-Diwaniya, Iraq. However, researchers announced that sheeppox and goat pox viruses are host-specific; molecular studies indicate that several genes may be associated with host preference (15).

Results of the phylogenetic analysis for the foremost time in this study region for P32 gene of sheep pox virus and two sequences gene deposited in the NCBI GenBank (PP992317.1 and PP992317.1), these sequences were observed to have a close evolutionary link and phylogenetic distinctiveness with the other sheep pox virus sequences in the GenBank NCBI of various nations, including Iraq (38), India (39,40), Germany (41), and Russia (11,42), with highly similarity (99.79-99.89%). The near link with Iraqi and other virus strains possibly explained the illegal and uncontrolled sheep movement and/or poor husbandry and prophylactic methods. Thus, it is recommended that farmers be aware of disease occurrence in susceptible animals. Moreover, restricting new entry animals' application of vaccination and eliminating clinical animals are priorities to prohibit more losses. It has been clear that the existence of outbreaks with severe morbidity and mortality authorized the requirement for prevention and control of sheep pox disease by orderly immunization and observation of the host specificity for the elaboration of specific vaccines in future for blocking the economic loss to the producing farms (18).

Conclusion

The current study revealed that PCR assay targeting the P32 region enables the recognition of the capripox virus in sheep and can be beneficial for later molecular epidemiology of this virus in different ruminants. Care should be

considered for preventing and controlling sheep pox disease by employing systematic vaccination and monitoring of the disease and minimizing losses to the farming community.

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Conflict of interest

The authors declared no competing interests.

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الكشف السريري والجزيئي والتحليل الوراثي لفيروس جدري الأغنام والماعز في الأغنام في مدينة الموصل، العراق

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الخلاصة

يتضمن جنس فيروسات كابريبوكس، فيروس جدري الأغنام، وفيروس جدري الماعز، وفيروس مرض الجلد العقدي، والتي تمثل إحدى عدوى الجدري الخطيرة التي لها أهمية اقتصادية ملحوظة في حقول الحيوانات. هدفت الدراسة إلى وصف العلامات السريرية لمرض جدري الأغنام، والكشف الجزيئي لفيروس جدري الأغنام وجدري الماعز في الأغنام المصابة طبيعياً ضد الجين P32 من جنس فيروسات كابريبوكس، ولأول مرة التحري عن التحليل الوراثي لفيروسات جدري الأغنام والماعز في الأغنام في مدينة الموصل، العراق. أظهرت النتائج السريرية الحمى ٤٠-٤١ م° والإسهال ووفاة بعض الحيوانات المصابة. كما تميزية الآفات الجلدية بالاحمرار والحطاطات والبثرات وقشور سوداء جافة منتشرة على الجسم. تراوح معدل الإصابة بالمرض والوفيات في الأغنام بين ١٠-٤٠% و٢-٦% على التوالي. فضلا عن الحصول على ١٠ تسلسلات محلية لفيروس جدري الأغنام، وبعدها تم تحليل التسلسل الفردي باستخدام (BLASTn)، ولم يتم الكشف عن فيروس جدري الماعز في الأغنام المصابة. تم تسجيل تسلسلين تحت الأرقام التسلسلية (PP992317.1 وPP992318.1) في بنك الجينات. تشير النتائج إلى أن التسلسلات في هذه الدراسة كانت مماثلة لتلك التسلسلات في العراق والهند وألمانيا وبنسبة تشابه ٩٩,٨٩%، ومع روسيا وبنسبة تشابه ٩٩,٧٩%. ولذلك ينصح المزارعون بضرورة الحذر من حدوث المرض في الحيوانات الحساسة، مع تقييد دخول الحيوانات الجديدة وتطبيق التطعيم والقضاء على الأغنام المصابة السريرية له الأولوية لمنع المزيد من الخسائر.

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