Archives of Razi Institute, Vol. 78, No. 2 (2023) 505-513

DOI: 10.22092/ARI.2022.359034.2359

Original Article

First Molecular Investigation of lumpy Skin Disease in Buffaloes Using the Qualitative and Quantitative Polymerase Chain Reaction Assays, Iraq

Mohammed Aboud, A[1 *], Kareem Luaibi, O[1]

1. Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Received 13 June 2022; Accepted 10 July 2022
Corresponding Author: ali.mohammed1200a@covm.uobaghdada.edu.iq

Abstract

Lumpy skin disease (LSD) is one of the most important infectious bovine diseases in Iraq in the last 10 years; however, the current study represents the first investigation to confirm the disease in buffaloes as well as ticks with estimation the association of positivity to clinical vital signs and risk factors. A total of 150 buffaloes were subjected for blood sampling, skin lesions and ticks. All the collected samples; 150 blood, 13 skin lesions, and 29 tick samples, were examined molecularly using the conventional and real-time PCR assays. The total positive results of blood, skin and ticks by conventional PCR were 5.33, 7.69 and 0%, respectively; while for real-time PCR, it was 15.33, 7.69 and, 0%, respectively. Insignificant differences were showed between values of temperature, pulse and respiratory rates of LSD positive and negative buffaloes by the conventional and real-time PCR assays. The association of positive conventional PCR results to risk factors (age, sex and region) was revealed a significant increase in prevalence and risk of LSD in buffaloes aged < 1 year; but for gender, insignificant variation in prevalence but not risk was seen between females and males. In case of different geographical region, significant higher prevalence was reported in Wasit; while, buffaloes of Maysan and Wasit were appeared at higher risk than those of Dhi-Qar. Regarding real-time PCR, insignificant differences were found between values of < 1, 1-4 and > 4-8 years age old, but not in group of >8 that showed a significant decline in positivity (0%). For sex, insignificant variation in prevalence, but not risk, was seen between females and males. Concerning region, buffaloes of Wasit province were recorded a significant higher values of prevalence and risk than other regions. LSD in buffaloes is mainly sub-acute, and PCR appeared to be a suitable diagnostic method in detection of infection; however, furthermore studies are necessary.

Keywords: Real-Time PCR, Envelop Protein (P32) Gene, Blood, Skin Lesion, Ticks

1. Introduction

Lumpy skin disease (LSD) is an infectious bovine disease, which infecting mainly cattle and to less extent buffaloes as a result of exposure to a viral species known as the lumpy skin disease virus (LSDV), belongs to Capripoxvirus genus in the subfamily Chordopoxvirinae and the family Poxviridae (1). The first describing of clinical syndrome that including high fever, 0.5-5 cm skin nodules in whole skin or subcutaneous tissue with enlargement of the superficial lymph-nodes, and then, development of ulcerative skin lesions, excessive lacrimation and nasal discharge, was restricted to sub-Saharan Africa, especially in Zambia in 1929, and considered initially to be the result of either poisoning or a hypersensitivity to insect bites (2, 3). Between 1929 to 1986, more cases and panzootic infections was reported in African countries such as Botswana (1943-1945), Republic of South Africa

(1949), Kenya (1957), Sudan (1972), Tanzania, Zimbabwe, Somalia, Cameron (1981-1986) and Egypt (1988) (4). According to the World Organization from Animal Health (OIE), LSD outbreaks have been suspected or confirmed in the Middle East countries such as Kuwait (1991), Lebanon (1993), Yemen (1995), United Arab Emirates (2000), Bahrain (2003), and Oman (2010) (5). Incursion of LSD was reported for the first time in Iraq, Turkey and Iran in 2013, indicating that the disease has a potential for further spread to the Asian and European countries (6, 7).

The disease can be transmitted from infected animals to susceptible hosts through blood, nasal discharge, lacrimal secretions, semen and saliva as well as through the infected milk to sucking calves resulting in severe economic losses due to chronic debility in affected animals, reduced milk production, poor growth, infertility, abortions, severe damage to hides and mortalities that could be reach 20% (8). Further, LSDV isolation from flies (*Culicoides*, *Glossina*, *Tabanidae* and *Stomoxys*) and mosquitoes has been suggested the mechanical transmission of the virus by these vectors (9).

Worldwide, the tentative diagnosis of disease is usually based on the characteristic clinical signs (10). However, the application of laboratory molecular assays such as polymerase chain reaction (PCR) have revealed a high reliability, sensitivity and specificity in detection and confirmation of the virus in suspected clinical specimens or biopsies (11, 12). In Iraq, LSD outbreaks were reported in cattle among different regions; and consequently, a number of studies have been performed to confirm the LSD in clinically infected animals using PCR assay and histology (10, 13). However, no studies or reports were found about the occurrence of disease in buffaloes or even vectors. Hence, the present study was carried out to confirm the LSD in buffaloes existed mainly in southern parts of Iraq in particular Wasit, Dhi-Qar and Maysan province using conventional and real-time PCR assays. Molecular examination of tick samples that collected from the infested buffaloes with estimation the association of positive findings to clinical vital signs and risk factors were aimed, also.

2. Materials and Methods

2.1. Study Animals

Totally, 150 unvaccinated LSD-buffaloes of different ages, both sexes and many regions were selected equally from three Iraqi provinces; Wasit, Dhi-Qar and Maysan. The selection of study animals was based on the information of veterinarians and owners about the recent appearance of clinical LSD cases in cattle and/or buffaloes existed at these areas. Initially, all study buffaloes were subjected to clinical examination to detect of pursued skin lesions and enlargement in lymph nodes, with measurement of vital signs (temperature, pulse and respiratory rates) and collection of tick samples if available. From each study animal, 5 ml of jugular venous blood was collected, aseptically using a disposable syringe into an anticoagulant EDTA tubes and transported to the laboratory.

2.2. Molecular Examination

Following the manufacturer instructions of the two DNA Extraction Kits, iNtRON Biotechnology (Korea) and Genaid (USA), DNAs were extracted from the samples of blood and ticks, respectively. The concentration (ng/μL) and purity of extracted DNAs were measured at an absorbance of 260 /280 nm using the Nanodrop system (Thermo-Scientific, UK).

For conventional PCR, the mastermix tubes were prepared using the Maxime PCR Premix Kit (iNtRON Biotechnology, Korea) at a total volume of 20μL through targeting the *Envelop Protein 32* (*P32*) gene [(F: 5´-AGGTTCGCGAAATTTCAGATGT-3´) and (R: 5´-TCCCCCTG TACGAATACA-3´)]. The Thermal Cycler conditions were included 1 cycle initial denaturation (95ºC / 5 minutes); 35 cycles of denaturation (95ºC / 30 seconds), annealing (60ºC / 30 seconds) and extension (72ºC / 1 minute); and 1 cycle final extension (72ºC / 5 minutes). The PCR products were analyzed by electrophoresis of the stained

Agarose gel with Ethidium Bromide at 100 volt and 80Am for 1 hour. The positive samples were detected at an amplicon size of ~499bp.

For real-time PCR, GoTaq qPCR Master Mix Kit (iNtRON Biotechnology, Korea) at a final volume of 25µL through targeting the *Envelop Protein 32* (*P32*) gene [(F: 5´-AGAGCTAACGA AATGAAAAACGGT-3´) and (R: 5´-TGAGCCATTTTTCCAACTC-3´)]. The qPCR conditions were involved 1 cycle pre-denaturation (95ºC / 5 minutes), 40 cycles denaturation (95ºC / 20 seconds) and annealing/extension with detection (58ºC / 1 minute), and melt cycle (65-90ºC). The qPCR data analysis was carried out in BioRad Real Time PCR Analysis Software by calculation of the threshold cycle number (CT value) the positive amplification and negative control sample.

2.3. Statistical Analysis

The findings of present study were analyzed using the GraphPad Prism version 6.0.1.298 (*GraphPad Software Inc., USA*) software. Chi-square (x^2) was applied to detect significant differences between values of clinical signs of positive and negative animals by molecular assays. Odds ratio was applied to estimate statistical association between age and sex factors and positive results by both CR assays. Values were represented as mean±standard errors (M±SE) or percentage (%), and the statistical differences in study results were considered significant at a level of (P<0.05).

3. Results

The total positive results for conventional PCR examination of 150 buffaloes was 8 (5.33%) using the blood samples. Among totally 13 suspected LSD skin lesions, only 1 (7.69%) sample was found to be positive. Among 29 tick samples collected from the 29 infested buffaloes, the findings revealed no positive samples in these samples (Figures 1 and 2).

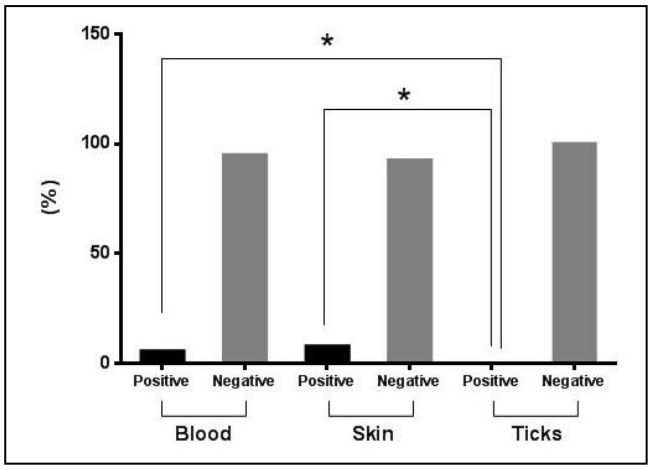

Figure 1. Total results for conventional PCR examination of 150 blood samples, 13 skin lesions and 29 tick samples using the qualitative conventional PCR assay.
Significance * (P<0.05)

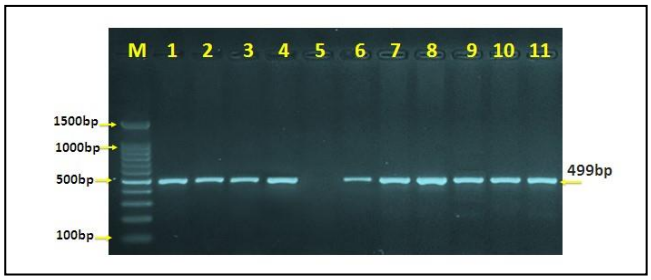

Figure 2. Electrophoresis of PCR products for *envelope protein (P32)* gene at 499bp using 1.5% Agarose gel stained with Ethidium Bromide at 100 Volt, 80 Am for 1 hour.
M: Ladder marker (100-1500bp); Lane (4): Positive Control; Lane (5): Negative Control; Lane (1): Positive skin lesion sample; Lanes (2, 3, 6, 7, 8, 9, 10 and 11): Positive blood samples

The application of real-time PCR assays revealed that the total positive results for testing 150 buffaloes was 23 (15.33%) in the blood samples, and 1 (7.69%) positive sample in a totally 13 suspected LSD skin lesions. However, no positive samples were found through testing of 29 tick samples (Figure 3). The amplification of target DNA was detected using the fluorescent molecules, and the cycle threshold (Cq) number of amplification of positive samples was ranged from 20 to 21 (Figure 4). The melting curve and melting peak for *envelope*

protein (*P32*) gene in LSDV positive samples was calculated using 2x SYBR Green DNA polymerase mastermix, and the chart was generated by plotting relative fluorescence (RFU) *vs*. cycle number (Figure 5 and 6).

In comparison between both molecular assays, the positive findings of real-time PCR were increased significantly ($P<0.029$) in blood samples (15.33%) compared to conventional PCR (5.33%), but not in values of skin lesion ($P>0.05$), (Figure 7).

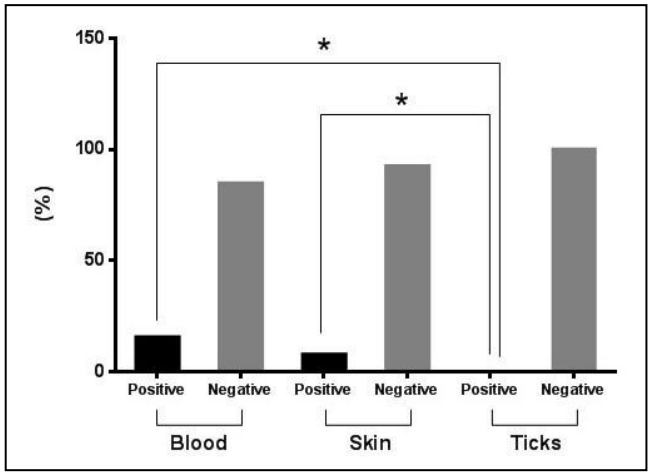

Figure 3. Total results for real-time PCR examination of 150 blood samples, 13 skin lesions and 29 tick samples using the qualitative conventional PCR assay.
Significance * ($P<0.05$)

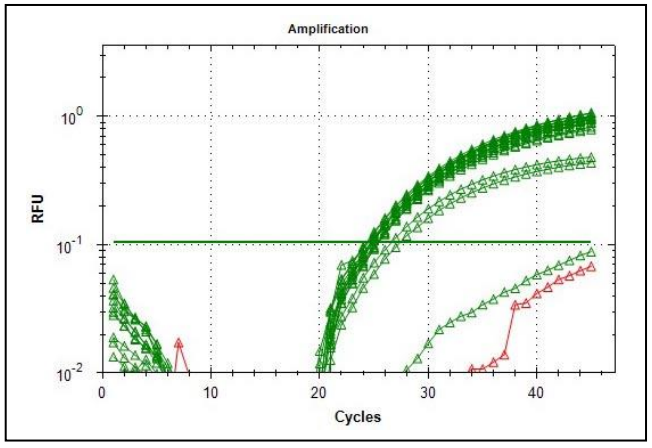

Figure 4. Amplification plot for *envelope protein* (*P32*) gene in LSDV positive samples using 2x SYBR Green DNA polymerase mastermix. The chart is generated by plotting relative fluorescence (RFU) vs. cycle number

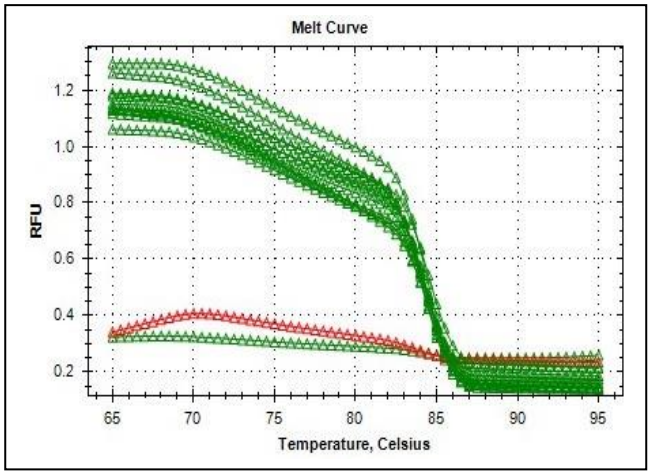

Figure 5. Melting curve for *envelope protein* (*P32*) gene in LSDV positive samples using 2x SYBR Green DNA polymerase mastermix. The chart is generated by plotting relative fluorescence (RFU) *vs*. cycle number

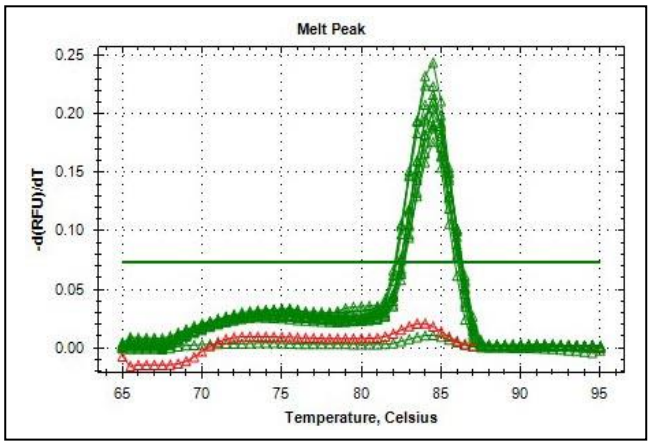

Figure 6. Melting peak for *envelope protein* (*P32*) gene in LSDV positive samples using 2X SYBR Green DNA polymerase mastermix

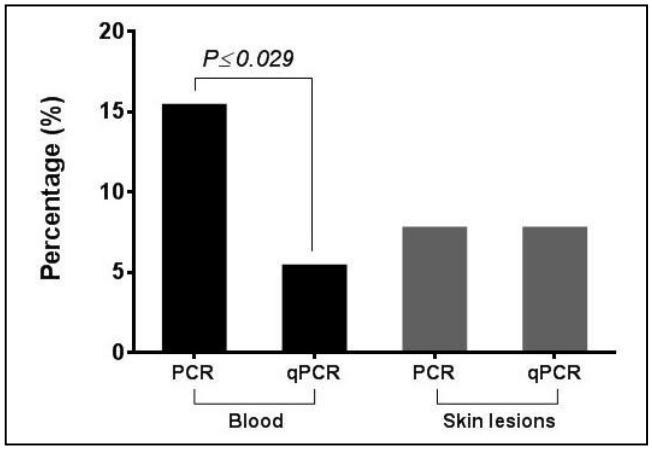

Figure 7. Total positive results of blood and skin lesion samples by the conventional and real-time PCR assays

The lack of significant differences (P>0.05) was observed between the total findings of temperature, pulse and respiratory rates of LSD positive and negative buffaloes by the conventional and real-time PCR (Table 1).

Statistical correlation between the positive findings of conventional PCR and the groups of risk factors was revealed a significant variation (P<0.05) between their values (Table 2). For age factor, the percentage of positive results was increased significantly (P<0.0381) in the groups of animals aged <1 year (6.94%), 1-4 years (3.7%) and >4-8 years (5.26%) when compared to buffaloes aged > 8 years (0%). However, values of Odds ratio and relative risk of buffaloes aged < 1 year (1.865 and 1.792, respectively) were increased significantly in comparison with the values of other age groups; 1-4 years (0.575 and 0.592, respectively), >4-8 years (0.991 and 0.985, respectively) and > 8 years (0 and 0, respectively). Regarding the sex factor, although no significant differences (P<0.515) were found between the positive findings of female (5.93%) and male (3.13%), values of Odds ratio and relative risk of females (1.95 and 1.895, respectively) appeared significantly higher than observed in males (0.513 and 0.528, respectively). Concerning with the findings of region factor, there was significant elevation (P<0.0344) in percentage of positive buffaloes to LSD as well as the Odds ratio in Wasit province (12% and 6.8, respectively) in comparison with those reported in Maysan (4% and 0.65%, respectively) and Dhi-Qar (0% and 0%, respectively) provinces. However, values relative risk were increased significantly (P<0.0395) in provinces of Maysan (6.67) and Wasit (6) compared to Dhi-Qar (0) province.

In this study, the findings of age factor were showed that the values of positive LSD were increased significantly (P<0.036) in buffaloes aged < 1 year (10.06%), 1-4 years (14.82%) and >4-8 years (10.53%) when compared to buffaloes aged > 8 years (0%). However, values of Odds ratio and relative risk reported a significant increase (P<0.05) in buffaloes aged < 1 year (1.497 and 1.414, respectively) when compared to values of other age groups; 1-4 years (0.941 and 0.947, respectively), >4-8 years (0.618 and 0.658, respectively) and >8 years (0 and 0, respectively). For sex factor, no significant differences (P<0.086) were seen between positive findings of females (16.1%) and males (12.5%); however, there were significant increases (P<0.0201) in values of Odds ratio and relative risk of females (1.343 and 1.288, respectively) than males (0.745 and 776, respectively). Among study regions, the findings of positivity, Odds ratio and relative risk of Wasi province (28%, 3.933 and 3.11, respectively) were increased significantly when compared to values of Dhi-Qar (4%, 0.157 and 0.19, respectively) and Maysan (14%, 0.858 and 0.875, respectively), (Table 3).

Table 1. Total results of vital signs among study animals (Total No.: 150)

Sign	Conventional PCR		Real-time PCR		*P*-value
	Negative	Positive	Negative	Positive	
Temp.	37.83±0.19	37.95±0.3	37.79±0.18	38.03±0.27	NS
Pulse rate	74.58±5.36	76.33±4.61	74.51±4.92	76.29±4.45	NS
Resp. rate	30.29±3.27	33.1±3.38	30.24±3.13	32.78±3.26	NS

S: Significance (P<0.05); NS: Non-significant (P>0.05)

Table 2. Association of positive results by conventional PCR to risk factors

Factor	Group	Total No.	Positive No. (%)	Odds ratio	Risk
Age (Year)	< 1	72	5 (6.94%)	1.865	1.792
	1 - 4	54	2 (3.7%)	0.575	0.592
	>4 - 8	19	1 (5.26%)	0.991	0.985
	>8	5	0 (0%)	0	0
	P-value		0.0381 S	0.0217 S	0.0239 S
Sex	Female	118	7 (5.93%)	1.95	1.895
	Male	32	1 (3.13%)	0.513	0.528
	P-value		0.0515 NS	0.016 S	0.0211S
Region	Wasit	50	6 (12%)	6.8	6
	Dhi-Qar	50	0 (0%)	0	0
	Maysan	50	2 (4%)	0.65	6.67
	P-value		0.0344 S	0.0016 S	0.0395 S

S: Significance ($P<0.05$); NS: Non-significant ($P>0.05$)

Table 3. Association of positive results by real-time PCR to risk factors

Factor	Group	Total No.	Positive No. (%)	Odds ratio	Relative risk
Age (Year)	< 1	72	13 (10.06%)	1.497	1.414
	1 - 4	54	8 (14.82%)	0.941	0.947
	>4 - 8	19	2 (10.53%)	0.618	0.658
	>8	5	0 (0%)	0	0
	P-value		0.036 S	0.0256 S	0.0368 S
Sex	Female	118	19 (16.1%)	1.343	1.288
	Male	32	4 (12.5%)	0.745	0.776
	P-value		0.086 NS	0.0147 S	0.0201
Region	Wasit	50	14 (28%)	3.933	3.11
	Dhi-Qar	50	2 (4%)	0.157	0.19
	Maysan	50	7 (14%)	0.858	0.875
	p-value		0.0178 NS	0.00193 S	0.00158 S

S: Significance ($P<0.05$); NS: Non-significant ($P>0.05$)

4. Discussion

Worldwide, there were a few data available about the occurrence and prevalence of LSD in buffaloes as well as the role of different vectors in transmission of infection between bovine animals; while in Iraq, this study appeared to be the first one carried out in buffaloes and in their infested ticks. In comparison with our findings, variable LSD positive results were reported using different diagnostic assays; 28.2% in South Africa using ELISA (14), 0% in Egypt by virus isolation and PCR (15) and 15.2% in other Egyptian study (16). The lack of positive findings in study tick samples may be caused either by the low numbers of tested tick samples or indicated that this vector is not implicated in transmission of LSDV. Other explanation is that ticks have a low chance to transmit the virus as a result of short period of existence on bodies of infested buffaloes because these animals are covered usually with water in rivers, lakes or even marshes. Although, the application of both PCR assays had targeting the Envelop protein (P32) gene, the findings of real-time PCR assay in blood samples were significantly than

reported by conventional PCR assay. Caliendo, Schuurman (17) demonstrated that the quantitative molecular assay is more sensitive than the qualitative, since the quantitative assay having a lower limit of sensitivity and gave lower viral load values at all concentrations tested. Other studies have mentioned for the ability of real-time PCR to overcome many problems found in conventional PCR such as low sensitivity and resolution, non-automated, poor precision, short dynamic range and size-based discrimination only (18, 19).

In this study, values of the vital signs; temperature, pulse and respiratory rates, were showed an insignificant variation between values of positive and negative animals obtained by both conventional and real-time PCR assays. Our findings were in contrast to the results of the only Iraqi study performed on acutely infected buffaloes (18, 19). However, insignificant variation in values of vital signs between positive and negative animals could be attributed to that most of these animals were infected sub-acutely. Considering the disease burden, cattle are found to be more sensitive to the illness compared to buffaloes or other ruminants (20).

Association between positive results of molecular PCR assays and risk factors were reported a significant variation in their values. For conventional PCR, we showed that animals of <1 year age old were more sensitivity to LSD than other age groups, and this in consistent with that reported Ayelet, Haftu (21). While, the findings of real-time PCR were seen that buffaloes of <1 year, 1-4 and > 4-8 years age old were more sensitive to LSD than those of >8 years. The prevalence of LSD in different age groups might be attributed to the diminished maternal immunity in calves of less than 1 year, or exposing of adult cattle to stress factors of pregnancy, lactation, and fattening. Constable, Hinchcliff (1) reported that all ages are susceptible to the causative virus, except animals recovered recently from an attack, in which case there is a solid immunity lasting for about 3 months. Also, very young calves, lactating and malnourished bovine animals develop more severe clinical disease during outbreaks. Abd Elmohsen, Selim (22) demonstrated in cattle that the disease prevalence in older animals (12-50 months) was higher than in young animals (3-12 months) attributing these results to the low frequency of exposure because calves are kept at home away from biting insects. Faris, El-Bayoumi (16) found that animals aged 1-2 years old and over 2 years have a higher prevalence of LSD than young animals of age less than one year as a result of exposure to stress factors.

In both conventional and real-time PCR assays, no significant variation was showed between the positive females and males suggesting that both genders are susceptible to infection. However, statistical analysis of results revealed that females were at a higher risk of LSD than males, and this might be attributed to that females have increased stress factors than males including lactation and pregnancy (10). Also, the number of existed males at each farm, or even tested males night be reflected negatively on the total positive results in the current study.

We showed that the prevalence rate and risk of LSD were fluctuated among different localities. The high prevalence rate of positivity and risk observed in Wasit province might be explained by the method of selection of study animals because study buffaloes of this province were subjected selectively for collection of samples. Also, Veterinarian and owners of Wasit province were provided much more helpful during collection of animals' data and then selection of suspected buffaloes with LSD. In many study areas, recent outbreaks in cattle livestock were targeted to detect study buffaloes since direct contact could play a role in transmission of infection from diseased cattle to sensitive buffaloes. Other explanations include the spread of cultivated areas and swamplands might result in a rapid propagation of insect population, or might be as a result of farmers' refusal to vaccinate their animals (16, 23).

This study provides data indicating infection by LSDV in buffalo population in Iraq; however, the role of these animals in the epidemiology of LSD is still not clear. The application of molecular PCR assays through targeting an *Envelop protein* (*P32*) gene shows a high efficacy in detection of disease especially with the real-time PCR assay. Blood represents the sample of choice instead of skin nodules since most infected buffaloes were revealed mild or sub-acute symptoms. The role of tick in transmitting of LSDV to positive LSD buffaloes was not seen in the current study; therefore, furthermore long-term molecular studies are necessary to implicate the role of ticks or other vectors such as mosquitoes and flies in transmission of infection or even in life cycle of LSDV. Additionally, control strategies should be included the consideration of the risk factors identified in this study.

Authors' Contribution

Study concept and design: A. M. A. and O. K. L.
Acquisition of data: A. M. A.
Analysis and interpretation of data: O. K. L.
Drafting of the manuscript: O. K. L.
Critical revision of the manuscript for important intellectual content: A. M. A. and O. K. L.
Statistical analysis: A. M. A.
Administrative, technical, and material support: A. M. A.

Ethics

The current study was approved by and carried out under the license of the Scientific Committee of the Department of Internal and Preventive Veterinary Medicine and the Scientific Committee of the College of Veterinary Medicine, University of Baghdad (Baghdad, Iraq).

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors have deeply indebted and grateful to the staff and the Head of Department of Internal and Preventive Medicine (College of Veterinary Medicine, University of Baghdad) for their help and support.

References

1. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats: Elsevier; 2017.
2. Al-sabawy HB, AL-Hamdany EK, AL-Sultan AA, RDAM SA. A High Light On Lumpy Skin Disease In Iraq And The Middle East: A Review Article. J Appl Vet Sci. 2020;5(2):94-103.
3. Al-Salihi KA, Hassan IQ. Lumpy Skin Disease in Iraq: Study of the Disease Emergence. Transbound Emerg Dis. 2015;62(5):457-62.
4. Abdulqa HY, Rahman HS, Dyary HO, Othman HH. Lumpy skin disease. Reprod Immunol. 2016;1(4):25-31.
5. Tuppurainen ES, Oura CA. Review: lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. Transbound Emerg Dis. 2012;59(1):40-8.
6. Gharban HAJ, Al-Shaeli SJJ, Al-Fattli HHH, Altaee MNK. Molecular and histopathological confirmation of clinically diagnosed lumpy skin disease in cattle, Baghdad Province of Iraq. Vet World. 2019;12(11):1826-32.
7. Sevik M, Dogan M. Epidemiological and Molecular Studies on Lumpy Skin Disease Outbreaks in Turkey during 2014-2015. Transbound Emerg Dis. 2017;64(4):1268-79.
8. Abutarbush SM. Lumpy Skin Disease (Knopvelsiekte, Pseudo-Urticaria, Neethling Virus Disease, Exanthema Nodularis Bovis). In: Bayry J, editor. Emerging and Re-emerging Infectious Diseases of Livestock. Cham: Springer International Publishing; 2017. p. 309-26.
9. Sprygin A, Pestova Y, Wallace DB, Tuppurainen E, Kononov AV. Transmission of lumpy skin disease virus: A short review. Virus Res. 2019;269:197637.
10. Gharban HAJ, Yousif AA. Serological and Molecular Phylogenetic Detection of Coxiella burnetii in Lactating Cows, Iraq. Iraqi J Vet Med. 2020;44:42-50.
11. Ochwo S, VanderWaal K, Ndekezi C, Nkamwesiga J, Munsey A, Witto SG, et al. Molecular detection and phylogenetic analysis of lumpy skin disease virus from outbreaks in Uganda 2017-2018. BMC Vet Res. 2020;16(1):66.

12. Sudhakar SB, Mishra N, Kalaiyarasu S, Jhade SK, Hemadri D, Sood R, et al. Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: Epidemiological features and molecular studies. Transbound Emerg Dis. 2020;67(6):2408-22.
13. Molla W, Frankena K, Gari G, Kidane M, Shegu D, de Jong MCM. Seroprevalence and risk factors of lumpy skin disease in Ethiopia. Prev Vet Med. 2018;160:99-104.
14. Fagbo S, Coetzer JA, Venter EH. Seroprevalence of Rift Valley fever and lumpy skin disease in African buffalo (Syncerus caffer) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa. J S Afr Vet Assoc. 2014;85(1):e1-e7.
15. Elhaig MM, Selim A, Mahmoud M. Lumpy skin disease in cattle: Frequency of occurrence in a dairy farm and a preliminary assessment of its possible impact on Egyptian buffaloes. Onderstepoort J Vet Res. 2017;84(1):e1-e6.
16. Faris DN, El-Bayoumi K, El-Tarabany M, Kamel ER. Prevalence and risk factors for lumpy skin disease in cattle and buffalo under subtropical environmental conditions. Adv Anim Vet Sci. 2021;9(9):1311-6.
17. Caliendo AM, Schuurman R, Yen-Lieberman B, Spector SA, Andersen J, Manjiry R, et al. Comparison of quantitative and qualitative PCR assays for cytomegalovirus DNA in plasma. J Clin Microbiol. 2001;39(4):1334-8.
18. Houghton SG, Cockerill FR. Real-time PCR: overview and applications. Surgery. 2006;139(1):1-5.
19. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonak J, Lind K, et al. The real-time polymerase chain reaction. Mol Aspects Med. 2006;27(2-3):95-125.
20. Das M, Chowdhury MSR, Akter S, Mondal AK, Jamal M. An updated review on lumpy skin disease: perspective of Southeast Asian countries. J Adv Biotechnol Exp Ther. 2021;4:322-33.
21. Ayelet G, Haftu R, Jemberie S, Belay A, Gelaye E, Sibhat B, et al. Lumpy skin disease in cattle in central Ethiopia: outbreak investigation and isolation and molecular detection of the virus. Rev Sci Tech. 2014;33(3):877-87.
22. Abd Elmohsen M, Selim A, Abd Elmoneim AE. Prevalence and molecular characterization of Lumpy Skin Disease in cattle. Benha Vet Med J. 2019;37(1):144-7.
23. Mhemid KM. Molecular detection of lumpy skin disease virus in cattle by polymerase chain reaction in Iraq: Khitam Mahdi Mhemid and Ibtesam Qasim Hassan. Iraqi J Vet Med. 2016;40(1):83-8.