Clinical and Molecular Detection of Malignant Catarrhal Fever in Buffalo Calves in Basrah, Iraq

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Abstract: *Background:* Malignant Catarrhal Fever is an acute, systemic, lymphoproliferative disease that affects Bovidae and Cervidae and other cloven-hoofed animals.

Objectives: The present work aimed to identify diseased buffalo calves based on clinical signs and to confirm the diagnosis by PCR.

Methods: The study included 76 native buffalo calves, aged 8 to 12 months, of both sexes. These calves exhibited high fever, panting, nasal discharge, conjunctivitis, lymphadenopathy, and corneal opacity. Additionally, 25 clinically healthy local native calves, both males and females, served as the control group.

Results: Of the 76 suspected calves, 66 (86.8%) were PCR-positive for MCF virus. Four isolates were sequenced and deposited in GenBank (LC918980–LC918983). Phylogenetic analysis showed close evolutionary relationships to international MCF virus isolates. Affected calves exhibited characteristic clinical signs, including leukocytosis predominantly due to lymphocytosis, altered coagulation indices, hypoglycemia, and hypoproteinemia, as well as significantly increased blood urea nitrogen, creatine kinase, and alkaline phosphatase levels.

Conclusions: Malignant Catarrhal Fever should not be underestimated; attention should always be paid to it because of its high infection rate, which often leads to the death of diseased animals and causes significant economic losses.

Keywords: Malignant catarrhal fever, Buffalo calves. Clinical signs, PCR, Phylogenetic analysis, Basra, Iraq.

INTRODUCTION

Malignant catarrhal fever (MCF) is an acute, systemic, mostly fatal lymphoproliferative disease that affects Bovidae and Cervidae and other cloven-hoofed animals, including pigs, deer, bison, and water buffalo [1, 2]. The disease is caused by 10 viruses from the Rhadinovirus genus (family Herpesviridae). Two of these viruses are carried by sheep, and five others have been found in these carrier animals [3, 4]. Additionally, two distinct and important groups of causative viruses were identified: the Alcelaphinae–Hippotraginae group and the Caprinae group. The Alcelaphine herpesvirus 1 (A1HV-1) is endemic worldwide in most wild animals and causes disease in these species. Furthermore, the ovine type of herpesvirus 2 (OvHV-2) was endemic in sheep [5, 6].

The disease ranges from an acute inflammatory condition with a short clinical course to a more chronic syndrome, occasionally presenting with skin involvement. In a clinical setting, the disease is characterized by fever, ropey salivation, and nasal and ocular discharge. However, specific lesions are usually detected in the buccal cavity and muzzle, along with enlarged lymph nodes, in animals affected by MCF [7, 8].

The disease is distributed globally across most continents, including North America, Africa, and Asia, affecting both domestic and zoo animals. It is also known by several synonyms, including Catarrhal Fever, Gangrenous coryza, Malignant Head Catarrh, and Snotsiekt [9, 10].

Diagnosis of MCF based solely on clinical signs and gross pathological examination cannot be relied upon, as these findings can be highly variable, although they are sometimes accepted. Nonetheless, the Polymerase Chain Reaction (PCR) exhibits higher sensitivity and specificity than serological and cytological tests for detecting the disease [3, 4, 6]. Additionally, the described real-time PCR assay provides a reliable method for diagnosing infection and quantifying the causative virus DNA in blood or saliva samples. It offers benefits such as faster processing, higher throughput, and a lower risk of false-positive results compared to conventional PCR [11].

Malignant catarrhal fever has recently been suspected in buffalo calves in northern Basrah, Iraq; however, molecular confirmation of the disease in this region remains limited. Therefore, the present study aimed to characterize the clinical manifestations of affected buffalo calves and to confirm the infection using polymerase chain reaction (PCR), thereby providing epidemiological evidence of the disease in southern Iraq.

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MATERIALS AND METHODS

Area of the Study and Animals

The study was conducted on 76 local native buffalo calf breeds, aged 8-12 months, of both sexes, reared in northern Basrah, Iraq. The animals exhibited high fever, panting, and severe nasal discharge with mucus, along with conjunctivitis, lymphadenopathy, and corneal opacity, which could be unilateral or bilateral. Additionally, 25 clinically healthy local native buffalo calf breeds, both male and female, were included as the control group. Both groups underwent complete clinical and laboratory examinations using standard methods [12].

Collection of Samples

Twelve milliliters of blood were drawn from the calf's jugular vein (under aseptic precautions). Three milliliters of EDTA-anticoagulated blood were used for a complete blood count performed on the XP-300 Automated Hematology Analyzer (Sysmex America). Additionally, the differential leukocyte count was estimated from a Giemsa-stained blood smear [13]. Another three milliliters of blood mixed with Trisodium citrate (using plasma) were used to evaluate clotting factor indices according to Weiss *et al* [13]. Serum extracted from the blood samples used for biochemical analysis for the spectrophotometric evaluation of Glucose, Total protein, BUN, Creatine kinase, and Alkaline phosphatase using kits from Biolabo/ France. Serum samples, after separation from blood, were stored at -20 degrees Celsius until use.

Polymerase Chain Reaction and Amplification: DNA Assay

Viral transport medium (VTM) samples were processed for viral nucleic acid extraction using the Viral Nucleic Acid Extraction Kit II (VR100, GeneAid, China) according to the manufacturer’s protocol. Extraction was carried out using a silica membrane spin-column method, enabling highly efficient purification and recovery of viral DNA from clinical samples. The purified nucleic acids were eluted in RNase-/DNase-free water, quantified using a NanoDrop spectrophotometer, and stored at −20 °C until further PCR analysis.

The herpesvirus DNA polymerase gene contains multiple evolutionarily conserved amino acid motifs (A, B, and C). Targeting these regions, [11] developed a set of degenerate universal primers capable of amplifying a conserved fragment of approximately 215–315 bp across a broad spectrum of mammalian, avian, and reptilian herpesviruses. A nested PCR strategy was employed to enhance both detection sensitivity and specificity:

- Primary PCR used two forward primers (DFA and ILK) and one reverse primer (KG1).
- Secondary PCR used one forward primer (TGV) and one reverse primer (IYG).

Following nested amplification and gel electrophoresis on a 1% agarose gel, the secondary PCR products were directly sequenced by Sanger sequencing using the same primers as in the secondary PCR to confirm and identify the amplified viral fragments.

PCR one	DFA-IGAYTTYGCIAGYYTITAYCC ILK-ITCCTGGACAAGCAGCARIYSGCIMTIA KG1-IGTCTTGCTCACCAGITCIACICCYTT
PCR Two	TGV-ITGTAACTCGGTGTAYGGITTYACIGGIGT IYG-ICACAGAGTCCGTRTCICCRTAIAT

Primer sequences

Primary PCR	DFA-I	GAYTTYGCIAGYYTITAYCC
Primary PCR	ILK-I	TCCTGGACAAGCAGCARIYSGCIMTIA
Primary PCR	KG1-I	GTCTTGCTCACCAGITCIACICCYTT
Secondary PCR	TGV-I	TGTAACTCGGTGTAYGGITTYACIGGIGT
Secondary PCR	IYG-I	CACAGAGTCCGTRTCICCRTAIAT

Statistical Analysis

In SPSS, an unpaired t-test is used to perform statistical analysis and calculate the difference between diseased and healthy control buffalo calves. The significance level was set at $P<0.05$ [14].

RESULTS

Molecular detection of MCF virus was performed using nested PCR targeting the conserved region of the herpesvirus DNA polymerase gene in buffalo calves. Successful amplification was confirmed by 1% agarose gel electrophoresis, which showed specific bands of 215–315 bp in positive samples, whereas negative samples showed no amplification. Of 76 clinically suspected cases, 66 (86.8%) were PCR-positive, and 10 (13.2%) were negative. Sanger sequencing of the amplified products confirmed

specificity, revealing conserved sequences of the herpesvirus DNA polymerase gene. Overall, nested PCR provided sensitive and specific detection of MCF virus, enabling reliable diagnosis even in samples with low viral load. As illustrated in Figures **1** and **2**, several analysis lanes displayed positively amplified DNA bands at the predicted molecular size. No obvious bands were observed in negative samples, validating the assay and ruling out non-specific amplification. The size of the amplified products was estimated using a molecular weight marker (DNA ladder) (100 bp to 1500 bp) located at the right side of the gel. Compared with the ladder, the observed PCR bands in positive samples were consistently within the expected size range, supporting our confidence in the amplification accuracy.

The bands showed slightly different intensities and sharpness across samples, possibly indicating differences in the original viral DNA concentration or template quality. The presence of sharply defined bands with no smear indicates good DNA integrity and good amplification conditions.

The gel electrophoresis results also corroborate the high detection rate observed by the nested PCR assay in this study, with MCF virus confirmed in 66/76 (86.8%) samples. The present study illustrates the

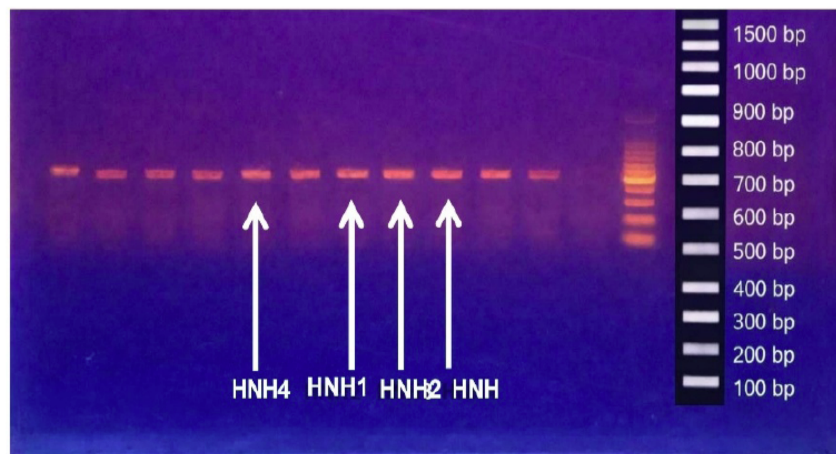

Figure 1: Agarose gel electrophoresis of nested PCR products targeting the herpesvirus DNA polymerase gene. Lanes (1–n) represent clinical samples from suspected buffalo calves. Positive samples show distinct bands at approximately 215–315 bp. Lane (M) represents the DNA molecular weight marker (100–1500 bp).

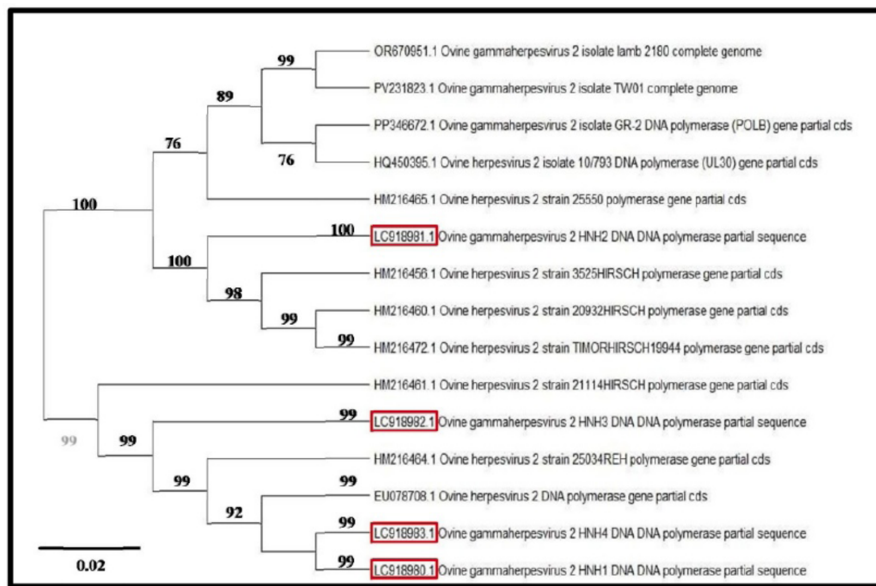

Figure 2: Phylogenetic tree illustrating the genetic relationship of local Ovine gammaherpesvirus 2. Isolates (LC918981.1, LC918982.1, LC918983.1, LC918980.1) (Red rectangle) are contrasted with previously reported strains based on DNA polymerase gene sequences.

Figure 3: Unilateral ocular opacity (grayish corneal opacity) in a calf buffalo infected with MCF.

specificity and sensitivity of a nested PCR technique for molecular diagnosis of MCF in buffalo calves.

Phylogenetic Analysis

Comparative phylogenetic analysis of OvHV-2 isolates using partial DNA polymerase (POLB/UL30) gene sequences showed that all study isolates clustered within the main OvHV-2 clade, confirming their identity as Ovine herpesvirus 2 and their close relationship to globally reported strains, indicating strong genetic conservation.

Isolates LC918981.1 (HNH2), LC918982.1 (HNH3), LC918983.1 (HNH4), and LC918980.1 (HNH1) formed a distinct subcluster within the OvHV-2 group, supported by high bootstrap values, suggesting a common local lineage.

These sequences were closely similar to reference strains (e.g., the HM2164 series and EU078708.1), with short branch lengths indicating low genetic divergence and high conservation of the DNA polymerase gene. Minor branching differences, supported by moderate-to-high bootstrap values, may reflect subtle geographic or evolutionary variation. Overall, the phylogenetic topology was well supported, confirming the identity of OvHV-2 and indicating that the DNA polymerase gene is a stable molecular marker for phylogenetic analysis.

Diseased calves showed signs of depression and refused to eat (95.45%), exhibited copious mucous nasal discharge with mouth breathing and ropiness of saliva (92.4%), congestion of ocular mucous membranes (89.3%), enlargement of superficial lymph nodes (86.3%), unilateral ocular opacity (corneal opacity with a grayish color) with lacrimation, Figure **3** (75.7%), and diarrhea with mild dehydration (69.8%). Table **1**.

On the other hand, diseased buffalo calves show a significant rise ($P<0.05$) in body temperature, heart rate, and respiratory rate when compared with the control group Table **2**.

Hematological analysis of diseased buffalo calves infected with MCF virus shows a significant increase ($P<0.05$). in total leukocyte count due to a significant lymphocytosis in diseased buffalo calves compared to the control group Table **3**.

Regarding the clothing factor indices, the results indicated that the disease significantly altered these indices compared with healthy control calves (Table **4**).

In the present study, biochemical analysis indicates a significant decrease ($P<0.05$) in glucose and total protein levels in diseased buffalo calves compared with the control group. Conversely, values of BUN, Creatine

Table 1: Clinical signs exhibited by (66) diseased buffalo calves with MCF

Clinical signs	No. of affected animals	%
Depression and refused to eat	63	95.4
Copious mucous nasal discharge with mouth breathing	61	92.4
Ropiness of saliva	61	92.4
Congestion of ocular mucus membranes	59	89.3
Enlargement of superficial lymph nodes	57	86.3
Unilateral ocular opacity with lacrimation	50	75.7
Diarrhea with mild dehydration	44	69.8

Table 2: The Body Temperature, Heart Rate, and Respiratory Rate of Diseased Buffalo Calves and the Control Group

The parameters	Control group n=25	Diseased buffalo calves n = 66	Significance
The body temperature C	39.12 ± 0.45	41 ±1.7*	(P<0.05)
Heart rate/min.	63.3 ±2.7	121.2 ±16.6*	
Respiratory rate/min.	21.65 ±3.24	87.5 ±5.7*	

*(P<0.05).

Table 3: Hematological Changes of Diseased Buffalo Calves and the Control Group

parameters	Control group n=25	Diseased buffalo calves n = 66	Significance
RBC $\times 10^6$	7.51± 1.33	7.53 ± 1.46	(p > 0.05)
Hb g/dl	13.3 ± 1.77	13.22± 2.23	
PCV %	34.5 ± 3.51	35.41±8.12	
TLC $\times 10^3$	10.77 ± 5.45	15.47 ± 8.45 *	(p < 0.05)
Lymphocytes/ Absolute	4550 ± 141.14	9752 ± 431.65 *	
Neutrophiles/ Absolute	4782 ± 322.31	4325.21 ± 433.17	(p > 0.05)
Monocytes/ Absolute	540 ± 322	550 ± 310	
Eosinophils/ Absolute	380 ± 20	388 ± 22	
Basophils/ Absolute	879± 78	80 ± 55	

*(P<0.05).

Table 4: The Indices of Clotting Factors of Diseased Buffalo Calves with MCF and the Control Group

parameters	Control group n=25	Diseased buffalo calves n = 66	Significance
Total platelet count x10^3	578.41 ± 78.45	361.91 ± 65.95*	(P<0.05)
Platelet volume /fl	11.123 ± 6.16	17.12 ± 3.76*	
Platelet distribution width %	14.678 ± 1.62	23.77 ± 7.65*	
Fibrinogen time /sec.	18.89± 2.91	26.25 ± 6.19*	
Prothrombin time /sec.	14.18 ± 1.71	31.86 ± 6.91*	
Activated partial thromboplastin time/sec/	51.55± 5.32	77.66± 11.18*	

*(P<0.05).

Table 5: Biochemical Analysis of Diseased Buffalo Calves with MCF and the Control Group

parameters	Control group n=25	Diseased buffalo calves n = 66	Significance
Glucose mg/dl	90±4.87	62± 7.86*	(P<0.05)
Total protein g/dl	6.34±1.66	4.65± 1.56*	
BUN mg/ dl	30.67± 2.76	71.55± 8.21*	
Creatine kinase U/ L	99.87±3.88	155.76± 7.87*	
Alkaline phosphatase U/L	31.34± 3.87	68.11± 8.65*	

*(P<0.05).

kinase, and alkaline phosphatase significantly increased (P<0.05) in infected buffalo calves with MCF than in the control group Table **5**.

DISCUSSION

Malignant Catarrhal Fever is a serious, often fatal disease affecting cattle, buffaloes, and other cloven-hoofed animals, causing significant economic losses and should not be overlooked [1, 15].

In this study, MCF diagnosis was confirmed by PCR, which provides higher sensitivity and specificity than conventional serological and cytological methods. PCR amplifies specific viral DNA sequences, making it the preferred molecular method for detecting the MCF virus [16].

The high nested-PCR positivity rate among clinically suspected buffalo calves underscores the reliability of molecular diagnosis for OvHV-2 in field settings. PCR remains the gold standard for confirming SA-MCF because virus isolation is impractical [11]. Negative results in some suspected cases may reflect low viral load, suboptimal sampling timing, DNA degradation, or other diseases with similar clinical signs [6]. Therefore, accurate diagnosis requires combining clinical evaluation with molecular testing.

Phylogenetic analysis further confirmed that all local sequences (LC918981.1, LC918982.1, LC918983.1, and LC918980.1) clustered within the OvHV-2 clade, supporting their identification as SA-MCF viruses. This supports the use of the DNA polymerase gene for species-level identification, although its conservation limits its utility for detailed transmission tracing [11, 17-19].

Among the local isolates, LC918981.1 (HNH2) clustered near reference strains HM216456.1, HM216460.1, and HM216472.1, indicating affiliation with a conserved global OvHV-2 lineage while representing a distinct local Basra variant within the broader SA-OvHV-2 group. LC918982.1 (HNH3) showed a different placement, forming a sister branch to a subcluster containing HM216464.1, EU078708.1, HNH4, and HNH1, suggesting limited genetic heterogeneity among Basra isolates rather than a single uniform sequence type. The closest relationship was observed between LC918983.1 (HNH4) and LC918980.1 (HNH1), which formed a tight sister pair, indicating a recent common ancestor and supporting local co-circulation of closely related OvHV-2 variants rather than repeated detection of an identical strain. Overall, all isolates grouped within the classical OvHV-2 clade, confirming their role in the SA-MCF cycle and consistent with previous Iraqi and international reports of OvHV-2 infection in buffalo and other susceptible hosts.

Clinical Manifestations

Diseased buffalo calves exhibit acute clinical manifestations, as reported in the literature [1, 12, 20]. The increased body temperature may reflect elevated levels of pyrogens released from lysed cells, followed by stimulation of thermoregulatory centers that trigger fever crises [21]. On the other hand, Lymphadenopathy, or swollen lymph nodes, results from cellular proliferation within the node, indicating reactive hyperplasia in response to infection, from infiltration by abnormal cells such as macrophages that may be laden with lipids, or from inflammatory responses that cause an influx of immune cells. The lymph node's role as a filter in the lymphatic system exposes it to antigens and cells, triggering immune responses or allowing migrating cells to accumulate, leading to enlargement [1, 22].

In the present study, anorexia and abstention from eating were found in 95.4% of diseased buffalo calves. Reduced appetite in most viral infections may be attributed to decreased ability to taste and smell foods [20]. As changes in appetite are associated with alterations in the concentrations of amino acid-derived neurotransmitters in the brain, further decreased feed intake at high body temperature could explain the depression observed in diseased calves [1, 21].

It was shown that abnormal saliva ropiness in cattle and buffalo, which causes a thick, stringy texture, often results from increased mucin content or altered saliva composition, and can be a sign of underlying issues, oral irritations that lead to excessive salivation. The increased ropiness is not a specific disease but a symptom indicating a condition that causes excessive fluid production and changes in the saliva's physical properties [23].

Unilateral ocular opacity and lacrimation observed in diseased buffalo calves may result from an inflammatory response within the cornea, causing edema (swelling) and cell damage that impair the cornea's transparency. The excessive tear production, or lacrimation, is the body's effort to clear irritants and protect the damaged eye [1, 12, 24]. Additionally,

diarrhea seen in diseased buffalo calves with MCF stems from a combination of malabsorptive, hypersecretory, and inflammatory processes. The virus can attach to immune cells such as lymphocytes and macrophages, leading to immunosuppression and damage to lymphoid tissue. The resulting intestinal damage, including villous atrophy and inflammation, hampers nutrient absorption and disrupts water and electrolyte balance, causing severe dehydration, acidemia, and death [12, 25].

It has been documented that the MCF virus can affect the white blood system. In Malignant Catarrhal Fever in cattle, lymphocytosis is caused by viral proteins that induce the survival and multiplication of latently infected lymphocytes, primarily CD8+ T cells, and potentially reprogram them into activated, proliferating cells. These viral mechanisms protect infected lymphocytes from programmed cell death (apoptosis), increasing their numbers and contributing to the systemic lymphoproliferation that characterizes MCF [13, 26].

It was thought that MCF might alter clotting factor responses in diseased animals [12, 27]. Where the coagulation system's mechanism can be altered, leading to the development of disseminated intravascular coagulation (DIC). Moreover, hypocoagulation and hypercoagulation are predominant conditions associated with disseminated intravascular coagulation syndrome, which primarily depend on platelet aggregation speed at different stages of the disease [28]. Hereby, petechial and/or ecchymotic hemorrhages observed on the mucous membranes of diseased animals and in the internal organs of the carcass reflect a decreased platelet count resulting from the release of endogenous mediators, such as platelet-activating factor, in inflammatory disorders [27]. On the other hand, it has been shown that Hyperfibrinogenemia, a quantitative defect characterized by abnormally high fibrinogen levels (as observed in the present study), is typically an acquired condition resulting from inflammation, infection (especially in the acute or advanced stages), and liver disease. Less commonly, it can also be a quantitative defect found in cases of congenital hypofibrinogenemia [29].

In the present study, a significant decrease in glucose levels (hypoglycemia) was observed in diseased buffalo calves with MCF, likely due to reduced glucose intake from decreased feeding (abstaining from eating) and impaired nutrient absorption caused by intestinal damage. Additionally, increased glucose utilization by the immune system to combat the infection and impaired liver function, which hinders gluconeogenesis, contribute to this drop. Severe organ damage, especially to the liver, and systemic inflammation further deplete glycogen stores and inhibit the body's ability to maintain blood sugar levels [1, 12, 30]. Moreover, diseased buffalo calves exhibited mild hypoproteinemia, as evidenced by a significant decrease in total protein levels compared with the control group. This may result from starvation, diarrhea, and other digestive issues affecting these animals, as shown by their refusal to eat. Fever and malabsorption also play important roles [31]. On the other hand, Khalphallah *et al.* [32] noted that hypoproteinemia results from decreased protein synthesis, as systemic inflammation impairs the liver's ability to synthesize proteins, including albumin. It can also result from increased protein loss, either through a protein-losing enteropathy (intestinal leakage) caused by viral damage and inflammation or via renal losses.

Boonperm *et al.* [33]. explained the significantly elevated BUN values in animals affected by MCF as pre-renal azotemia, resulting from reduced blood flow to the kidneys and decreased urea filtration. This can occur due to dehydration (common with fever and anorexia caused by viral disease), tissue catabolism that increases protein breakdown for energy, or when the infection infects a weakened animal, further increasing ammonia and urea synthesis. Moreover, direct renal damage from the virus itself is also possible [1, 12]. Additionally, increased creatine kinase (CK) in animals with MCF infections is primarily due to virus-induced muscle damage and inflammation, which leads to breakdown of muscle cell membranes and release of intracellular CK into the circulation. The virus directly harms muscle tissue, and the host's immune response can also contribute to inflammation and cellular disruption, leading to elevated serum CK levels indicative of muscle damage [25, 34]. Conversely, it has been suggested that, in viral infections, alkaline phosphatase (AP) can have both pro- and anti-inflammatory roles, primarily by dephosphorylating nucleotides and other inflammatory stimuli, thereby generating adenosine, an anti-inflammatory molecule. AP also modulates immune responses by degrading NETs (Neuroendocrine tumors), which are important in inflammation and autoimmunity, and by dephosphorylating inflammatory factors. In cattle and buffalo, viral infections can increase AP activity in specific organs and secretions, such as the liver and

nasal mucosa, in response to inflammation. However, its role in viral infections remains complex and not fully understood [1, 12].

CONCLUSIONS

The findings of this study confirm the ongoing circulation of malignant catarrhal fever (MCF) in southern Iraq and demonstrate its association with severe clinical signs and significant pathological changes in affected cattle. These results underscore the importance of early diagnosis, active surveillance, and separating susceptible animals from potential reservoir hosts, particularly sheep, to reduce disease transmission and minimize economic losses.

ETHICAL CONSIDERATIONS

This article considers all ethical principles. However, after scientific verification, the committee responsible for animal ethics at the University of Basrah's College of Veterinary Medicine's animal ethics committee gave the green light for this research to proceed, according to the college’s letter No. 93/37 dated 2025.

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CONFLICT OF INTEREST

The participants in this scientific work (authors) confirm that there is no conflict of interest of any kind.

AUTHORS' CONTRIBUTIONS

Authors reviewed and agreed on their individual contributions before and during the submission of their article. ALI Y. ATSHAN, the first author, was responsible for collecting and analyzing samples in the laboratory. Additionally, the second author (corresponding author), ALSAAD K.M., was responsible for the statistical analysis and for writing the entire article.

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DATA AVAILABILITY

All the data supporting the findings of this research study are available within the manuscript.

HUMAN AND ANIMAL RIGHTS

This scientific research was conducted under the supervision of the Human and Animal Rights Ethics Committee of the University of Basrah, College of Veterinary Medicine. Iraq, via its letter No.96/37/2025.

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