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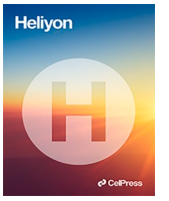

Research article

Molecular identification of different toxinogenic strains of *Clostridium perfringens* and histo-pathological observations of camels died of per-acute entero-toxaemia

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A R T I C L E I N F O

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A B S T R A C T

Enterotoxaemia is a severe disease caused by *Clostridium perfringens* and render high mortality and huge economic losses in livestock. However, scanty information and only few cases are reported about the presence and patho-physiology of enterotoxaemia in camels. The bacterium induces per-acute death in animals due to rapid production of different lethal toxins. The necropsy of camels (per-acute = 15, acute = 3) was conducted at 18 outbreaks of enterotoxaemia in camels in the desert area of Bahawalpur region. At necropsy, the serosal surfaces of visceral organs in the abdominal, peritoneal and thoracic cavities were found to have petechiation with severe congestion. Moreover, both the cut-sections of different visceral organs and the histo-pathological analysis revealed the pathological lesions in heart, lungs, kidneys, spleen, small and large intestines. Grossly, the kidneys were severely congested, hyperemic, swollen and softer in consistency. Under the microscope, different sections of kidneys indicated that the convulated and straight tubules were studded with erythrocytes. In the intestines, there were stunting fusion of crypts and villi. Similarly, various histo-pathological ailments were also observed in the heart, lungs and spleen. At blood agar, the collected samples showed beta hemolytic colonies of *C. perfringens* that appeared as medium sized rods microscopically and stained positively on Gram staining. Multiplex PCR revealed *C. perfringens* type A (α and β_2 genes) and D (epsilon gene) and the deaths were found to be significantly higher due to *C. perfringens* type D compared to those by *C. perfringens* type A. Hence, it has been concluded that enterotoxaemia in camel affects multiple organs and becomes fatal, if occurred due to *C. perfringens* type D.

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1. Introduction

In Southern Punjab, the desert area of Cholistan lies between longitudes 69°52′ to 75°24′E and latitudes 27°42′ 29°45′N and is part of the three districts (Bahawalpur, Rahim Yar Khan and Bahawalnagar). The livestock animals especially camel are the main cornerstones for livelihoods of the people of Cholistan. In Pakistan, the camel is mainly found in the sandy deserts of Thal, Thar and Cholistan [1]. With 21 breeds and estimated 1.1 million camels (Pakistan Economic Survey 2022–23, Finance Division Government of Pakistan, Islamabad), Pakistan is the 9th producer of camels in the world after Somalia, Sudan, Ethiopia, Niger, Mauritania, Chad, Kenya and Mali [2]. The camel is still the most economical and efficient animal thriving in the desert environment and its meat and milk are being used as a new source of food, to meet the rapidly increasing requirements [3]. Moreover, camel is also a potential source of bones, hide, hair and manure being used in different industries [1]. Since, the camel uniquely adapts to the hot environment, it contributes significantly to the food supply better than other domestic animals that are affected by heat and scarcity of feed and water in such harsh regions [4]. Regardless of the importance of camels in the livelihood of people of desert areas, poor husbandry practices, insufficient water bodies, decreased availability of fodder, hot and humid climatic conditions, seasonal migration of dairy herds and deficit animal health monitoring services like rapid and reliable diagnosis of different ailments along with inappropriate treatment facilities are not only the major issues for livestock farmers but also support the incidence of different diseases in the dairy animals [5]. Therefore, these factors decrease the efficiency of immune system and hence, favor the rapid multiplication and proliferation of various infectious agents and spread of different pathogens among the animals causing severe outbreaks of different diseases, especially entero-toxaemia [6]. However, there is a huge gap of knowledge regarding outbreaks and diagnosis of the causative agent of this disease in camels.

Entero-toxaemiais usually caused by different species of *Clostridium perfringens*; an anaerobic, sporulating and Gram-positive bacterium that causes per-acute and severe clinical signs of the disease, resulting in sudden death of animals in acute cases [7]. *C. perfringens* is categorized into seven toxino-types (A to G) on the basis of production of four major lethal toxins including alpha (cpα), beta (cpβ), epsilon (etx) and iota (itx), along with production of other toxins like perfringolysin, β_2 toxin and enterotoxin [8]. Among the various types of *C. perfringens*, type A is ubiquitous in nature and is normally present in the intestines of healthy animals like sheep, goat, cattle [9–13] and chicken [14,15]. Any aggression in the gut-associated lymphoid tissue and the residing microbiota, leads to an imbalance in the gastric defense mechanism and the immune complex, that ultimately promotes a bacterial dysbiosis and an intestinal inflammatory process by *C. perfringens*, either clinically or sub-clinically [16]. Consequently, ultimately *C. perfringens* causes the disease in cattle [9–11,17], sheep [11,18,19], goat [8,18,20,21], horse [11,22–23], dog [11], camel [13,24–26] and poultry [11,14,27].

There are only few reports of the disease in old [26,28] and New [29] world camels. Amongst these, the acute and sub-acute forms of entero-toxaemia have been reported in camels in Mongolia and these 2 outbreaks of the disease were also found to be predisposed by *Trypanosoma evansi* and Salmonella spp. leading to death of animals in the acute cases [28]. Similarly, *C. perfringens* type D was found in an outbreak in camel calves at Saudi Arabia causing acute catarrhal enteritis [26]. While, *C. perfringens* types C and D have been reported in llama at veterinary teaching hospital of Colorado State University [29]. In the current study, we have observed about 18 outbreaks of enterotoxaemia in camels in the tropical and sub-tropical climatic conditions. Out of these outbreaks, a total of 14 outbreaks were occurred in the camels kept with Cholistani cattle and buffaloes at different livestock farms located in the Bahawalpur region, while the other 4 outbreaks had been occurred at the camel herds kept at the Cholistan desert. All the camels were usually kept on sandy floor under similar husbandry practices and were offered lush green fodder and concentrate mixed ration and were also brought-out for grazing on daily basis. The animals were observed daily for the visible physical signs of any disease and the sick animals showing abnormal symptoms were immediately placed separately and treated with anti-pyretic, antibiotics, anti-allergics and multi-vitamins. For the camels died of entero-toxaemia, the post-mortem was conducted, the necropsy lesions were observed, the histo-pathology of different affected organs was analyzed and then the involvement of different toxigenic genes of clostridium was confirmed by molecular diagnosis.

2. Materials and methods

2.1. Collection of samples and histo-pathological observations

The necropsy was carried out on each camel within 1–2 h after the death. After gross examination, the tissue samples of kidneys, lungs, intestine, liver, spleen and heart were immediately collected and preserved by immersion in 10% neutral buffered formalin (pH = 7.3) for histo-pathological observations. All the collected tissues of 0.5–1.0 cm thick were processed by using paraffin sectioning technique and 4–5 μm thick histological sections were stained by Hematoxylin and Eosin staining technique, as previously described [30–33].

2.2. Bacterial isolation and identification

Intestinal tissue samples were collected from the diseased animals and stored at 4 °C till further processing. Initially, cooked meat broth media was used to enrich the intestinal samples for the presence of *C. perfringens*. After 24 h of anaerobic incubation at 37–42 °C in a CO_2 incubator, the inoculum was streaked on 5% Columbia sheep blood agar (Oxoid, CM0331) plates, anaerobically at 37 °C for further 24 h. Later on, the obtained colonies were gram stained for further identification [34].

Table 1
Oligonucleotide primers for detection of *C. perfringens* toxino-types.

Toxin gene	Primers	Primer sequence (5′-3′)	Product size
cpa (α-toxin)	CPA F	GCTAATGTTACTGCCGTTGA	324 bp
	CPA R	CCTCTGATACATCGTGTAAG	
cpb (β-toxin)	CPB F	GCGAATATGCTGAATCATCTA	195 bp
	CPB R	GCAGGAACATTAGTATATCTTC	
etx (ε-toxin)	ETX F	TGGGAACTTCGATACAAGCA	376 bp
	ETX R	AACTGCACTATAATTTCCTTTTCC	
iap (ι-toxin)	IA F	AATGGTCCTTTAAATAATCC	272 bp
	IA R	TTAGCAAATGCACTCATATT	
cpb_2 (β_2-toxin)	CPB2 F	AAATATGATCCTAACCAACAA	548 bp
	CPB2 R	CCAAATACTCTAATCGATGC	

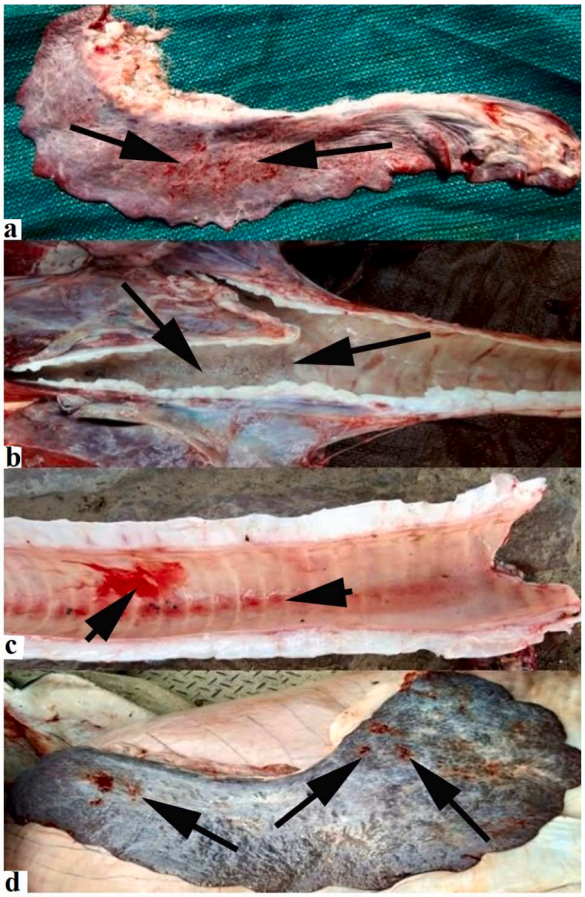

Fig. 1. Necropsy of spleen (a) showing severe congestion (arrow) and cut-section of trachea (b) that was found to be severely hypremic (arrow head) and contained frothy exudates (arrow). Spleen (d) was congested and showing extensive petechial hemorrhages (arrow heads) at serosal surfaces.

2.3. DNA extraction and quantification

The bacterial cells collected from the obtained pure colonies were mixed in sterile water and centrifuged at 6000×*g* for 4–5min. After centrifugation, the pellet was suspended in 200 μL of cold Tris EDTA buffer and then extraction of DNA was performed using EZ-10 Spin Column Extraction kit (Bio-Basic, Canada), as described earlier [35,36]. The quantity and purity of the obtained DNA was assessed by using NanoDrop ND-1000 spectrophotometer (Wilmington, NC) [37].

2.4. Molecular characterization via simple and multiplex PCR

For confirmation of bacterial isolates of *C. perfringens*, the isolated DNA from pure colonies was subjected to PCR using primers

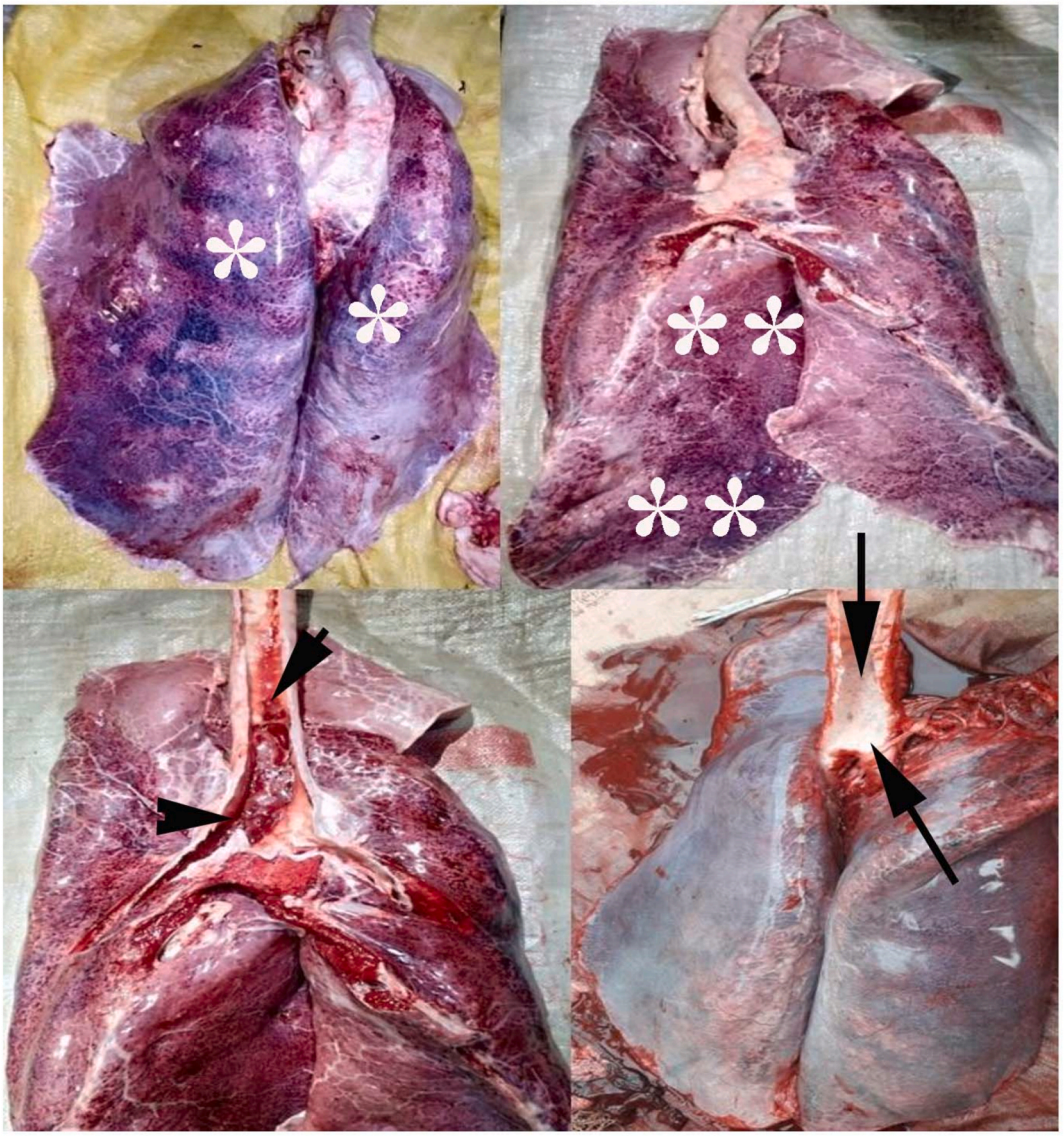

Fig. 2. Necropsy of lungs and cut-section of trachea of different camels died due to enterotoxaemia. The lungs were severely congested (**), darker in color, edematous, enlarged and exhibited consolidation (*). Extensive inflammatory exudate (arrow head) or frothy material was also observed at the bifurcation of the trachea (arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

specific for alpha gene (*cpa*), as previously described [37–39]. The confirmed isolates were then subjected to multiplex PCR [8] to determine the particular toxino-types involved in causing the disease. Briefly, the reaction mixture for mPCR contained taq polymerase (1.2U), PCR buffer (1×), $MgCl_2$ (4 mM), dNTPs (250 μM), forward and reverse primers (0.12 μM each) for alpha (α), beta (β), epsilon (ε), iota (i) and β_2 genes (Table 1) and DNA (1.5–2 μL; 100–200 ng/μL). The reaction conditions were: pre-denaturation at 95 °C for 10 min (one cycle), denaturation at 94 °C for 45 s, annealing at 55 °C for 90 s, extension at 72 °C for 90 s for a duration of 35 cycles, followed by final extension at 72 °C for 10 min. The amplified PCR products were run on 1.5–2.0% agarose gel in a gel electrophoresis system [35,40] and seen under UV light source by using Alpha Imager Mini Imaging System (CA, USA).

3. Results

3.1. Occurrence of the disease and clinical picture

The history of the infection revealed that per acute (15) and acute (3) deaths were occurred in the camels. In the per-acute cases, no clinical signs were observed whereas different clinical ailments such as anorexia, fever, respiratory distress and watery diarrhea were

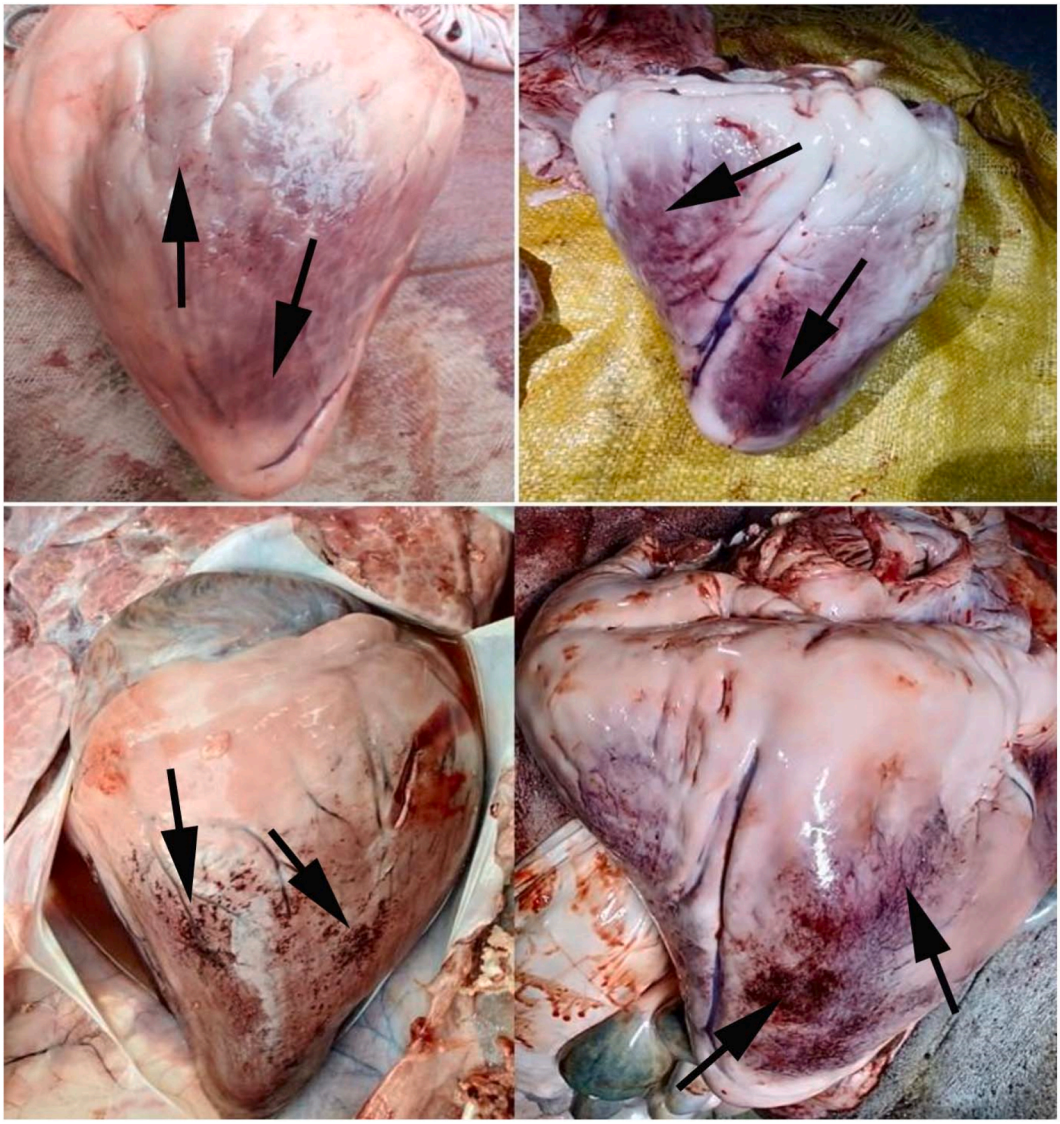

Fig. 3. Necropsy of heart of the camels died due to enterotoxaemia showing different gross pathological lesions such as hyperemic, congested and edematous myocardium, focal and multifocal petechial hemorrhages at serosal surfaces along with the presence of necrotic foci (arrow) at right and left ventricles.

observed in the acute cases.

3.2. Necropsy and post-mortum observations

Grossly, trachea was found to be hyperemic and contained frothy exudates in all the cases. The spleen exhibited congestion, having pin-point to ecchymosis and petechial hemorrhages at visceral and parietal serosal surfaces (Fig. 1). The lungs, in most of the cases, showed pleural adhesions and the cut-sections of the lungs contained protein-rich pleural exudate, especially at the junction of trachea and bronchi. The lungs appeared darker in color, severely congested, enlarged, having solid consistency and exhibited severe pulmonary edema (Fig. 2).

After de-skinning of the carcasses, abundant light yellow to straw color fluid was observed in the abdominal cavity and strands of fibrinous materials were observed on serosal membrane of small and large intestines. The ballooning of the intestines, multifocal ulcers, severe congestion, diffused hemorrhages and fibrino-necrotic enteritis were the prominent lesions recorded in the intestine of camels. The cut sections of small intestine showed thickened mucosa, hemorrhages, severe mucosal and serosal reddening and yellowish pseudo-membranes. Jejunum and ilium of small intestine contained abundant brown red watery and gelatinous contents with foul smelling. The large intestine was severely congested and showed ulcerative lesions. The heart in all cases, was congested and focal and multifocal petechial hemorrhages were observed at the serosal surfaces of the heart (Fig. 3). Sero-sanguineous pericardial

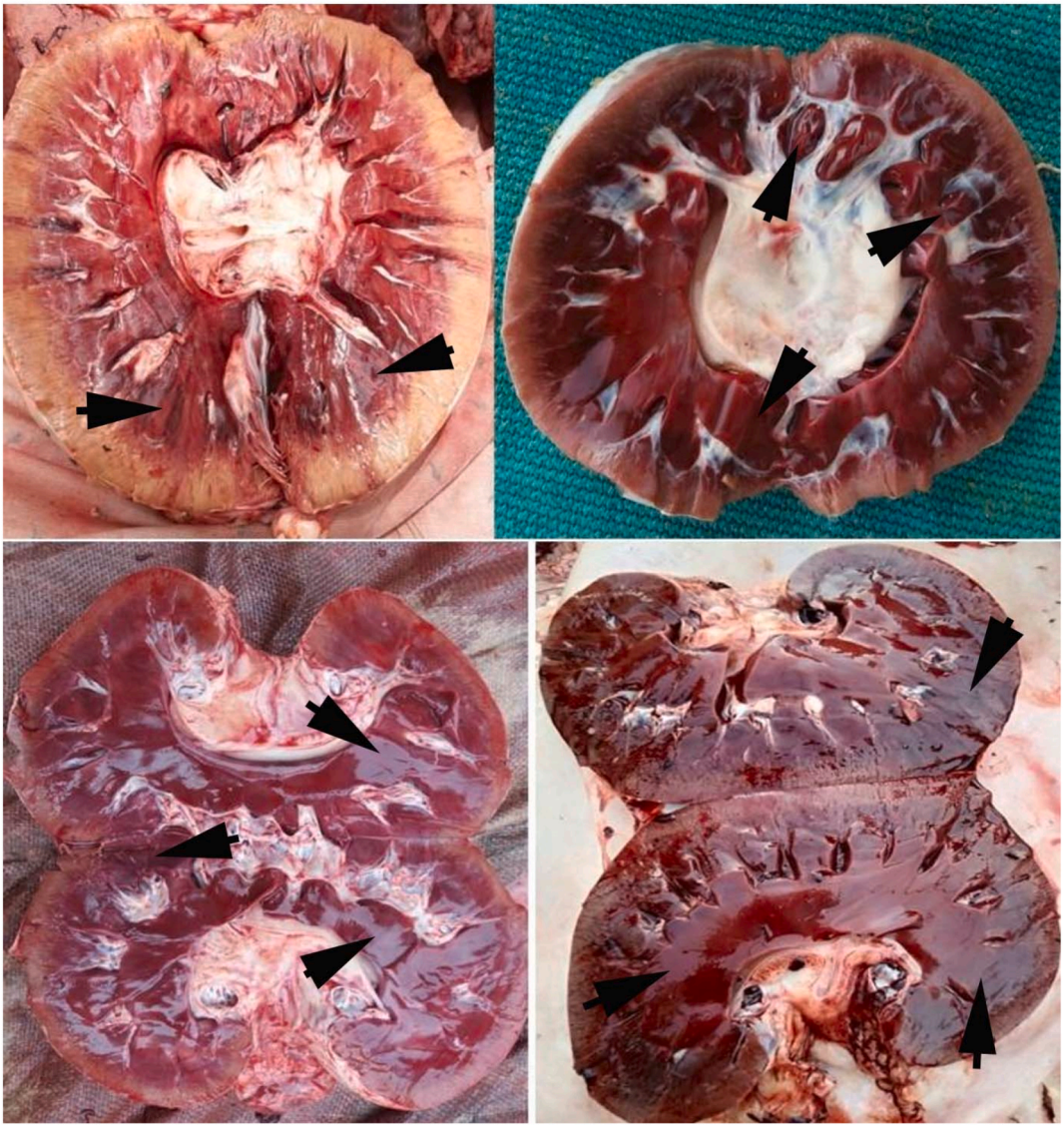

Fig. 4. Necropsy and cut-section of kidneys of the camels died due to enterotoxaemia. The kidneys were consolidated and darker in color. In the cut sections, kidneys exhibited severe edema, congestion and hyperemia (arrow head). There observed diffused hemorrhages both at mucosal and serosal surfaces. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

fluid was the characteristic lesion observed in all the cases at the time of necropsy. The heart contained clotted blood in both right and left ventricles, the myocardium was found to be hyperemic and congested along with focal and multifocal petechial hemorrhages and necrotic foci at the serosal surfaces. Kidneys in most of the cases were consolidated and darker in color and the cut-sections exhibited severe edema, congestion and hyperemia and there found diffused hemorrhages both at mucosal and serosal surfaces of the kidneys (Fig. 4).

3.3. Histo-pathological observations

At microscopic observations, the lungs of all the cases showed severe congestion, atelectasis, emphysema, broncho-pneumonia, interstitial edema and hemorrhages. In sub-acute cases, the lungs of camels exhibited extensive microscopic changes like broncho-interstitial pneumonia, hemorrhages, fibrinous exudate, embolic pneumonia, eosinophilic proteinaceous material and inflammatory exudate within the alveoli, bronchi and bronchioles (Fig. 5). Histological observations of different sections of spleen of different camels exhibited lymphoid depletion, congestion, hemorrhages and necrosis of white and red pulp. The histo-pathological analysis of intestinal sections showed degeneration and necrosis of villi, congestion, sub-mucosal edema and hemorrhages in the lamina propria and sub-mucosa (Fig. 6). In few sub-acute cases, fusion of villi, degeneration of crypts of villi, necrosis and detachment of epithelium of villi

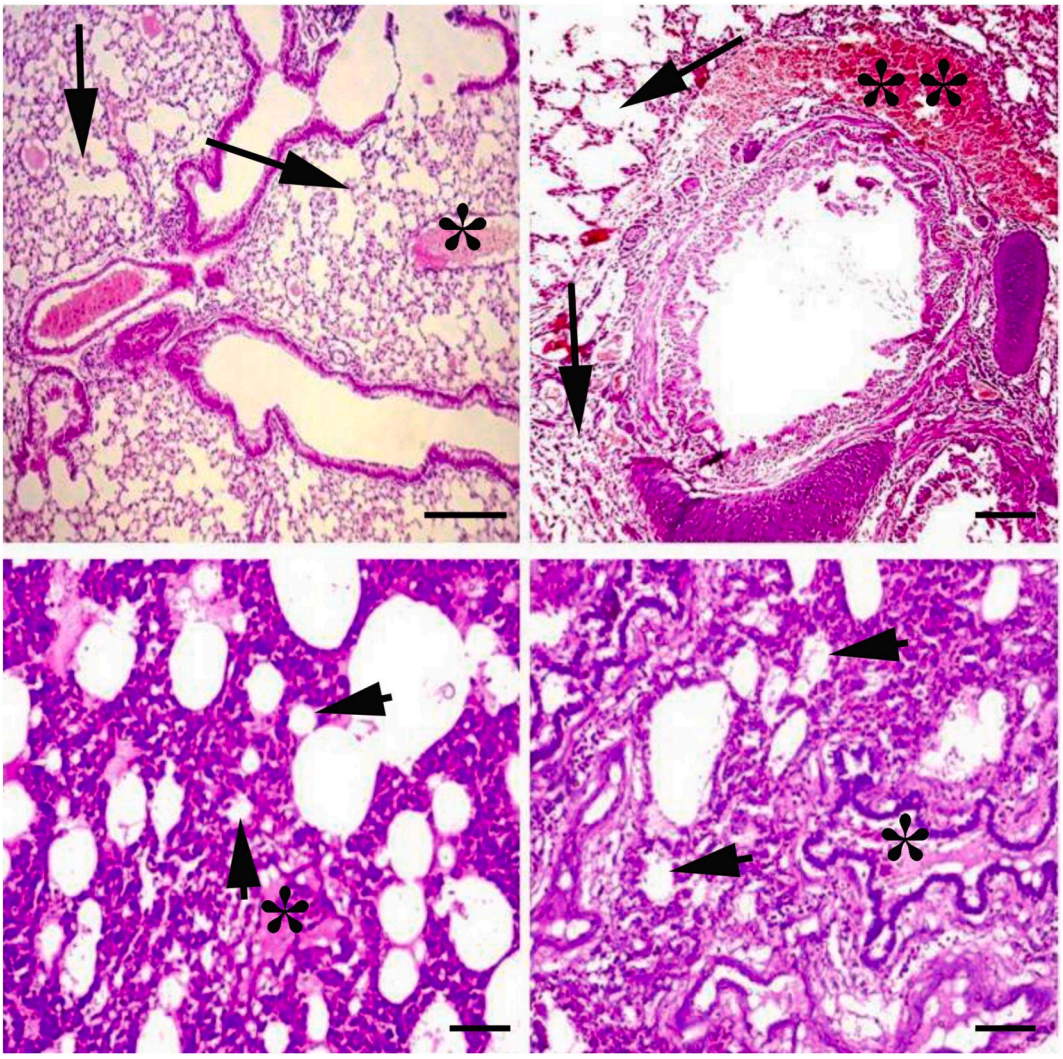

Fig. 5. Histo-pathological observations of lungs of the camel died due to enterotoxaemia. The lungs showed severe congestion, atelectasis (arrow head), broncho-pneumonia, interstitial edema (*) and hemorrhages in all cases. The lungs exhibited extensive hemorrhages (**), broncho-interstitial pneumonia (arrow), fibrinous exudate, eosinophilic proteinaceous material and inflammatory exudate within the alveoli, bronchi and bronchioles. Hematoxylin and Eosin staining, Scale bar = 100 μm.

and increased inter-cellular spaces were observed in both small and large intestines. The mucosa and sub-mucosa of intestines were diffusely infiltrated by inflammatory cells especially neutrophils (Fig. 6). Microscopic observations of heart revealed coagulative necrosis, edema, hemorrhages, myocarditis, cellular infiltration, congestion, necro-hemorrhagic myocarditis and degeneration of the cardiac myocytes (Fig. 7). Histo-pathological analysis of different sections of kidneys of camels in acute and sub-acute cases exhibited necrosis of renal tubular epithelial cells, congestion, increased urinary spaces, necrosis of renal tubules, hemorrhages and severe degeneration of glomeruli and of renal tubular epithelium (Fig. 8). Moreover, severe congestion, hemorrhages, degeneration, increased sinusoidal spaces, coagulative necrosis and neutrophilic hepatitis were observed in the hepatic sections of different animals died due to enterotoxaemia (Fig. 9).

3.4. Bacterial isolation and identification

The blood agar plates after 24 h showed beta hemolytic isolated colonies which were confirmed morphologically as *C. perfringens*. Microscopically, gram positive medium sized rods were observed after routine Gram staining procedure.

3.5. Molecular toxino-typing of isolates

All isolates were screened to confirm the involvement of particular toxino-type of clostridium causing sudden deaths in the affected

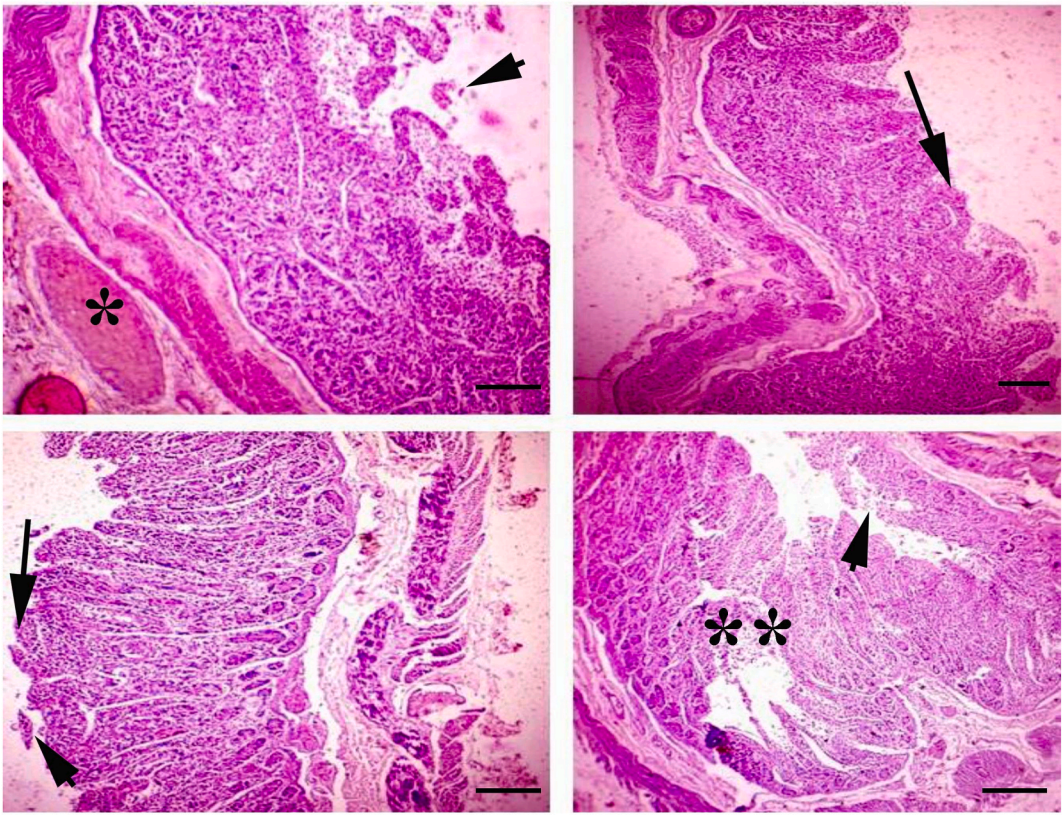

Fig. 6. Histo-pathological observations of intestine of the camels died due to enterotoxaemia. At histo-pathology, the intestinal sections showed degeneration (**), fusion of villi (arrow) and necrosis of villi (arrow head), congestion, edema and hemorrhage in lamina propria (*) and sub-mucosa. The mucosa and sub-mucosa of small and large intestines were diffusely infiltrated with inflammatory cells. Hematoxylin and Eosin staining, Scale bar = 100 μm.

camels. The specific bands of alpha + epsilon (n = 8) toxin genes confirmed the presence of *C. perfringens* type D while bands of alpha (n = 3) and alpha + beta 2 (n = 7) genes depicted the presence of *C. perfringens* type A. None of the isolates were found to be of types B, C and E (Table 2, Fig. 10).

4. Discussion

In Cholistan, dense population of camels has been reared since decades and are mainly used for milk, meat, transport and draught purposes. Infectious diseases are the main threat to the health and production of camels in the region and among these diseases, enterotoxaemia is the major disease caused by *clostridium* that causes huge economic losses to the livestock including camel. There is only limited information available in the literature about the cases of enterotoxaemia in camels [26,28,29] and for the first time, here we have described a complete picture of the per-acute deaths of camels died of enterotoxaemia, their necropsy lesions, histopathology of different visceral organs and molecular diagnosis of different toxinogenic genes involved in the infection.

The outbreaks reported in our study had been found to be caused by *C. perfringens* type A and D. Since, type A is usually present in the intestines of healthy sheep, goat, cattle, camel [9–13] and poultry [14,15], hence, this type can cause the infection in these animals at any time, due to inappropriate immune system caused by environmental stress or poor management practices. Moreover, type D of the bacterium is also previously reported to be involved in causing enterotoxaemia in sheep, goats, buffaloes, cattle and camels [8,41]. Similarly, type A has also been reported to cause per-acute and acute enterotoxaemia in breeding and racing camels [13] while all the four (A, B, C and D) types, with predominantly type A (75.2%), have been found to cause enterotoxaemia along with diarrhea in young and adult camels [24,25]. Among the reported cases of the disease in camel (26,28–29], the sub-acute forms of enterotoxaemia and death in acute cases have been caused by *C. perfringens* types C and D in Mongolia [28], while type D was isolated from the camel calves at Saudi Arabia causing acute catarrhal enteritis [26] and types C and D have also been found in llama [29]. Hence, all these studies also report the presence of *C. perfringens* types A and D, as we have observed both these types in the current outbreaks.

It is quite well known that the history, clinical ailments, necropsy lesions and the histo-pathological investigations are of vital and reliable tools for the presumptive diagnosis of the diseases in animals [8]. However, the confirmation of different toxigenic strains and involvement of related toxinogenic genes require isolation and characterization using standard laboratory techniques. Different clinical outcomes of the acute cases of enterotoxaemia include chocolate colored urine, opisthotonus, depression, anorexic, head down

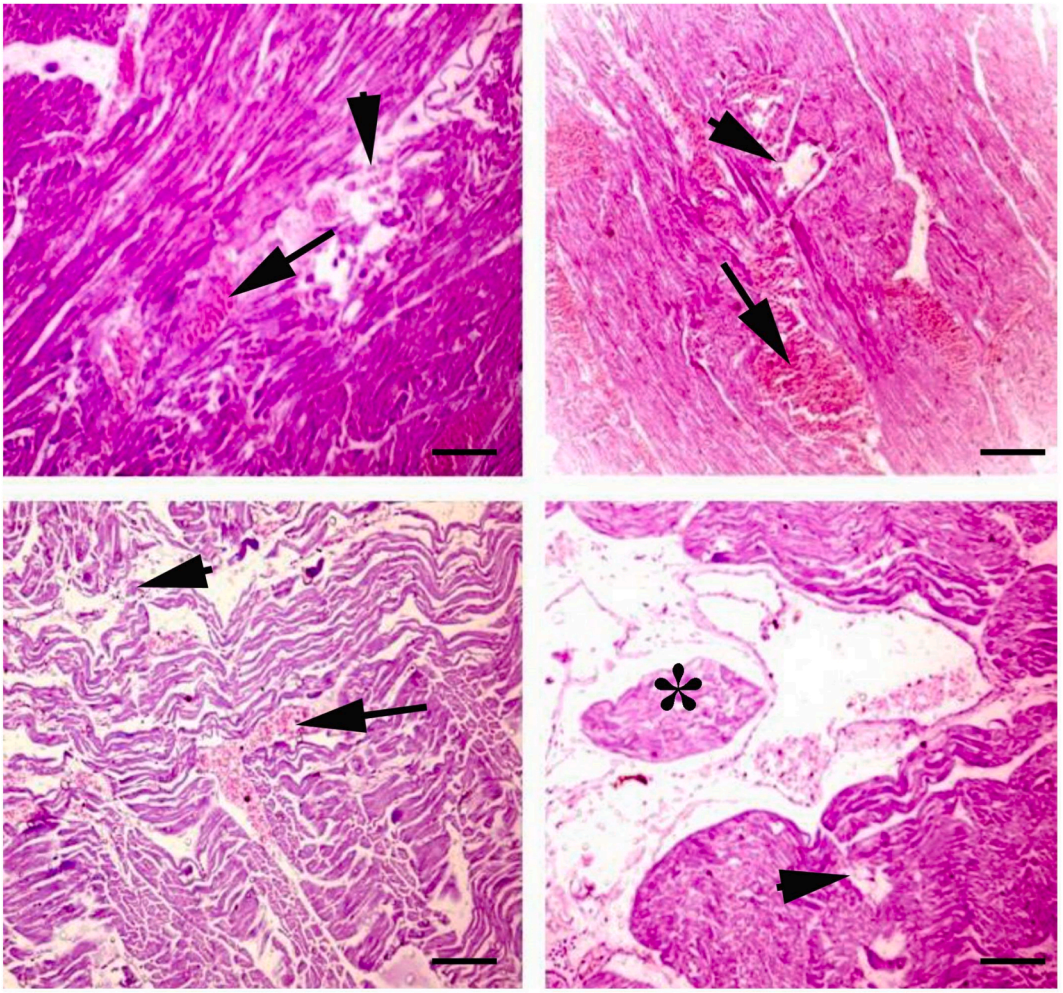

Fig. 7. Histo-pathological observations of heart of the camel died due to enterotoxaemia. Histologically, the heart revealed coagulative necrosis, edema, hemorrhages (arrow), myocarditis, cellular infiltration, congestion alongwith degeneration and breakdown of the cardiac myofibers (arrow head). Hematoxylin and Eosin staining, Scale bar = 250 μm.

and staying towards corners caused by clostridium have been observed in domestic [cattle: 9,42, sheep and goat: 8,18,20,21,43,44] and wild [45–48] animals. Moreover, in some cases, the camels showed extension of legs and a stretched neck. These clinical disorders might be related to effects of bacterial toxins on the brain causing neuronal degeneration [49]. Previously, it has also been reported that the haemorrhagic lesions on small intestine are regarded as suitable lesions for the exact diagnosis of enterotoxaemia while counting of *C. perfringens* in intestinal passages cannot be solely considered and advised for discriminative diagnosis of this disease [50]. Similarly, different gross lesions like thickening of intestine and multifocal to segmental hemorrhages at small intestinal serosa and necro-hemorrhagic enteritis have also been examined in horses due to *C. perfringens* [22].

Previously, the detailed pathological lesions like intestinal ballooning, myocardial hemorrhages, congested and edematous lungs, hydro-pericardium, hydro-thorax, congested and hyperemic liver, petechial hemorrhages on spleen and swollen, soft and hemorrhagic kidneys had been found on necropsy in the goats infected with enterotoxaemia [8]. Similarly, intestinal ballooning and blood-stained intestinal contents that lead to bloody diarrhea in camels died of enterotoxaemia have also been reported [24]. Moreover, acute catarrhal enteritis and acute myocardial degeneration were observed in camels due to *C. perfringens* [26] and the bacterium was also recovered from the intestinal contents of camels died from per-acute and acute enterotoxaemia [13]. In the same context, different pathological lesions like hydropericardium, myocardial hemorrhages, swollen soft kidneys, edematous and congested lungs, intestinal ballooning and congested liver have also been reported in small ruminants [46,51]. However, no such data were previously available regarding the diagnosis of enterotoxaemia in camels kept under desert environment and our study is the first detailed report of the clinical signs along with histo-pathological lesions in different visceral organs.

Earlier studies are also of the view that histo-pathological changes are reliable and useful tools for the diagnosis of enterotoxaemia [46,51]. Hence, different characteristic and prominent microscopic histo-pathological lesions in various visceral organs like intestine [Camel: 26; Cattle: 17; Sheep: 44,52; Goat: 8,44], stomach [Cattle: 17], liver [Camel: 26], lungs [Cattle: 17; Goat: 8], kidneys [Camel:

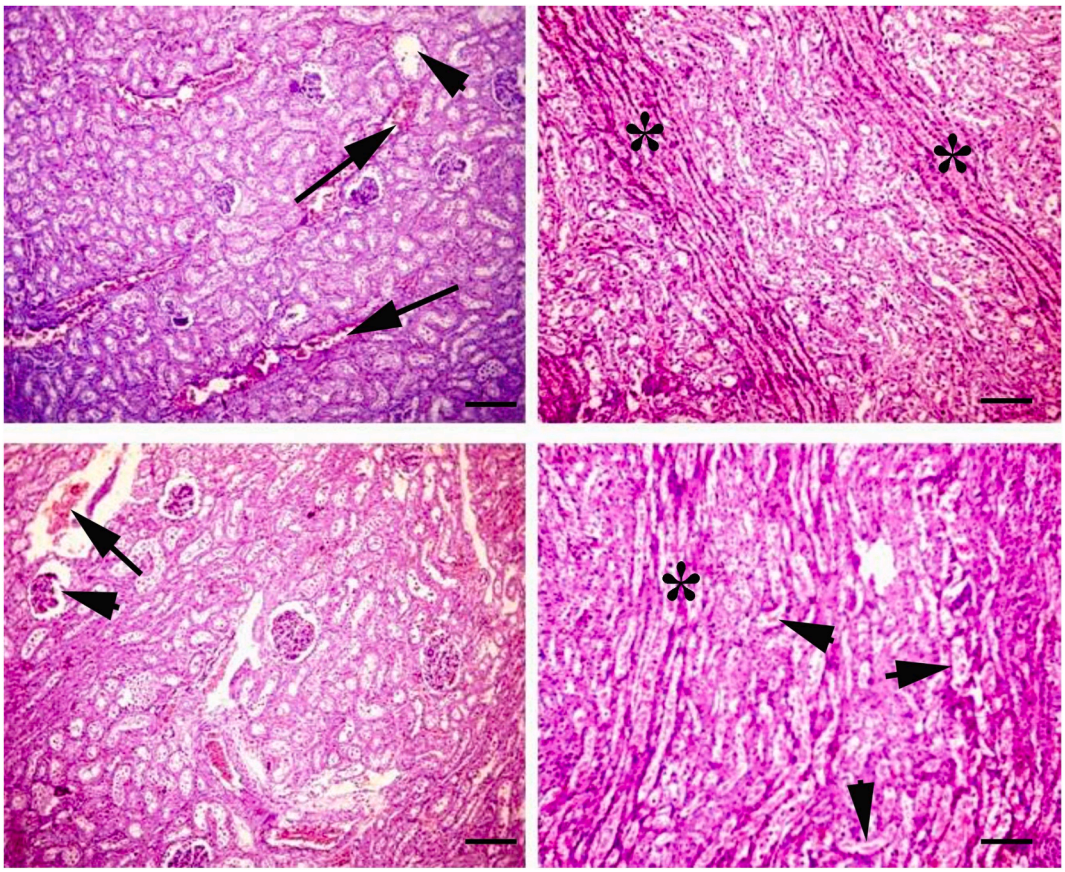

Fig. 8. Histo-pathological observations of kidneys of the camel died due to enterotoxaemia. At histo-pathological analyses, the kidneys exhibited necrosis of renal tubular epithelial cells, congestion, increased urinary spaces, necrosis of renal tubules (arrow head), hemorrhages (arrow) and severe degeneration of glomeruli (*) and of renal tubular epithelium. Hematoxylin and Eosin staining, Scale bar = 250 μm.

26; Cattle: 17; Goat: 8], heart [Camel: 26; Cattle: 17] and brain [Cattle: 17; Sheep: 44,52] have been reported in the animals affected with enterotoxaemia. The histo-pathology of stomach and intestine depicted the characteristic features of the disease caused by *C. perfringens* type A was isolated along with *Trypanosoma evansi* and Salmonella spp. [28]. We also have described characteristic histo-pathological lesions like hemorrhages, congestion and presence of fibrinous exudate in the lungs, intestine, liver and kidney, broncho-interstitial pneumonia, degeneration and coagulative necrosis of intestinal villi, cardiac myocytes, renal glomeruli and tubular epithelial and hepatocytes and infiltration of inflammatory cells in the cardiac, hepatic and pulmonary interstitial tissues. These microscopic observations in correlation with clinical necropsy picture lead us to the definitive diagnosis of enterotoxaemia caused by Clostridial species.

In our study, the bacterium isolated from different organs like compartments of stomach and intestines of breeding and racing camels suffering from per-acute and acute enterotoxaemias has been found to be *C. perfringens* type A. On the basis of involvement of different toxigenic genes in the diagnosis of enterotoxaemia due to *C. perfringens* in animals, it has been investigated that epsilon toxin has an involvement of 56% in the pathological alterations in bovines [17]. It has also been recorded that β_2 and ε-toxin (epsilon) are strongly associated toxins produced by *C. perfringens* type D causing intestinal disorders in goats and play a crucial role in causing enterotoxaemia [53]. Enteritis, abomasitis and necrosis of abomasal or of intestinal mucosa have been observed due to clostridium bacteria in cattle calves [54]. Moreover, a study has investigated that in horses different genes including beta2-toxin (cpβ_2) gene are of vital importance for the detection of toxigenic *C. perfringens* causing intestinal disorders and pathogenesis of typhlocolitis [23]. The presence of different toxin genes of type A, B, C and D have been confirmed by PCR in sheep and goats [19,55] and the isolates of types A and D harboring cpe and cpβ_2 toxic genes causing intestinal disorders have been identified using molecular techniques [55]. In this context, initially the bacterial species was identified on the basis of simple PCR and then multiplex PCR was conducted to identify and further confirm different toxino-types of the bacterium, by using different primers specific for these toxino-types. Hence, the presence of *C. perfringens* type A (cpα, cpβ_2) along with type D (etx) have been illustrated in the gel electrophoresis of multiplex PCR products of samples collected from the dead animals.

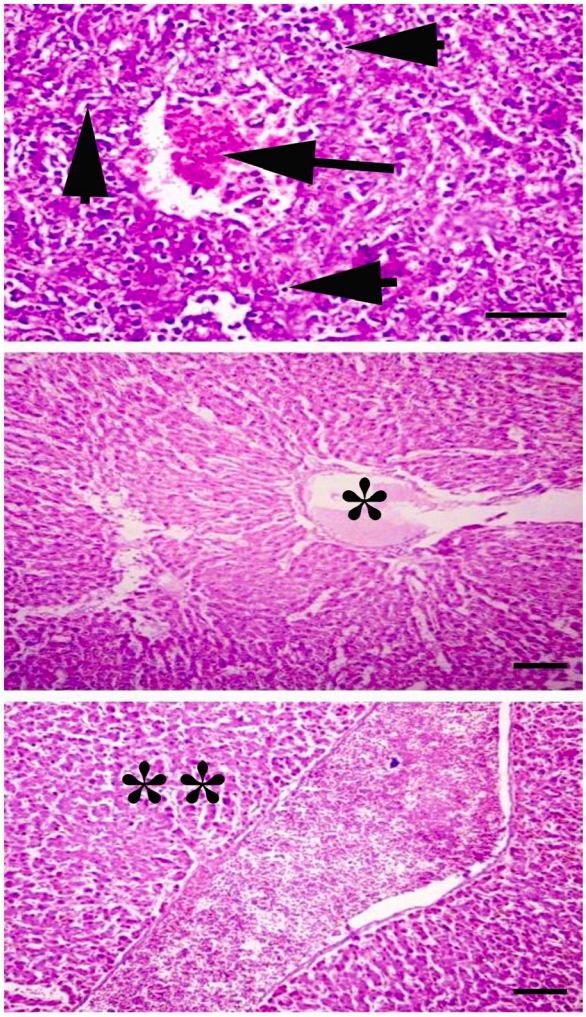

Fig. 9. Histo-pathological observations of liver of the camel died due to enterotoxaemia. Microscopically, there observed severe congestion, hemorrhages (arrow), edema (*), degenerated and increased sinusoidal spaces (**), coagulative necrosis (arrow head) and neutrophilic hepatitis in the hepatic histo-pathological sections. Hematoxylin and Eosin staining, Scale bar = 250 μm.

Table 2
Toxin types reported from enterotoxaemia outbreak in camels.

Types of *C. perfringens*	Toxins	Positive samples	
		Number	%
A	cpα	3	16.67
A	cpα+cpβ$_2$	7	38.89
D	cpα+etx	8	44.44

C. perfringens type A: cpα+cpβ$_2$, *C. perfringens* type D: etx

5. Conclusions

To the best of our knowledge, the current study is the first detailed and comprehensive investigation of this infection in camel. The study described the per-acute deaths of camels died of enterotoxaemia, characteristic necropsy lesions in trachea, spleen, liver, heart, lungs, intestine and kidneys on cut-sections of the organs and severe histo-pathological alterations on microscopic observations in these organs depicted the presence of clostridial bacteria. The results of beta hemolytic colonies on blood agar, Gram staining and PCR revealed that the isolated bacterium has been found to be clostridium, while multiplex PCR further confirmed the involved toxinotypes as A and D. Hence, we have confirmed that this bacterium is the causative agent of the severe infection and ultimately death of the desert camels due to per-acute cases of enterotoxaemia.

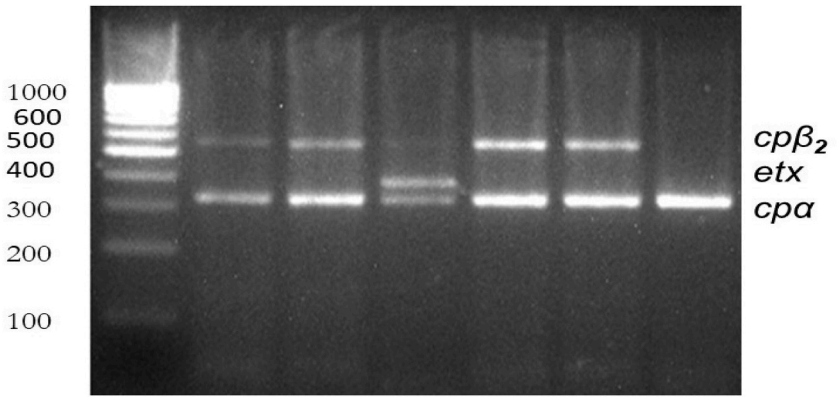

Fig. 10. Gel electrophoresis of the multiplex PCR products depicting different toxino-genes (*cpα, etx, cpβ2* genes) of *C. perfringens*. Lane 1: DNA molecular weight marker; lanes 2–3, 5–6: *C. perfringens* type A (*cpα, cpβ2*); lane 4: *C. perfringens* type D (*etx*); lane 7: *C. perfringens* type A (*cpα*).

Data availability statement

All the data for this study will be available from the corresponding author upon request.

CRediT authorship contribution statement

Hafiz Muhammad Ali: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Shujaat Hussain:** Writing – review & editing, Methodology, Formal analysis. **Muhammad Zishan Ahmad:** Writing – review & editing, Formal analysis. **Abu Baker Siddique:** Writing – review & editing, Investigation. **Sultan Ali:** Writing – review & editing, Data curation. **Mudassar Mohiuddin:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Muhammad Ehsan:** Writing – review & editing, Resources, Funding acquisition. **Muhammad Nadeem:** Writing – review & editing, Validation. **Abdul Qayyum:** Writing – review & editing, Investigation, Data curation. **Riaz Hussain:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Data curation, Conceptualization. **Iahtasham Khan:** Writing – review & editing, Validation, Funding acquisition. **Dunia A. Al-Farraj:** Writing – review & editing, Validation, Funding acquisition. **Enshad Alzaidi:** Writing – review & editing, Validation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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