Research Article

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Isolation and Genotyping Study of *Clostridium Perfringens* From Broiler Farms Infected with Necrotic Enteritis in Sulaimania Province

Sazan Qadr Amin[1], Nawzad Rasheed Abdulrahman[1*] and Sadat Abdulla Aziz[2]

[1]Department of Anatomy and Histopathology, College of Veterinary Medicine, University of Sulaimani, Kurdistan Region, Iraq.

[2]Department of Microbiology, College of Veterinary Medicine, University of Sulaimani, Kurdistan Region, Iraq

*Corresponding author: nawz_ra@yahoo.com

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Abstract

The study was conducted to isolate and toxinotype the suspected cases of *Clostridium perfringens* infections of broiler farms in Sulaimania province. A total of 108 samples were collected from intestinal contents, mucosal scraping, and hemorrhagic lymphoid nodules from suspected cases of necrotic enteritis in broilers. The result of isolated and identified bacteria were revealed that 63 (58%) out of 108 samples were positive for C. *perfringens*. The results revealed that the isolates were only positive for alpha and beta2 toxin genes. Phylogenetic and DNA sequence analysis of *cpa* and *cpb2* gene showed that *cpa* genes were highly identical to isolates from broiler in Iran, poultry stool and broiler in Brazil, and blue calves in Belgium. While *cpb2* gene is closely related to the isolates of broiler in Iran, India and isolates of goat in Pakistan. The results indicated that the causative agent of necrotic enteritis in broiler farms in the region was mainly due to *C. perfringens* type A infection

Keywords: *Clostridium perfringens;* Characterization; Necrotic enteritis

عزل ودراسة التنميط الجيني لجرثومة المطثيات البرفنجية المعزولة من فروج اللحم المصاب بخمج الامعاء التنخري في محافظة السليمانية

الخلاصة

اجريت هذه الدراسة لعزل ومعرفة نوع الذيفان من الحالات المشتبه اصابتها بجرثومة المطثيات البرفرنجية من فروج اللحم في محافظة السليمانية. كان عدد عينات الدراسة 108 عينة من محتوى الامعاء ومسحة الطبقة المخاطية للامعاء والعقيدات اللمفاوية من الحالات المشتبه اصابتها بخمج الامعاء التنخري في فروج اللحم المفحوصة من اصل 108 عينة مفحوصة كانت 63 عينة (58%) موجبة ومصابة بجرثومة المطثيات البرفرنجية. وكشفت النتائج أن العزلات كانت إيجابية فقط لجينات الذيفانين ألفا وبيتا 2. وكانت تحصيلة التحليل الجيني للجينات cpa وcpb2 أن الجين cpa مشابه لجينات الجرثومة المعزولة من فروج اللحم في ايران والعجول في بلغاريا. في حين الجين cpb2 متشابه مع عزلات من الماعز في باكستان. بينت النتائج أن الجرثومة المسبب لالتهاب الأمعاء التنخري لفروج اللحم في الاقليم يرجع اساسا الى جرثومة المطثيات البرفرنجية A.

Introduction

Necrotic enteritis is the most common clostridial enteric disease in poultry, which typically occurs in broiler aged between two to six weeks (1) that caused by *Clostridium perfringens*, which is a Gram-positive, rod-shaped, spore forming, anaerobic bacteria. They are widely distributed in the environment and as a part of the normal gastrointestinal tract flora of pathogen (3). The organism has been classified traditionally into five toxinotypes (A- E) basis on the production of toxins including; alpha (α), beta (β), epsilon (ε) and iota (ι) (4). But this typing system was recently expanded to include two additional types, such as type F strains produce enterotoxin and alpha-toxin; type G strain produces alpha and NetB (5). *C. perfringens* is responsible for many histotoxic and enterotoxic

diseases in humans and animals (6).

In poultry, *C. perfringens,* especially type A and type C, can cause both clinical and subclinical form of necrotic enteritis (7). The coccidial pathogen is the most important known predisposing factor that enhances the induction of necrotic enteritis by damaging the intestinal epithelium, allowing *C. perfringens* to penetrate and replicate rapidly to produce sufficient amount of the toxins that causes the disease (8.9). In addition, some dietary components such as fish meal, high level of indigestible-non-starch polysaccharides, dysbacteriosis although have been widely accepted as predisposing factors (10).

There was not any data about the type of the *C. perfringens* that caused necrotic enteritis in broiler farms in Kurdistan region. Accordingly, the aims of the study were to isolate and toxinotype the suspected cases of *Clostridium perfringens* in broiler farms, basis on the presence or absence of the major and the minor toxin genes, including alpha (*cpa*), beta (*cpb*), epsilon (*etx*), and iota (*itx*) toxins, beta2, necrotic enteritis toxin B (*netB*), using selective medium, multiplex and uniplex PCR.

Materials and methods

Samples collection:

The samples from intestinal content, mucosal scraping, and hemorrhagic lymphoid tissue were aseptically collected from 108 diseased broiler aged between two to six weeks from 18 farms in Sulaimania province from August 2018 to May 2019. The samples were collected from farms where the birds had not been taken any antibiotic supplementation for about 72 hrs before sampling. Diseased broiler were characterized by having suspected clinical signs of necrotic enteritis, including depression, ruffled feathers, and diarrhea. The samples were taken from most affected parts of the intestines and collected in sterile plastic bags and processed immediately for isolation and identification of *C. perfringens*

Isolation and identification *C. perfringens*:

The samples directly inoculated into tubes that contained freshly prepared cooked meat broth and incubated at 37°C for 24 hrs in an anaerobic jar (BD company, U.S.A) with gaspacks (Thermo scientific, U.S.A) were used to create the anaerobic condition. A loopful of inoculated fluid medium was streaked on sheep blood agar (Himedia, India) supplied with cycloserine (400 mg/1L), at 37°C for 24 hrs under anaerobic conditions. For the purpose of bacterial purification, the single bacterial colonies were picked up and inoculated into tryptose sulfite cycloserine agar (Quelab, Canada) with and without egg yolk, which was incubated at 37°C for 24 hrs under strict anaerobic condition. The isolated colonies were further characterized by looking at the morphology of the bacteria using Gram stain.

DNA extraction:

The boiling technique was used to extract DNA from all the isolates (11). Briefly, one well-isolated colony was selected, and suspended in 100µl of (Milli Q) water in an Eppendorf tube, incubated at 100°C for 15 min. using thermo-shaker (Biotech, Spain), and exposed to a pulsatile vortex (5-6 times/15 min.). Then cooled down and centrifuged (Maan lab, Sweden) at 14.000 × *g* for 10 min. Finally, the supernatant was kept, and the precipitate was discarded.

Multiplex and Uniplex PCR:

The multiplex PCR reaction was performed in a thermo-cycler (Techne ® Prime/ U.K) by adding 1µl of the primers mix (Table 1), 5 µl DNA into 10 µl PCR master mix cocktail (Genetbio Inc.). Then the volume was completed to the total reaction volume of 20 µl with ultrapure water. Uniplex PCR for detecting Beta2, NetB, and Alpha toxin gene was set in a

total reaction volume of 20 µl by mixing 1µl of the forward and reverse primers of each gene separately (Table 2), 5 µl DNA and the volume was completed with ultrapure water. The PCR amplification was as follow: initial denaturation was 95 °C for 5 min, denaturation at 95°C/30 sec, annealing at 55°C/30 sec, and extension at 72°C/1 min. The final extension was at 72°C/10 min. DNA bands were visualized on 1.5% agarose gel (Ingenius/ U.S.A).

DNA sequence analysis and Phylogenic Tree:

Both sense and antisense strands of the amplified DNA sequences were sequenced using Sanger DNA sequencer (Macrogen Co., Korea). The obtained DNA sequences subjected to DNA analysis using Clustal Omega (Multiple Sequence Alignment) and NCBI nucleotide blast. Phylogram was created using MEGA-X software. The amino acid sequences and their corresponding codons were predicted using the ExPASy Server (https://web.expasy.org/translate/). The obtained DNA sequences of both alpha and beta-2 toxin genes recorded in the National Center for Biotechnology Information (NCBI) under different accession numbers, including MN224676, MN224677, MN224678, and MN224679 for alpha-toxin, and MN239885، MN239886 and MN239887 for beta-2 toxin genes.

Results and Discussion

Isolation and identification *C. perfringens*:

In this study, 63 (58 %) of the isolates out of 108 samples suspected cases were positive for *C. perfringens*. The isolation and characterization of bacterial colonies were revealed basis on the cultural properties of the isolated bacteria on cooked meat broth, blood agar, and TSC agar with and without egg yolk.

Culturing of isolates on sheep blood agar produced small to medium-sized smooth-colonies, which had a gray and glistening appearance, surrounded by an inner zone of complete hemolysis due to theta toxin and an outer zone of partial hemolysis due to alpha toxin (Fig. 1). The bacteria produced a typical round and flat colonies, which had smooth and black color on tryptose sulfite cycloserine agar (Fig. 1). While the growth of the organism on TSC agar with egg yolk produced opalescence around the black colonies as a result of the breaking down of lecithin indicates to lecithinase activity (Fig. 1). The isolates were Gram-positive bacilli appearance (Fig. 1).

Multiplex and Uniplex PCR:

The multiplex PCR reaction showed that almost all isolates were positive for *C. perfringens* alpha-toxin gene and negative for other toxin-forming genes (Fig. 2). This result indicated that the isolated type was *C. perfringens* type A, because of the presence of alpha-toxin gene alone, which is only present in *C. perfringens* type A.

The uniplex PCR results reconfirmed the presence of the alpha-toxin gene; besides, the *cpb2* gene (548 bp) was detected in 20 from 63 isolates of the *C. perfringens* type A (Fig. 3). However, all *cpa* gene-positive samples were negative for the NetB toxin gene.

DNA sequence analysis and Phylogenic Tree:

The DNA sequence analysis of the partially sequenced *C. perfringens*, which had been isolated from different broiler farms, using conventional software, including Clustal Omega (Multiple Sequence Alignment), NCBI nucleotide blast, MEGA-X software, and ExPASy Server showed that *C. perfringens* type A alpha gene (Fig. 5) (MN224677 and MN224678, and MN224676 and MN224679), had 100% to 99.22% homology respectively. The phylogenic tree analysis showed that the first two sequences were 100% similar to that of taxon

L43548.1 (isolated from blue calves in Belgium), and 99.87% identical to that of taxon JQ071544.1 (isolated from poultry stool in Brazil). Still, it has a low rate identity of 84.18% with AF204209.1, which has been isolated from a diseased swan in the UK (Fig. 4). The other two sequences MN224676 and MN224679 were 100% similar to taxon X13608.1, L43547.1, and KT020614.1, which isolated from veterinary isolate, blue calves and broiler chicken in the UK, Belgium and Brazil respectively. However, the sequences were 99.68% identical to taxon GU581194 (isolated from the intestinal tract of diseased broiler chicken in Iran), and 84.31% homology with taxon AF204209.1 which was isolated from a diseased swan in the UK (Fig. 4).

The DNA sequence of the isolates showed that there were several nucleotides substitutions within the sequence of the partially sequenced alpha-toxin genes (MN224677 and MN224678, MN224676 and MN224679) at the site 164 (C with T), 266 (T with C), 263 (A with C), 605 (C with A), 613 (A with G) and 783 (G with A) (Fig. 5). Codons at the site 164, 266, 263, and 783 were replaced with alternative codons for the same amino acids. While, nucleotides substitutions at the site 605 and 613 altered the corresponding codons that lead to the replacement of the amino acid alanine (MN224676 and MN224679) with aspartate (MN224677 and MN224678), and threonine (MN224676 and MN224679) with alanine (MN224677 and MN224678) (Fig. 6).

Similar to the alpha gene, *C. perfringens* type A beta2 toxin gene was amplified from 20 of alpha-toxin gene positive isolates. Beta2 toxin gene was found to be highly conserved. The sequence analysis showed that there was only one nucleotide substitution at site 428 of the Beta2 toxin gene (MN239887) (Fig. 8). The substitution of the nucleotide Guanine (MN239886 and MN239885) with adenine that leads to the alteration of the corresponding codon (UGU to UAU) and replacement of the amino acid cysteine with tyrosine (Fig. 9). The phylogenic analysis of *cpb2* partially sequenced gene revealed that MN239886 and MN239885 were 100% identical with the sequence MF471365.1 (isolated from broiler chicken in India), AY884037.1 (from an unknown source), GU581183.1 (isolated from diseased broiler chicken in Iran) and MF191716.1 (isolated from goat in Pakistan). The sequence homology with the other taxons was ranged between 93.75% to 99.75%, respectively (Fig. 7). The MN239887 sequence of the isolate was 100% identical to that of the taxon KX924463.1 isolated from gout in Pakistan and taxon GU581182.1 and GU581185.1, which had been isolated from broiler chicken in Iran. Also, the sequence homology with other sequences were ranged between 93.50% to 99.50%, respectively (Fig. 7).

Discussion

Isolation and identification *C. perfringens*:

Clostridium perfringens infections are of economic concern in poultry production, resulting in gastrointestinal dysbacteriosis and necrotic enteritis (15). Proper characterization and identification of the causative agent's disease are very crucial in minimizing economic losses due to the clostridial infections in the poultry.

The characteristic of the colonial morphology, which included, appearance of inner zone of complete hemolysis due to theta toxin and an outer zone of incomplete hemolysis due to alpha toxin on sheep blood agar and blackish colonies on TSC agar which is due to reduction of sulfite to sulfide by *C. perfringens* which in turn react with iron and form a black iron sulfide precipitate, is concurred with observation by other authors (16, 17). The growth of the isolates on medium contained egg yolk produced opalescence around the colony due to the breaking down of lecithin by alpha toxin (14). Microscopically the bacteria characterized by having gram-positive rod-shaped with blunt ends

after being stained with gram stain.

Multiplex and Uniplex PCR:

Alpha and beta2 toxin genes, which have been detected in the present study, appear to be associated with the virulence of the bacteria. Alpha toxin is one of the most important lethal, hemolytic, and dermonecrotic toxins produced by *C. perfringens*, is considered to be the major virulence factor and lethal toxin in the pathogenesis of necrotic enteritis (15, 18). The beta2 toxin was found to have in-vitro cytotoxicity and be lethal in mice (19). Although it seems to be associated with enteric diseases in piglet (20), horses (21). Although Beta2 toxin gene was isolated from sheep dysentery and an African elephant ulcerative enteritis (22, 23).

NetB toxin gene is plasmid coded, pore-forming toxin plays a crucial role in the pathogenesis of necrotic enteritis in broiler (24). The isolates in the present study were found to be negative for *netB* gene similar to that which have been reported by Nakano *et al*. (25) and Merati *et al*. (26).

DNA sequence analysis and Phylogenic Tree:

The *cpa* sequence (100% to 99.22%) similarity with the recorded sequences, including isolated *C. perfringens cpa* gene from blue calves in Belgium (L43548.1 and L43547.1), veterinary isolate in UK (X13608.1), broiler in Iran (GU581194), poultry stool (JQ071544.1) and broiler (KT020614.1) in brazil, and *cpb2* gene of *C. perfringens* homology with *C. perfringens* isolates from broiler in India (MF471365.1), Iran (GU581183.1, GU581182.1, and GU581185.1), goat in Pakistan (MF191716.1 and KX924463.1), with a minor point mutations which were observed in the present study might be related to the fact that all isolates were obtained from broiler of the same age group at a limited geographical region, they appears to be epidemiologically related (27). Although those homologies with other various isolates from different countries might be associated with a substantial border and a large trade relation with other countries, especially, regarding the sources of chicks, and litter in the farms. Eggshell fragments, chick fluff, and paper pads in commercial hatcheries were reported to be contaminated with *C. perfringens* (28). The wild migratory birds might have a contribution in introducing the *C. perfringens* strains across countries. Wild birds have been reported as a reservoir of *C. perfringens* and might have a role in the transmission of the bacteria (29). The ration of animal origin contained high protein, particularly in fish meal followed by meat, bone meal and dry fish, was detected to be contaminated with *C. perfringens* (30). Also, a high level of *C. perfringens* contamination was found in processed animal proteins (31). It has been revealed that *C. perfringens* strains of mammalian species can cause necrotic enteritis in chickens (32).

Nucleotides substitutions that altered the corresponding codons were expected to be followed by replacement of the amino acid alanine with aspartate, and threonine with alanine in the polypeptide chain of alpha toxin protein, and replacement of the amino acid cysteine with tyrosine in the structure of beta2 toxin protein that might be associated with the alteration of the structural and functional properties of alpha and beta2 toxin in *C. perfringens* (33). Meanwhile, it was found that induced substitution of amino acid with another amino acid by point mutation alters the activity and function of the alpha-toxin protein (34) and epsilon toxin (35). However, further study is needed to investigate the role of the predicted amino acid alterations on *C. perfringens* virulence.

Table 1: Primers used for multiplex PCR to detection of the types of *C. perfringens* toxin genes

Primer	Sequence	Gene	Size of product	Annealing temp.	Reference
Alpha	5`-GTTGATAGCGCAGGACATGTTAAG-3` 5`-CATGTAGTCATCTGTTCCAGCATC-3`	*cpa*	400	55 °C	(11)
Beta	5`-ACTATACAGACAGATCATTCAACC-3` 5`-TTAGGAGCAGTTAGAACTACAGAC-3`	*cpb*	236	55 °C	
Epsilon	5`-ACTGCAACTACTACTCATACTGTG-3` 5`-CTGGTGCCTTAATAGAAAGACTCC-3`	*etx*	541	55 °C	
Iota	5`-GCGATGAAAAGCCTACACCACTAC-3` 5`-GGTATATCCTCCACGCATATAGTC-3`	*iap*	317	55 °C	

Table 2: Uniplex PCR primer sets for detection of *C. perfringens* toxins.

Toxin	Primer sequence (5´-3´)	Gene	Size	Annealing temp.	Reference
Alpha	5`AGTCTACGCTTGGGATG AA-3` 5`TTTCCTGGGTTGTCCATTTC-3`	*cpa*	900	55 °C	(12)
Net-B	5`-GCTGGTGCTGGAATAAATGC-3` 5`-TCGCCATTGAGTAGTTTCCC-3	*netB*	383	55 °C	(13)
Beta2	5`-AAATATGATCCTAACCAACAA-3` 5`-CCAAATACTCTAATCGATGC-3	*cpb2*	548	55 °C	143)

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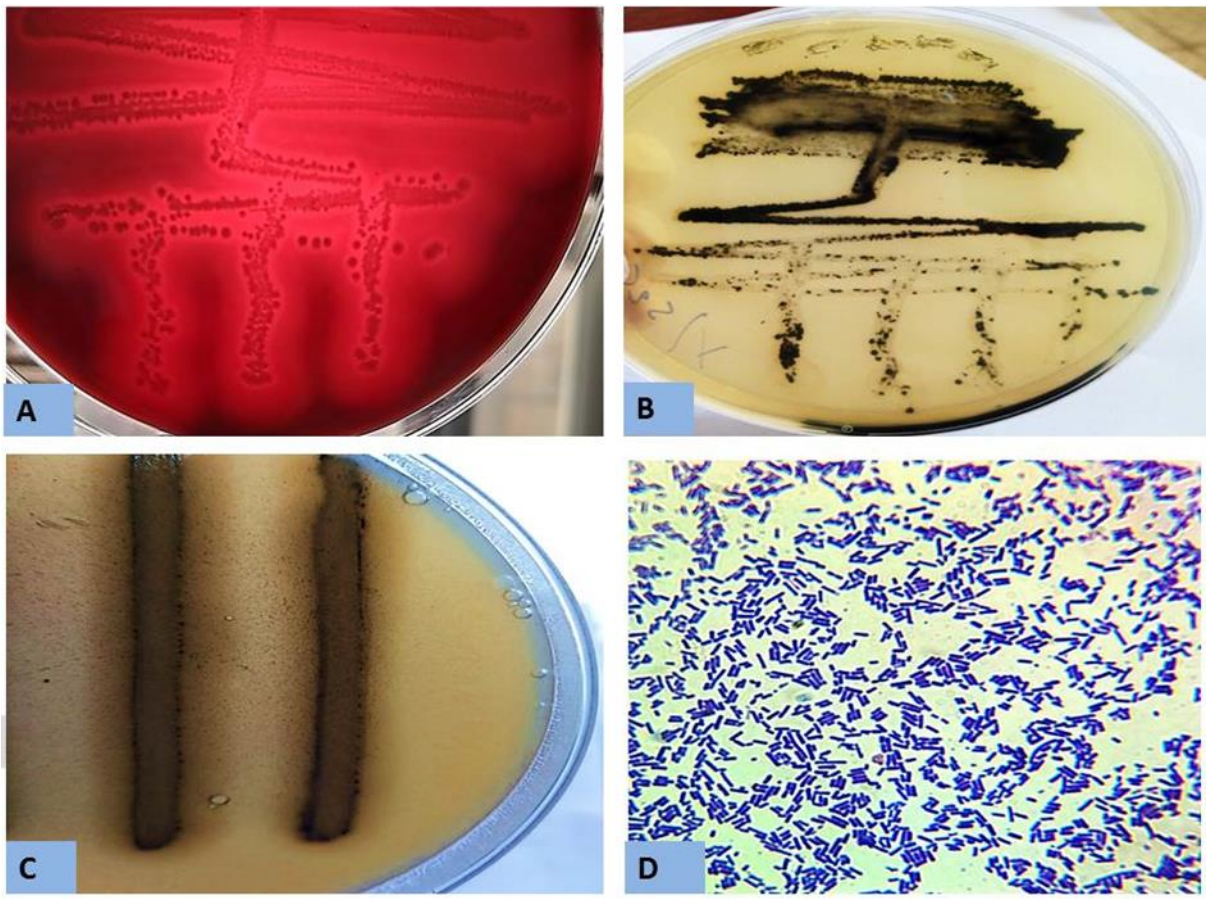

Figure 1: A; *C. perfringens* culture on blood agar. **B**; TSC agar culture. **C**; TSC agar with egg yolk. **D**; photograph of *C. perfringens* stained with gram stain under oil immersion (100X).

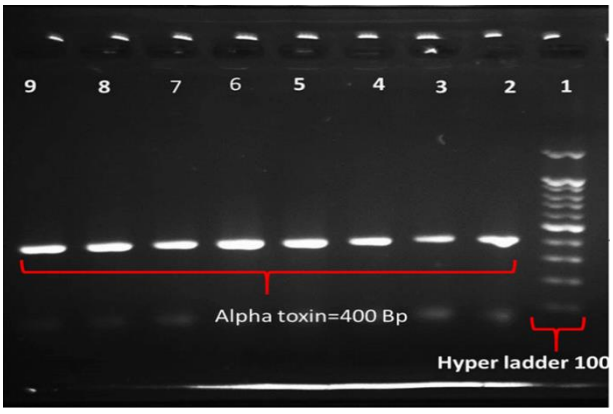

Figure 2: Multiplex PCR of *C. perfringens* isolates. Lane 1: hyper DNA ladder 100. Lane 2-9: *C. perfringens* types, which is only positive for type A.

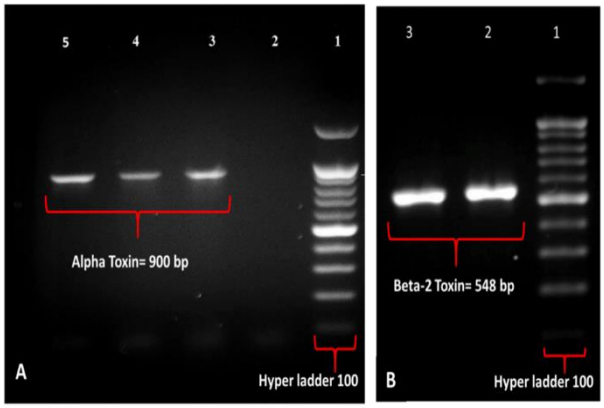

Figure 3: Panel A: Lane 1: Hyper ladder 100. Lane 3, 4 and 5: amplified alpha-toxin gene (900 bp). Panel B: Lane 1: Hyper ladder 100. Lane 2 and 3: amplified beta2 toxin gene (548 bp).

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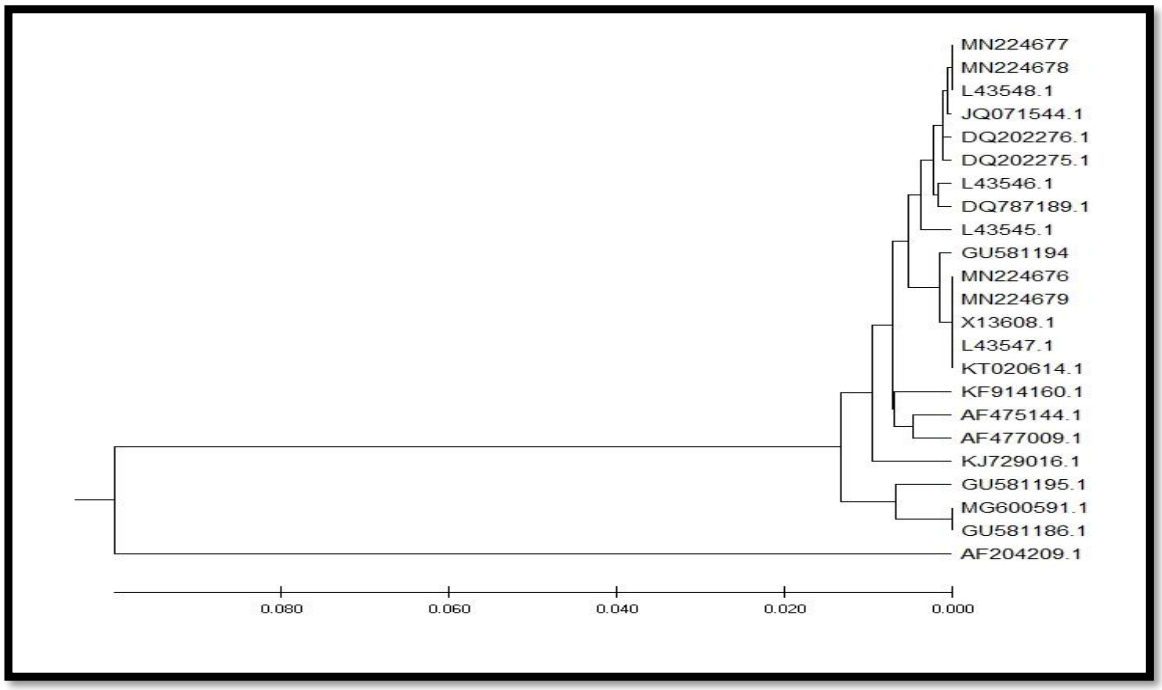

Figure 4: Phylogenic tree of partially sequenced *Clostridium perfringens* type A alpha toxin genes. The phylogram were created using MEGA-X software.

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GU581194.1      ------------------------------------------------------------ 0
L43547.1        TGCTATGATTGTAACTCAAGGGGTTTCAATCTTAGAAAATGATCTGTCCAAAAATGAACC 176
MN224676        -----------------------------------AAATGATCTGTCCAAAAATGAACC 24
MN224679        -----------------------------------AAATGATCTGTCCAAAAATGAACC 24
X13608.1        TGCTATGATTGTAACTCAAGGGGTTTCAATCTTAGAAAATGATCTGTCCAAAAATGAACC 960
KT020614.1      ------------------------------------------------------------ 0
MN224677        -----------------------------------AAATGATCTGTCTAAAAATGAACC 24
MN224678        -----------------------------------AAATGATCTGTCTAAAAATGAACC 24
JQ071544.1      ----------------------------------ATGATCTGTCTAAAAATGAACC 2
GU581194.1      ------------------------------------------------------------ 0
L43547.1        AGAAAGTGTAAGAAAAAACTTAGAGATTTTAAAAGAGAACATGCATGAGCTTCAATTAGG 236
MN224676        AGAAAGTGTAAGAAAAAACTTAGAGATTTTAAAAGAGAACATGCATGAGCTTCAATTAGG 84
MN224679        AGAAAGTGTAAGAAAAAACTTAGAGATTTTAAAAGAGAACATGCATGAGCTTCAATTAGG 84
X13608.1        AGAAAGTGTAAGAAAAAACTTAGAGATTTTAAAAGAGAACATGCATGAGCTTCAATTAGG 1020
KT020614.1      ------------------------------------------------------------ 0
MN224677        AGAAAGTGTAAGAAAAAACTTAGAGATTTTAAAAGAGAACATGCATGAGCTTCAATTAGG 84
MN224678        AGAAAGTGTAAGAAAAAACTTAGAGATTTTAAAAGAGAACATGCATGAGCTTCAATTAGG 84
JQ071544.1      AGAAAGTGTAAGAAAAAACTTAGAGATTTTAAAAGAGAACATGCATGAGCTTCAATTAGG 84
GU581194.1      ------------------------------------------------------------ 0
L43547.1        TTCTACTTATCCAGATTATGATAAGAATGCATATGATCTATATCAAGATCATTTCTGGGA 296
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MN224676        TTCTACTTATCCAGATTATGATAAGAATGCATATGATCTATATCAAGATCATTTCTGGGA 144
MN224679        TTCTACTTATCCAGATTATGATAAGAATGCATATGATCTATATCAAGATCATTTCTGGGA 144
X13608.1        TTCTACTTATCCAGATTATGATAAGAATGCATATGATCTATATCAAGATCATTTCTGGGA 1080
KT020614.1      ------------------------------------------------------------ 0
MN224677        TTCTACTTATCCAGATTATGATAAGAACGCCTATGATCTATATCAAGATCATTTCTGGGA 144
MN224678        TTCTACTTATCCAGATTATGATAAGAACGCCTATGATCTATATCAAGATCATTTCTGGGA 144
JQ071544.1      TTCTACTTATCCAGATTATGATAAGAACGCCTATGATCTATATCAAGATCATTTCTGGGA 142
GU581194.1      ------------------------------------------------------------ 0
L43547.1        TCCTGATACAGATAATAATTTCTCAAAGGATAATAGTTGGTATTTAGCTTATTCTATACC 356
MN224676        TCCTGATACAGATAATAATTTCTCAAAGGATAATAGTTGGTATTTAGCTTATTCTATACC 204
MN224679        TCCTGATACAGATAATAATTTCTCAAAGGATAATAGTTGGTATTTAGCTTATTCTATACC 204
X13608.1        TCCTGATACAGATAATAATTTCTCAAAGGATAATAGTTGGTATTTAGCTTATTCTATACC 1140
KT020614.1      ------------------------------------------------------------ 0
MN224677        TCCTGATACAGATAATAATTTCTCAAAGGATAATAGTTGGTATTTAGCTTATTCTATACC 204
MN224678        TCCTGATACAGATAATAATTTCTCAAAGGATAATAGTTGGTATTTAGCTTATTCTATACC 204
JQ071544.1      TCCTGATACAGATAATAATTTCTCAAAGGATAATAGTTGGTATTTAGCTTATTCTATACC 202
GU581194.1      ------------------------------------------------------------ 0
L43547.1        TGACACAGGGGAATCACAAATAAGAAAATTTTCAGCATTAGCTAGATATGAATGGCAAAG 416
MN224676        TGACACAGGGGAATCACAAATAAGAAAATTTTCAGCATTAGCTAGATATGAATGGCAAAG 264
MN224679        TGACACAGGGGAATCACAAATAAGAAAATTTTCAGCATTAGCTAGATATGAATGGCAAAG 264
X13608.1        TGACACAGGGGAATCACAAATAAGAAAATTTTCAGCATTAGCTAGATATGAATGGCAAAG 1200
KT020614.1      ------------------------------------------------------------ 0
MN224677        TGACACAGGGGAATCACAAATAAGAAAATTTTCAGCATTAGCTAGATATGAATGGCAAAG 264
MN224678        TGACACAGGGGAATCACAAATAAGAAAATTTTCAGCATTAGCTAGATATGAATGGCAAAG 264
JQ071544.1      TGACACAGGGGAATCACAAATAAGAAAATTTTCAGCATTAGCTAGATATGAATGGCAAAG 262
GU581194.1      ------------------------------------------------------------ 0
L43547.1        AGGAAACTATAAACAAGCTACATTCTATCTTGGAGAGGCTATGCACTATTTTGGAGATAT 476
MN224676        AGGAAACTATAAACAAGCTACATTCTATCTTGGAGAGGCTATGCACTATTTTGGAGATAT 324
MN224679        AGGAAACTATAAACAAGCTACATTCTATCTTGGAGAGGCTATGCACTATTTTGGAGATAT 324
X13608.1        AGGAAACTATAAACAAGCTACATTCTATCTTGGAGAGGCTATGCACTATTTTGGAGATAT 1260
KT020614.1      ------------------------------------------------------------ 0
MN224677        AGGAAACTATAAACAAGCTACATTCTATCTTGGAGAGGCTATGCACTATTTTGGAGATAT 324
MN224678        AGGAAACTATAAACAAGCTACATTCTATCTTGGAGAGGCTATGCACTATTTTGGAGATAT 324
JQ071544.1      AGGAAACTATAAACAAGCTACATTCTATCTTGGAGAGGCTATGCACTATTTTGGAGATAT 322
GU581194.1      ------------------------------------------------------------ 0
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L43547.1        AGATACTCCATATCATCCTGCTAATGTTACTGCCGTTGATAGCGCAGGACATGTTAAGTT 536
MN224676        AGATACTCCATATCATCCTGCTAATGTTACTGCCGTTGATAGCGCAGGACATGTTAAGTT 384
MN224679        AGATACTCCATATCATCCTGCTAATGTTACTGCCGTTGATAGCGCAGGACATGTTAAGTT 384
X13608.1        AGATACTCCATATCATCCTGCTAATGTTACTGCCGTTGATAGCGCAGGACATGTTAAGTT 1320
KT020614.1      ------------------------------------------------------------ 0
MN224677        AGATACTCCATATCATCCTGCTAATGTTACTGCCGTTGATAGCGCAGGACATGTTAAGTT 384
MN224678        AGATACTCCATATCATCCTGCTAATGTTACTGCCGTTGATAGCGCAGGACATGTTAAGTT 384
JQ071544.1      AGATACTCCATATCATCCTGCTAATGTTACTGCCGTTGATAGCGCAGGACATGTTAAGTT 382
GU581194.1      ----------------------------------------AACACAGCAGGTTGCAAAAC 20
L43547.1        TGAGACTTTTGCAGAGGAAAGAAAAGAACAGTATAAAATAAACACAGCAGGTTGCAAAAC 596
MN224676        TGAGACTTTTGCAGAGGAAAGAAAAGAACAGTATAAAATAAACACAGCAGGTTGCAAAAC 444
MN224679        TGAGACTTTTGCAGAGGAAAGAAAAGAACAGTATAAAATAAACACAGCAGGTTGCAAAAC 444
X13608.1        TGAGACTTTTGCAGAGGAAAGAAAAGAACAGTATAAAATAAACACAGCAGGTTGCAAAAC 1380
KT020614.1      ----------------------------------------------CAGCAGGTTGCAAAAC 16
MN224677        TGAGACTTTTGCAGAGGAAAGAAAAGAACAGTATAAAATAAACACAGCAGGTTGCAAAAC 444
MN224678        TGAGACTTTTGCAGAGGAAAGAAAAGAACAGTATAAAATAAACACAGCAGGTTGCAAAAC 444
JQ071544.1      TGAGACTTTTGCAGAGGAAAGAAAAGAACAGTATAAAATAAACACAGCAGGTTGCAAAAC 442
                                                ****************

GU581194.1      TAATGAGGCTTTTTATACTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 80
L43547.1        TAATGAGGCTTTTTATACTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 656
MN224676        TAATGAGGCTTTTTATACTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 504
MN224679        TAATGAGGCTTTTTATACTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 504
X13608.1        TAATGAGGCTTTTTATACTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 1440
KT020614.1      TAATGAGGCTTTTTATACTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 76
MN224677        TAATGAGGATTTTTATGCTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 504
MN224678        TAATGAGGATTTTTATGCTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 504
JQ071544.1      TAATGAGGATTTTTATGCTGATATCTTAAAAAACAAAGATTTTAATGCATGGTCAAAAGA 502
                ******** ******* ****************************************

GU581194.1      ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 140
L43547.1        ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 716
MN224676        ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 564
MN224679        ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 564
X13608.1        ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 1500
KT020614.1      ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 136
MN224677        ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 564
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MN224678        ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 564
JQ071544.1      ATATGCAAGAGGTTTTGCTAAAACAGGAAAATCAATATACTATAGTCATGCTAGCATGAG 562
                ************************************************************

GU581194.1      TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 200
L43547.1        TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 776
MN224676        TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 624
MN224679        TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 624
X13608.1        TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 1560
KT020614.1      TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 196
MN224677        TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 624
MN224678        TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 624
JQ071544.1      TCATAGTTGGGATGATTGGGATTATGCAGCAAAGGTAACTTTAGCTAACTCTCAAAAAGG 622
                ************************************************************

GU581194.1      AACAGCGGGATATATTTATAGATTCTTACACGATGTATCAGAGG---------------- 244
L43547.1        AACAGCGGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 836
MN224676        AACAGCGGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 684
MN224679        AACAGCGGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 684
X13608.1        AACAGCGGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 1620
KT020614.1      AACAGCGGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 256
MN224677        AACAGCAGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 684
MN224678        AACAGCAGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 684
JQ071544.1      AACAGCAGGATATATTTATAGATTCTTACACGATGTATCAGAGGGTAATGATCCATCAGT 682
                ****** ***********************************

GU581194.1      ------------------------------------------------------------ 244
L43547.1        TGGAAAGAATGTAAAAGAACTAGTAGCTTACATATCAACTAGTGGTGAGAAAGATGCTGG 896
MN224676        TGGAAAGAATGTAAAAGAACTAGTAGCTTACATATCAACTAGTGGTGAGAAAGATGCTGG 744
MN224679        TGGAAAGAATGTAAAAGAACTAGTAGCTTACATATCAACTAGTGGTGAGAAAGATGCTGG 744
X13608.1        TGGAAAGAATGTAAAAGAACTAGTAGCTTACATATCAACTAGTGGTGAGAAAGATGCTGG 1680
KT020614.1      T----------------------------------------------------------- 257
MN224677        TGGAAAGAATGTAAAAGAACTAGTAGCTTACATATCAACTAGTGGTGAGAAAGATGCTGG 744
MN224678        TGGAAAGAATGTAAAAGAACTAGTAGCTTACATATCAACTAGTGGTGAGAAAGATGCTGG 744
JQ071544.1      TGGAAAGAATGTAAAAGAACTAGTAGCTTACATATCAACTAGTGGTGAAAAAGATGCTGG 742
GU581194.1      ------------------------------------------------------------ 244
L43547.1        AACAGATGACTACATGTATTTTGGAATCAAAACAAAGGATGGAAAAACTCAAGAATGGGA 956
MN224676        AACAGATGACTACATGTATTT---------------------------------------- 765
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MN224679        AACAGATGACTACATGTATTT---------------------------------------765
X13608.1        AACAGATGACTACATGTATTTTGGAATCAAAACAAAGGATGGAAAAACTCAAGAATGGGA1740
KT020614.1      ------------------------------------------------------------257
MN224677        AACAGATGACTACATGTATTT---------------------------------------765
MN224678        AACAGATGACTACATGTATTT---------------------------------------765
JQ071544.1      AACAGATGACTACATGTATTTTGGAATCAAAACAAAGGATGGAAAAACTCAAGAATGGGA802
```

Figure 5: Alignment of partially sequenced alpha toxin gene using Cluster omega multiple sequence alignment software.

```
L43547.1      MKRKICKALICAALRTSLWAGASTKVYAWDGKIDGTGTHAMIVTQGVSILENDLSKNEPE   60
MN224676      ----------------------------------------------------NDLSKNEPE   9
MN224679      ----------------------------------------------------NDLSKNEPE   9
MN224677      ----------------------------------------------------NDLSKNEPE   9
MN224678      ----------------------------------------------------NDLSKNEPE   9
                                                                    *********

L43547.1      SVRKNLEILKENMHELQLGSTYPDYDKNAYDLYQDHFWDPDTDNNFSKDNSWYLAYSIPD   120
MN224676      SVRKNLEILKENMHELQLGSTYPDYDKNAYDLYQDHFWDPDTDNNFSKDNSWYLAYSIPD   69
MN224679      SVRKNLEILKENMHELQLGSTYPDYDKNAYDLYQDHFWDPDTDNNFSKDNSWYLAYSIPD   69
MN224677      SVRKNLEILKENMHELQLGSTYPDYDKNAYDLYQDHFWDPDTDNNFSKDNSWYLAYSIPD   69
MN224678      SVRKNLEILKENMHELQLGSTYPDYDKNAYDLYQDHFWDPDTDNNFSKDNSWYLAYSIPD   69
              ************************************************************

L43547.1      TGESQIRKFSALARYEWQRGNYKQATFYLGEAMHYFGDIDTPYHPANVTAVDSAGHVKFE   180
MN224676      TGESQIRKFSALARYEWQRGNYKQATFYLGEAMHYFGDIDTPYHPANVTAVDSAGHVKFE   129
MN224679      TGESQIRKFSALARYEWQRGNYKQATFYLGEAMHYFGDIDTPYHPANVTAVDSAGHVKFE   129
MN224677      TGESQIRKFSALARYEWQRGNYKQATFYLGEAMHYFGDIDTPYHPANVTAVDSAGHVKFE   129
MN224678      TGESQIRKFSALARYEWQRGNYKQATFYLGEAMHYFGDIDTPYHPANVTAVDSAGHVKFE   129
              ************************************************************

L43547.1      TFAEERKEQYKINTAGCKTNEAFYTDILKNKDFNAWSKEYARGFAKTGKSIYYSHASMSH   240
MN224676      TFAEERKEQYKINTAGCKTNEAFYTDILKNKDFNAWSKEYARGFAKTGKSIYYSHASMSH   189
MN224679      TFAEERKEQYKINTAGCKTNEAFYTDILKNKDFNAWSKEYARGFAKTGKSIYYSHASMSH   189
MN224677      TFAEERKEQYKINTAGCKTNEDFYADILKNKDFNAWSKEYARGFAKTGKSIYYSHASMSH   189
MN224678      TFAEERKEQYKINTAGCKTNEDFYADILKNKDFNAWSKEYARGFAKTGKSIYYSHASMSH   189
              ******************** **:**********************************

L43547.1      SWDDWDYAAKVTLANSQKGTAGYIYRFLHDVSEGNDPSVGKNVKELVAYISTSGEKDAGT   300
MN224676      SWDDWDYAAKVTLANSQKGTAGYIYRFLHDVSEGNDPSVGKNVKELVAYISTSGEKDAGT   249
MN224679      SWDDWDYAAKVTLANSQKGTAGYIYRFLHDVSEGNDPSVGKNVKELVAYISTSGEKDAGT   249
MN224677      SWDDWDYAAKVTLANSQKGTAGYIYRFLHDVSEGNDPSVGKNVKELVAYISTSGEKDAGT   249
```

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MN224678      SWDDWDYAAKVTLANSQKGTAGYIYRFLHDVSEGNDPSVGKNVKELVAYISTSGEKDAGT  249
              ************************************************************

L43547.1      DDYMYFGIKTKDGKTQEWEMDNPGNDFMTGSKDTYTFKLKDENLKIDDIQNMWIRKRKYT  360
MN224676      DDYMY-------------------------------------------------------  254
MN224679      DDYMY-------------------------------------------------------  254
MN224677      DDYMY-------------------------------------------------------  254
MN224678      DDYMY-------------------------------------------------------  254
              *****

L43547.1      AFSDAYKPENIKIIANGKVVVDKDINEWISGNSTYNIK        398
MN224676      ------------------------------------        254
MN224679      ------------------------------------        254
MN224677      ------------------------------------        254
MN224678      ------------------------------------        254
```

Figure 6: Amino acid sequence of alpha toxin gene, created using Expasy bioinformatics software.

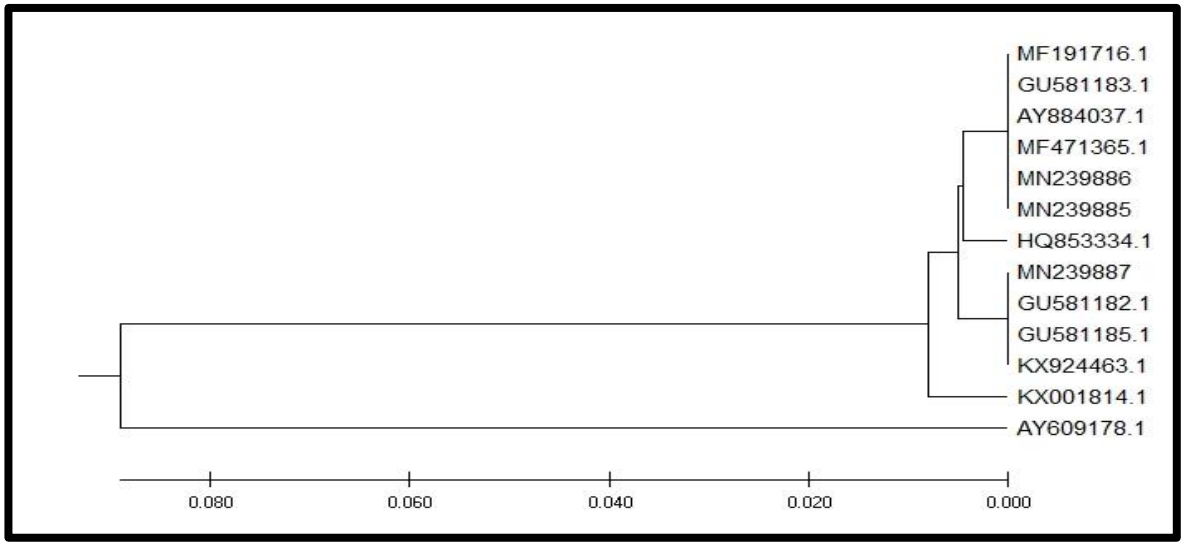

Figure 7: Phylogenic tree of partially sequenced *Clostridium perfringens* type A beta-2 toxin genes.

```
MF191716.1      AGTAAATGAAGGTAAATGTATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 143
GU581183.1      AGTAAATGAAGGTAAATGTATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 231
AY884037.1      AGTAAATGAAGGTAAATGTATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 239
MN239885        AGTAAATGAAGGTAAATGTATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 164
MN239886        AGTAAATGAAGGTAAATGTATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 164
MF471365.1      AGTAAATGAAGGTAAATGTATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 195
GU581185.1      AGTAAATGAAGGTAAATATATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 240
GU581182.1      AGTAAATGAAGGTAAATATATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 233
MN239887        AGTAAATGAAGGTAAATATATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 164
KX924463.1      AGTAAATGAAGGTAAATATATTCCTACACCAAGTTTCAGAACTCAAGTTTGTACATGGGA 228
```

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MF191716.1      TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 203
GU581183.1      TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 291
AY884037.1      TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 299
MN239885        TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 224
MN239886        TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 224
MF471365.1      TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 255
GU581185.1      TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 300
GU581182.1      TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 293
MN239887        TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 224
KX924463.1      TGACGAATTAAGTCAATATATTGGAGACGCTGTTAGTTTTACACGTTCTAGTAAATTTCA 288
                ************************************************************

MF191716.1      ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 263
GU581183.1      ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 351
AY884037.1      ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 359
MN239885        ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 284
MN239886        ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 284
MF471365.1      ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 315
GU581185.1      ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 360
GU581182.1      ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 353
MN239887        ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 284
KX924463.1      ATATAGTTCTAATACGATTACATTAAACTTTAGACAATATGCAACTTCTGGATCAAGATC 348
                ************************************************************

MF191716.1      CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 323
GU581183.1      CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 411
AY884037.1      CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 419
MN239885        CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 344
MN239886        CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 344
MF471365.1      CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 375
GU581185.1      CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 420
GU581182.1      CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 413
MN239887        CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 344
KX924463.1      CTTAAAGGTAAAATACAGTGTAGTAGACCATTGGATGTGGGGGGATGACATTAGAGCTTC 408
MF191716.1      TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAGGTTC 383
GU581183.1      TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAGGTTC 471
AY884037.1      TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAGGTTC 479
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MN239885        TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAG---- 400
MN239886        TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAG---- 400
MF471365.1      TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAGGTTC 435
GU581185.1      TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAGGTTC 480
GU581182.1      TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAGGTTC 473
MN239887        TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAG---- 400
KX924463.1      TCAATGGGTATATGGTGAAAATCCGGATTATGCTAGACAGATAAAATTATATCTAGGTTC 468
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Figure 8: Alignment of partially sequenced beta-2 toxin gene using Cluster omega multiple sequence alignment software.

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CP025503.1      MKKLIVKSTMMLLFSCLLCLGIQLPNTVKANEVNKYQSVMVQYLEAFKNYDIDTIVDISK 60
MN239885        ------------------------------------------------------------ 0
MN239886        ------------------------------------------------------------ 0
MN239887        ------------------------------------------------------------ 0
KX924463.1      ------------------------------------------------------------ 0
CP025503.1      DSRTVTKEEYKNMLMEFKYDPNQKLKSYEITGSRKIDNGEIFSVKTEFLNGAIYNMEFTV 120
MN239885        -------------------------------------------SVKTEFLNGAIYNMEFTV 18
MN239886        -------------------------------------------SVKTEFLNGAIYNMEFTV 18
MN239887        -------------------------------------------SVKTEFLNGAIYNMEFTV 18
KX924463.1      ---------------------YQKLKSYEITGSRKIDNGEIFSVKTEFLNGAIYNMEFTV 39
CP025503.1      SYIDNKLMVSNMNRISIVNEGKCIPTPSFRTQVCTWDDELSQYIGDAVSFTRSSKFQYSS 180
MN239885        SYIDNKLMVSNMNRISIVNEGKCIPTPSFRTQVCTWDDELSQYIGDAVSFTRSSKFQYSS 78
MN239886        SYIDNKLMVSNMNRISIVNEGKCIPTPSFRTQVCTWDDELSQYIGDAVSFTRSSKFQYSS 78
MN239887        SYIDNKLMVSNMNRISIVNEGKYIPTPSFRTQVCTWDDELSQYIGDAVSFTRSSKFQYSS 78
KX924463.1      SYIDNKLMVSNMNRISIVNEGKYIPTPSFRTQVCTWDDELSQYIGDAVSFTRSSKFQYSS 99
CP025503.1      NTITLNFRQYATSGSRSLKVKYSVVDHWMWGDDIRASQWVYGENPDYARQIKLYLGSGET 240
MN239885        NTITLNFRQYATSGSRSLKVKYSVVDHWMWGDDIRASQWVYGENPDYARQIKLYL----- 133
MN239886        NTITLNFRQYATSGSRSLKVKYSVVDHWMWGDDIRASQWVYGENPDYARQIKLYL----- 133
MN239887        NTITLNFRQYATSGSRSLKVKYSVVDHWMWGDDIRASQWVYGENPDYARQIKLYL----- 133
KX924463.1      NTITLNFRQYATSGSRSLKVKYSVVDHWMWGDDIRASQWVYGENPDYARQIKLYLGSGET 159
CP025503.1      FKNYRIKVENYTPASIKVFGEGYCY  265
MN239885        -------------------------  133
MN239886        -------------------------  133
MN239887        -------------------------  133
KX924463.1      -------------------------  159
```

Figure 9: Amino acid sequence of beta2 toxin gene, created using Expasy bioinformatics software.

Conclusion

The results of the present study indicated that the causative agent of necrotic enteritis in broiler farms in Sulaimania province is caused by *C. perfringens* type A, which is characterized by having alpha-toxin gene along with beta2 toxin gene.

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