



Epidemiological Pattern and Diagnostic Approaches to Enterotoxaemia Among Young Ruminants in Kafrelsheikh Governorate, Egypt

Yamen M. Hegazy^{1,4*}, Atef F. Oreiby¹, HasabAllah Abou ElGhait², Hegazy M.M², Alaa El-Meniesy³, Ramadan Selim¹, Mohamed A Salem⁴ and Magdy H. Al-gaabary¹

¹ Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, 33511, Egypt

² Animal Production Research Institute, Agricultural Research Center, 9 Nadi El-Seid, 12618, Dokki, Giza, Egypt.

³ Veterinary Serum Vaccine Research Institute, El - Sekka El - Beda st. Abbassia, Cairo, Egypt

⁴ Clinical Studies Department , Colleg Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Saudi Arabia

Enterotoxaemia is responsible for high mortalities among young ruminants with severe economic losses to farmers. Using a structured questionnaire, Farmers' knowledge and practices responsible for the spread of enterotoxaemia among ruminant species in 16 farms in Egypt were investigated. Also, 70 calves and lambs found to have died suddenly of haemorrhagic enteritis were examined post-mortem, and samples were collected from the intestine to isolate bacteriologically the causative agents. Toxins were identified in the intestinal contents of dead young ruminants and genes responsible for toxin production were identified in isolated *Clostridium perfringens* using multiplex PCR. Results showed a good knowledge of farmers on enterotoxaemia and they revealed that it is responsible for 25% of young ruminants' mortalities despite the high vaccination coverage. Farmers mentioned that most cases occur in the winter season and after Foot and Mouth disease (FMD) outbreaks. Most farmers do not follow quarantine measures for newly purchased animals and do not select only to buy vaccinated animals. *Cl. perfringens* type A and alpha toxin only were identified from two dead calves samples, while *Cl. perfringens* type D and alpha and epsilon toxins were identified from all other dead lambs and calves samples. In conclusion, control of enterotoxaemia has to be considered especially in winter seasons and known times of FMD outbreaks. *Cl. perfringens* type A and *Cl. perfringens* type D is the predominant causative agent of enterotoxaemia in calves and lambs in the study area.

Keywords: *Clostridium perfringens*, enterotoxaemia, epidemiology, ruminants, multiplex PCR.

Introduction

Enterotoxaemia is a major cause of mortalities in different young ruminant species; lambs, kids, and calves. It is caused by *cl. perfringens* and is characterized by sudden death associated with intestinal hemorrhage in affected animals. *Cl. perfringens* type A is the most common cause of enteritis, abomasitis, and enterotoxaemia in many animal mammalian species, especially calves [1,2,3]. *Cl. perfringens* type B causes necro-hemorrhagic enteritis, mostly in sheep, *cl. perfringens* type C causes necrotizing enteritis, while type D strains of

Cl. perfringens produce enterotoxaemia in sheep, goats, and cattle [4]. *Cl. perfringens* type E is considered an infrequent cause of enterotoxaemia in different ruminant species [5].

The clinical signs of enterotoxaemia appear after 30 min of toxin production characterized by anorexia, diarrhea, dehydration, frothing, and respiratory distress. Post-mortem findings include severe congestion and hemorrhage of the intestine with enlarged mesenteric lymph nodes, histological examination shows edema of the lungs, kidney, lymph nodes, and brain with lung hemorrhages and hemorrhagic enteritis [6]. Some calves and lambs

*Corresponding author: Yamen M. Hegazy, E-mail: yhegazy@kfu.edu.sa Tel.:00201016147930

(Received 12/11/2023, accepted 04/12/2023)

DOI: 10.21608/EJVS.2023.243259.1669

©2023 National Information and Documentation Center (NIDOC)

die before they develop diarrhea; but others develop colic, become depressed, and bloat.

Detection and identification of enterotoxaemia toxins types are critical to ensure a good understanding of the epidemiology of *Cl. perfringens* infections and be useful in the development of effective control of the disease. PCR has proven a reliable and sensitive diagnostic tool for the rapid identification of *Cl. perfringens* [7].

Cl. perfringens in a recent study in Egypt accounted for 95 % and 69% of calf diarrhea in winter and spring, respectively, and for 73% and 61% of calf mortalities in Qaliubiya governorate and 64% of these isolates were of type A [8]. Mortality rate due to *Cl. perfringens* is 8% at Behira governorate [9]. *Cl. perfringens* types A and B were isolated from 4 and 5.5%, diarrheic calves in the Behira governorate, respectively and the incidence of toxigenic and non-toxigenic strains were 81.9% and 18.1%, respectively [10].

The vaccination against enterotoxaemia using bacterin-toxoid vaccines is widely used to reduce enterotoxaemia prevalence but enterotoxaemia is still a common problem in livestock production [11]. There are other effective non-commercial vaccines such as nano-vaccines and recombinant vaccines which can be potential alternatives shortly to conventional vaccines [11].

Our aims of the current study are to describe the epidemiological pattern of enterotoxaemia among suddenly dead young ruminants suffering from post-mortem hemorrhagic enteritis in the Kafrelsheikh area, Egypt. Also, to determine the risk factors associated with enterotoxaemia in ruminant farms through farmer practices analysis. Furthermore, to isolate and identify *Cl. perfringens* associated with enterotoxaemia among young ruminants in the study area.

Materials and methods

Study area and samples

In the period between January and December 2021, a total of 16 ruminant farms in the Kafrelsheikh governorate suffered from sudden death among their young animals after a short period of hemorrhagic enteritis. The total number of young animals in these farms was 1908; 250 buffalo calves,

Identification of *Cl. perfringens* isolates

a- Culture character on 10% sheep blood and egg yolk agar media

The culture was anaerobically at 37 °C for 24 h. Observation for a double zone of hemolysis on blood agar and the surrounding zone of opalescence with

158 cow calves, and 1500 sheep lambs. A total of 70 young animals were found dead suddenly; 6 buffalo calves, 7 cow calves, and 57 sheep lambs. Intestinal lopes, kidney, and liver samples were collected immediately after death from sudden dead calves and lambs with hemorrhagic enteritis. Samples were placed in sterile separate polyethylene bags, labeled, and carried on ice to the laboratory.

Intestinal content was mixed with saline (3:1), then centrifuged at 3000 rpm for 15 min. 0.3 ml of supernatant fluid was injected into the Swiss mouse tail vein. Samples that resulted in the death of the injected mice within 24 h. were considered for further investigation.

A Questionnaire survey was carried out among the farm owners to determine their Knowledge, attitude, and practices against diseases caused by *Clostridia* spp. which are responsible for sudden death among calves and lambs. The questionnaire was developed in English and translated into Arabic, the main author of the article was responsible for filling it out upon meeting with farm owners. The questions were about the proportion of sudden death among calves every year in the farms, the suspected causes, detected clinical signs, postmortem findings, the response to treatment, the vaccination regimen in these farms, introducing new animals into the farm, hygienic measures taken for control of diseases caused by clostridia spp. and their effects, and finally the presence of quarantine in the farm.

All data collected across the questionnaire were stored on an Excel sheet and descriptive analysis was carried out.

Isolation of *Cl. perfringens* [12].

Swaps from each sample were inoculated into sterile freshly prepared cooked meat medium tubes and anaerobically incubated at 37 °C for 24 - 48 h for enrichment. Swaps from each incubated tube were streaked onto 200 µg/ml neomycin sulfate 10% sheep blood agar. The plates were anaerobically incubated at 37 °C for 24 - 48 h. Colonies that showed double zone hemolysis were considered suspected and were re-cultured for purification and further identification.

the formation of a pearly layer (indicating lecithinase production) on egg yolk agar was performed.

b- Microscopic examination

Smears from suspected colonies were stained with Gram's stain and examined under a light microscope to search for Gram-positive bacilli.

c- Nagler reaction:

It was performed according to Helal *et al.*[13] Suspected colonies were streaked over the surface of an egg yolk agar plate, which was divided into two halves, one containing antibodies against alpha toxin produced by *Cl. perfringens* and the other half not. The suspected colonies were streaked across the plate starting from the free half of the plate, then were incubated under anaerobic conditions at 37 °C for 48 h. Nagler’s reaction was positive in the free half of the plate and opalescence was formed but was inhibited on the other half of the plate which contains anti-alpha antibodies.

d- Polymerase chain reaction (PCR)

For further confirmation of *Cl. perfringens* isolates, a uniplex PCR targeting alpha toxin (which is produced from all types of *Cl perfringens*) was performed according to Yoo *et al.* [14] with some modifications. The Primer sequence is illustrated in Table 1 and PCR cycling conditions were the same as that is mentioned below for multiplex PCR of toxin-typing. A reaction volume of 25 µl composed of 12.5 µl PCR master mix (Takara, Japan), 20 pmol (1 µl) of each primer, 6 µl template DNA, and 4.5 µl PCR grade water was used.

TABLE 1. Oligonucleotide primers sequences used in the current study

Toxin	Primer	Sequence	Amplified product	Reference
Alpha toxin	F	GTTGATAGCGCAGGACATGTTAAG	402 bp	[14]
	R	CATGTAGTCATCTGTTCCAGCATC		
Beta toxin	F	ACTATACAGACAGATCATTCAACC	236 bp	
	R	TTAGGAGCAGTTAGAACTACAGAC		
Epsilon toxin	F	ACTGCAACTACTACTCATACTGTG	541 bp	
	R	CTGGTGCCTTAATAGAAAAGACTCC		
Iota toxin	F	GCGATGAAAAGCCTACACCACTAC	317 bp	
	R	GGTATATCCTCCACGCATATAGTC		

Typing of *Clostridium perfringens* toxins

Toxin typing was conducted by three different methods: mice neutralization test according to Smith and Holdeman (1969) [15], and multiplex PCR according to Yoo *et al.* [14] with some modifications in protocol and cycling conditions. DNA extraction was performed using QIAamp DNA Mini Kit (QIAGEN, United States. The primers of beta, epsilon, and iota toxins were used in the multiplex PCR. It was performed in a reaction volume of 50 µl composed of 25 µl PCR master mix, 20 pmol (1 µl) of each primer, 10 µl template DNA, and 9 µl PCR grade water. Cycling conditions were set as 94 °C for 5 min followed by 35 cycles of 94 °C for 30 sec, 55 °C for 40 sec, and 72 °C for 45 sec, then a final extension step at 72 °C for 10 min. PCR products were run into 1.5% agarose gel for 30 min and transferred to a UV cabinet for visualization of results.

Results

Epidemiological investigations

The examined suddenly dead young animals were showing hemorrhagic enteritis signs a few hours before death could be extended for 24-36 h. Affected calves were almost found dead within 24 to 36 h after the onset of clinical signs. Upon post-

mortem examination hemorrhagic enteritis with a blackish color portion in dead animals was found with signs of toxemia on the examined mucous membranes (Fig. 1 and 2).



Fig. 1. Calf showed a congested intestine (black arrow) with a diffuse intestinal darkish coloration (blue arrow)

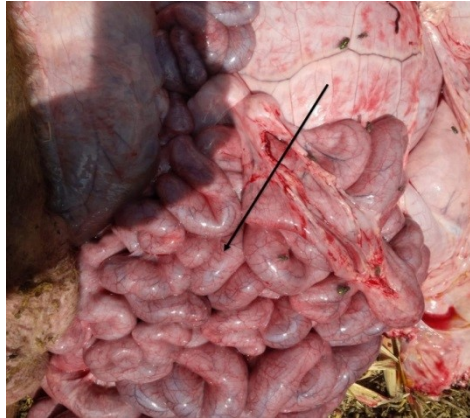


Fig. 2. Showed diffuse congestion of the intestine (black arrow) of sudden dead young lamb

The 16 farmers showed a good knowledge of *Clostridia* spp. as a causative agent of sudden death among their young ruminants, and they declared that

the mean parentage of sudden death among calves and lambs is 8.5% and 25% of this percentage is suspected to be due to enterotoxaemia, respectively. Out of the 16 farmers, 9 (56%) said that the fatality due to enterotoxaemia among their young animals is 100%. Most farmers declared that enterotoxaemia cases are prevalent in the winter season and after Foot and Mouth disease (FMD) outbreaks and it could affect animals despite high vaccination coverage (Table 2). Almost half of the farmers buy young animals from different sources and 44% of farmers do not ask about the vaccination status of such purchased animals and declare that enterotoxaemia occurs among these newly bought young animals (Table 2). There are no quarantine measures in almost 38% of examined farms/herds.

TABLE 2. Results of the answers of 16 farmers in Egypt regarding their practices towards enterotoxaemia

Questions	Answer	Number	%
Season of occurrence of enterotoxaemia	Winter and after FMD outbreaks	15	94%
Vaccination against enterotoxaemia	Yes	13	81%
Occurrence of enterotoxaemia in vaccinated young ruminants or those from vaccinated dams	Yes	13	81%
Buy young ruminants	Yes	9	56%
Ask on vaccination status among bought animals	Yes	4 / 9	44%
Occurrence of enterotoxaemia among bought animals	Yes	4 / 9	44%
Presence of quarantine in the farm/herd	Yes	6	38%

Isolation and identification of *Clostridia* spp.

Clostridium spp. were isolated from all samples of dead animals, and they all were identified using different identification tests as *Clostridium perfringens*. Uniplex PCR identified the alpha toxin gene in all isolates (Figure 3) while multiplex PCR identified the epsilon toxin gene in all isolates of all lambs and most calves and no other toxin gene was identified from dead animals (Figure 4). Therefore, all isolates from lambs and calves were identified as *Cl. perfringens* type D, while *Cl. perfringens* type A was identified from only 2 calves.

Identification of toxins using mice neutralization test revealed the identification of alpha toxin only from calves and alpha and epsilon toxins from lambs and calves as shown from 10 animals in Table 3.

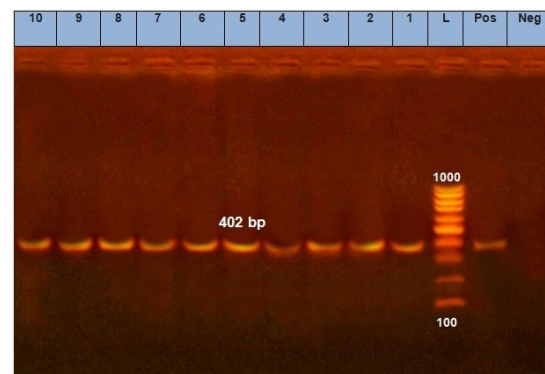


Fig. 3 Detection of *Clostridium perfringens* alpha toxin gene from *Clostridium perfringens* isolates.

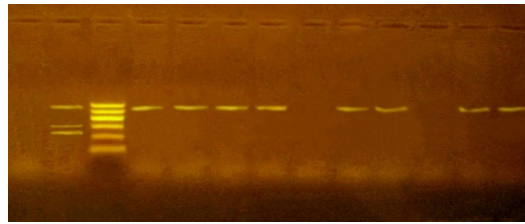


Fig. 4. Detection of *Clostridium perfringens* epsilon toxin gene amplified by multiplex PCR from *Clostridium perfringens* isolates of small intestine of dead small ruminants in Egypt

TABLE 3. Identification of *Clostridium perfringens* toxins from small intestine contents of 10 suddenly identified calves and lambs using toxin-antitoxin neutralization test

Sample	Alpha toxin	Beta toxin	Epsilon toxin	Iota toxin
1	+	-	+	-
2	+	-	+	-
3	+	-	-	-
4	+	-	+	-
5	+	-	+	-
6	+	-	-	-
7	+	-	+	-
8	+	-	+	-
9	+	-	+	-
10	+	-	+	-

Discussion

Clostridium bacteria produce eight classes of toxins and frequently found in the soil and water in most parts all over the world [16]. Enterotoxaemia in neonatal calves and lambs caused by *Cl. perfringens* represents an economic problem for farmers worldwide as it is associated with sudden death with lesions of hemorrhagic enteritis [17]. In the current study, the knowledge and practices of farmers were assessed for the first time in the study area – up to our knowledge- to identify weak points in enterotoxaemia control in a developing country that depends mainly on agriculture and farming as a major source of income. Farmers in the study area declared that almost 25% of neonatal mortalities are due to enterotoxaemia despite the regular vaccination regime being applied. This high-case fatality rate reported is lower than that reported by Omer et al. [18]. The reason behind this difference may be because our study depends on the farmers' observation which may lack the confirmation of causes of mortalities. The high case fatality rate of enterotoxaemia may be attributed to the incomplete effectiveness of the used vaccines in protection against enterotoxaemia [11]. Therefore, new vaccines have been developed and may offer another option to farmers shortly. Also, half of the farmers tend to buy animals without prior information on their vaccination status and these animals could be non-vaccinated animals and could be either infected or susceptible to enterotoxaemia. Almost one-third of the farmers do not have quarantine measures at all

for the newly purchased animals and this poses a critical point for the introduction of new infections to the farm/herd as the introduction of a case is responsible for keeping cases of enterotoxaemia in the environment [19]. The concentration of enterotoxaemia cases in the winter season and especially after FMD outbreaks agreed with the findings of Selim et al. [8] and Omer et al. [18] who found that the ability of the bacterium to survive in the environment especially in the winter season under unsuitable conditions is an important factor helps to initiate enterotoxaemia outbreaks.

The prevalence of enterotoxaemia caused by *Cl. perfringens* has been often reported in sheep and cattle throughout the world, therefore the cornerstone for epidemiological investigations and vaccine improvement is the accurate identification of *Clostridium perfringens* variants [20]. *Cl. perfringens* toxins recognition is very important because they are related to particular gastric and intestinal animal sicknesses. There are different methods for the identification of toxins of *Cl. perfringens* such as the Toxin-antitoxin neutralization test and Enzyme-Linked Immunosorbent assay (ELISA) and PCR. Toxin-antitoxin neutralization test takes a long time, costs money, tedious, and the monovalent method is unsuitable and immoral for applying to laboratory animals [21]. On the other hand, ELISA is laborious, cannot identify β_2 toxins, and requires activated spore-forming bacteria using a particular culture for the recognition of toxins [22]. PCR has become an

important research and diagnostic tool, as it is quick and able to directly recognize the bacterium in clinical samples, feasible, and lower cost in comparison to other tests. [20].

The current study showed that using multiplex PCR, *Cl. perfringens* type A is the only cause of enterotoxaemia in some calves and this agreed with the findings of Selim *et al.* [8] who isolated and identify such a microorganism from all specimens collected from suddenly dead calves in Egypt. On the other hand, our findings of identification of *Clostridium perfringens* type A and D as the most common isolates from enterotoxaemia cases of lambs come in agreement with the findings of Tutuncu *et al.* [23] in Turkey, Nazki *et al.* [24] in India, Moustafa *et al.* [25] and Moustafa *et al.* [26] in Egypt and Omer *et al.* [18] in Saudi Arabia and this contradicts with findings of Nayel *et al.* [27] who found that *Clostridium perfringens* type B was isolated from suspected enterotoxaemia cases of lambs.

Conclusions

In conclusion, the results of this study indicate that enterotoxaemia is endemic in Egypt and responsible for the high case fatality rate among calves, lambs, and kids especially in the winter season and following infectious disease outbreaks such as FMD. *Cl. perfringens* type A and type D are responsible for enterotoxaemia in calves and lambs, respectively in the study area. Moreover, there is a need for educational campaigns to the farmers to correct their hazardous practices which lead to the spread of enterotoxaemia in their flocks and farms.

Acknowledgment: NA

Conflicts of interest: The authors declared no competing interests.

Funding : No funding was obtained

References

1. Compiani, R., Grossi, S., Lucini, L. and Sgoifo Ross, C.A. Prevention of the main Clostridial diseases in cattle. *Large Animal Review*, **27**(1), 51-56 (2021).
2. Katharine, M.S., Callan, J.R and David, C.V. Clostridial abomasitis and enteritis in ruminants. *Veterinary Clinical North American Food and Animal Practice*, **34**(1), 155–184 (2018).
3. Mamsin, A. M., Barzani, K. Kh. and Mohammed, B. T. Genotyping and Phylogenetic Analysis of *Clostridium perfringens* Isolated from Domesticated Ruminants in Duhok Governorate, Iraq. *Egyptian Journal of Veterinary Sciences*, **54**(6), 1215–1226 (2023).
4. Uzal, F.A., Navarro, M.A., Jihong, L., Freedman, J.C., Shrestha, A. and McClane, B.A. Comparative pathogenesis of enteric clostridial infections in humans and animals. *Anaerobe*, **53**,11-20 (2018).
5. Goharia I.M., Navarro, M. A., Li, J., Shrestha, A., Uzal, F. and McClane, B. A. Pathogenicity and virulence of *Clostridium perfringens*. *Virulence*, **12**(1), 723–753 (2021).
6. Nasir, A.A., Younus, M., Rashid, A., Abdul Khaliq, A., Khan, E., Shah, S.H, Aslam, A., Ghumman M.A. and Joiya, M.H. Clinical pathological findings of *Clostridium perfringens* type D enterotoxaemia in goats and its hemolytic activity in different erythrocytes. *Iranian Journal of Veterinary Research*, **16**(1), 94 -99 (2015).
7. Das, A., Mazumder, Y., Dutta, B., Shome, B. and Bujarbaruah, K. Molecular typing of *Clostridium perfringens* isolated from diarrheic cattle. *Journal of Animal Sciences Advances*, **2**, 226-229 (2012).
8. Selim, A.M., Elhaig, M.M., Zakaria, I. and Ali, A. Bacteriological and molecular studies of *Clostridium perfringens* infections in newly born calves. *Tropical Animal Health and Production*, **49**(1), 201-205 (2017).
9. Nasr, M., Bakeer, N.M., Hammouda, H.A. and Omar, A. A. Epidemiological, Clinical and Bacteriological Studies on Bacterial Lamb Enteritis at Behera Province, Egypt. *Alexandria Journal of Veterinary Sciences*, **43**(1), 8-16 (2014).
10. Atwa, E.I., Sharaf, E.M. and Zakary, E.M. Bacterial diarrhea in newly born calves in Menoufeya governorate. *Assiut Veterinary Medical Journal*, **58**(135), 126 – 137 (2012).
11. Khiav, L.A. n and Azadeh, Z. Vaccination against pathogenic clostridia in animals: a review. *Tropical Animal Health and Production*, **53**(2), 284 – 296 (2021).
12. Abd Al-Tawab, A., El-Hofy, F.I., Khater, D.F. and Kotb, M.A. Typing of *Clostridium perfringens* isolated from some meat products by using PCR. *Benha Veterinary Medical Journal*, **29**(1), 118-123 (2015).
13. Helal, S.S., Khalaf, N.M., El Menisy, A.A. and Lebdah, M.A. *Clostridium perfringens* type A causing necrotic enteritis outbreaks among chickens in Egypt. *Zagazig Veterinary Journal*, **47**(4), 398-407 (2019).
14. Yoo, H.S., Lee, S.U., Park, K.Y. and Park, Y.H. Molecular typing and epidemiological Survey of prevalence of *Clostridium perfringens* types by multiplex PCR. *Journal of Clinical Microbiology*, **35**(1), 228-232 (1997).
15. Smith, L.D. and Holdeman, L. The pathogenic anaerobic bacteria, Springfield, Illinois, Charles C. Thomas, USA. pp 423 (1969).
16. Mirbehresi, H., Pirhadi, M., Shokri, S. and Esmaeili, A. Epidemiological aspects of poisoning Infectious-toxic bacteria of botulism on food poisoning in Iran; A review study. *Egyptian Journal of Veterinary Sciences*, **53**(1), 25–30 (2022).
17. Otter, A. and Uzal, F. A. Clostridial diseases in farm animals: 1. Enterotoxaemias and other alimentary tract infections. *In Practice*, **42**(4), 187-248 (2020).
18. Omer, S.A., Babiker, S.H., Aljulaifi, M.Z.N., Al-Olayan, E.M., Alagaili, A.N. and Mohammed, O.B.

- Epidemiology of enterotoxaemia in livestock in the Kingdom of Saudi Arabia. *Journal of King Saud University*, **32**(5), 2662-2668 (2020).
19. Maurya, V. "Bacterial Diseases of Goats." in Principles of Goat Disease and Prevention, John Wiley & Sons, Inc, Hoboken, New Jersey, USA. pp 95-110 (2023).
20. Mohammadabadi, M.R. Role of *clostridium perfringens* in pathogenicity of some domestic animals. *Journal of Advances in Agriculture*, **7**(3), 1117-1121 (2017).
21. Ahsani, M.R., Mohammadabadi, M.R. and Shamsaddini, B.M. *Clostridium perfringens* isolate typing by multiplex PCR. *Journal of Venomous Animals and Toxins including Tropical Diseases*, **16**(4), 573-578 (2010).
22. Baums, C.G., Schotte, U., Amtsberg, G. and Goethe, R. Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. *Veterinary Microbiology*, **100**(1-2), 11-6 (2004).
23. Tutuncu, M., Kiliçoğlu, Y., Guzel, M., Pekmezci, D. and Gulhan, T. Prevalence and toxin typing of *Clostridium perfringens* enterotoxins in small ruminants of Samsun Province, Northern Turkey. *Journal of Animal and Plant Science*, **28**(4), 1204-1207 (2018).
24. Nazki, S., Wani, S.A., Parveen, R., Ahangar, S.A., Kashoo, Z.A., Hamid, S., Dar, Z.A., Dar, T.A. and Dar, P.A. Isolation, molecular characterization and prevalence of *Clostridium perfringens* in sheep and goats of Kashmir Himalayas, India. *Veterinary World*, **10**(12), 1501-1507 (2017).
25. Moustafa, S., Zakaria, I., Moustafa, A., AboSakaya, R. and Selim, A. Molecular epidemiology and genetic characterization of *Clostridium perfringens* infections in lambs. *Microbial Pathogenesis*, **173**(A), 105822 (2022).
26. Moustafa, S., Zakaria, I., Moustafa, A., AboSakaya, R. and Selim, A. Bacteriological and serological investigation of *Clostridium perfringens* in lambs. *Scientific Reports*, **12**, 19715 (2022).
27. Nayel, M., El-Sify, A., Akram, S., Allaam, M. and Abdeen, E. Molecular Typing of *Clostridium perfringens* isolates from soil, healthy, and diseased sheep in Egypt by Multiplex PCR. *Journal of Veterinary Medical Research*, **22**(1), 53-57 (2013).

النمط الوبائي وطرق التشخيص للتسمم المعوي بين صغار المجترات في محافظة كفر الشيخ، مصر

يامن حجازي^{1,4*}، عاطف عربي¹، حسب الله أبو الغيط²، محمد حجازي²، علاء المنيسي³، رمضان سالم¹، محمد سالم⁴ و مجدي الجعبري¹

¹ قسم طب الحيوان (الأمراض المعدية)، كلية الطب البيطري، جامعة كفر الشيخ، كفر الشيخ، 33511، مصر

² معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، 9 نادي الصيد، 12618، الدقي، الجيزة، مصر.

³ معهد بحوث الأمصال واللقاحات البيطرية - ش السكة البيضاء العباسية، القاهرة، مصر.

⁴ قسم الدراسات السريرية، كلية الطب البيطري، جامعة الملك فيصل، الأحساء 31982، المملكة العربية السعودية

يعتبر التسمم المعوي مسؤولاً عن ارتفاع معدل الوفيات بين صغار المجترات مع خسائر اقتصادية فادحة للمزارعين. وباستخدام استبيان منظم، تم دراسة معارف المزارعين وممارساتهم المسؤولة عن انتشار مرض التسمم المعوي بين أنواع الحيوانات المجترية في 16 مزرعة في مصر. كما تم فحص عدد 70 عجلًا وحملًا ماتوا فجأة بسبب التهاب الأمعاء النزفي، وتم جمع عينات من الأمعاء لعزل العوامل المسببة جراثيمياً. تم التعرف على السموم في محتويات أمعاء المجترات الصغيرة الميئة وتم التعرف على الجينات المسؤولة عن إنتاج السموم في بكتريا كلوستريديا البرفرينجينز المعزولة باستخدام تفاعل البلمرة المتسلسل المتعدد. أظهرت النتائج معرفة المزارعين الجيدة بمرض التسمم المعوي وكشفت أنه مسؤول عن 25% من وفيات صغار المجترات على الرغم من إجراءات التحصن المكثفة ضد التسمم المعوي. وذكر المزارعون أن معظم الحالات تحدث في فصل الشتاء وخصوصاً بعد تفشي مرض الحمى القلاعية. لا يتبع معظم المزارعين إجراءات الحجر الصحي للحيوانات المشتراة حديثاً ولا يختارون شراء الحيوانات المحصنة فقط. تم التعرف على كلوستريديا البرفرينجينز من النوع A وسم ألفا فقط من عينتين من العجول الميئة، في حين تم التعرف على كلوستريديا البرفرينجينز من النوع D وسموم ألفا وإيسيلون من جميع عينات الحملان والعجول الميئة الأخرى. في الختام، يجب النظر في السيطرة على تسمم الدم المعوي خاصة في مواسم الشتاء والأوقات المعروفة لتفشي مرض الحمى القلاعية. كلوستريديا البرفرينجينز من النوع A و من النوع D هو العامل المسبب السائد لتسمم الدم المعوي في العجول والحملان في منطقة الدراسة.

الكلمات الدالة: كلوستريديا البرفرينجينز، التسمم المعوي، علم الأوبئة، المجترات، تفاعل البلمرة المتسلسل المتعدد.