Assiut University web-site: www.aun.edu.eg

NETB, A NEW TOXIN RELATED TO *CLOSTRIDIUM PERFRINGENS*-INDUCED AVIAN NECROTIC ENTERITIS IN BROILER CHICKENS

RAGAB FAROUK; OMAR AMEN; AHMED HASSAN AND RAGAB SAYED IBRAHIM

Department of Avian and Rabbit Diseases, Faculty of Vet. Medicine, Assiut University, Egypt.

Received: 5 October 2021; **Accepted:** 31 October 2021

ABSTRACT

Necrotic enteritis is well-defined as poultry disease which occures as a result of liberation of toxins from *Clostridium perfringens* type A, C, D, and G pathogenic strains. This study was performed to determine the prevalence rate of *C.perfringens* in broilers from different farms in Assiut Governorate and to examine the isolates for the existence of alpha, beta, epsilon, and netB gene. A sum of 100 intestinal specimens were compiled from diseased broiler chickens (3-6 W) which had clinical signs and post mortem lesions of Necrotic Enteritis and examined by conventional and molecular methods. *C.perfringens* was isolated from 52% (52/100) of the bacteriologically examined flocks. Only ten isolates among suspected isolates were examined by using uniplex and multiplex PCR. The results reported 100% positivity for cpa and netB gene in the examined isolates and neither cpb nor etx gene were detected and proved that all isolates were *Clostridium perfringens* type G.

Keywords: *Clostridium perfringens*, Broiler, Multiplex Polymerase Chain Reaction, NetB gene.

INTRODUCTION

Necrotic enteritis is a serious disease that affects the poultry industry all over the world (Ali and Islam, 2021). Globally, economic losses to the poultry industry due to NE are estimated to be more than $6 billion per year (Moore, 2016).

Corresponding author: Ragab Farouk
E-mail address: ragabfarouk@vet.aun.edu.eg
Present address: Department of Avian and Rabbit Diseases, Faculty of Vet. Medicine, Assiut University, Egypt.

NE is the most commonly caused by *Clostridium perfringens*. It's a rod-shaped, gram-positive, anaerobic spore-forming bacteria that's found throughout the gastrointestinal tract (Ali *et al*., 2020).

Approximately more than 17 exoproteins which known to be toxic are created by *Clostridium perfringens*, leading to a new toxigenic classification including seven types (A–G) according to group of six exotoxins which are released from bacteria. (Alpha, beta, epsilon, iota, CPE, and NetB). *C. perfringens* types A (alpha

toxin), C (beta, and alpha toxins), and G (Net toxin) are the most common causes of NE (Rood *et al*., 2018 and McMullin, 2020).

Recently, two novel types of toxins (F and G) were described, Type F generates enterotoxin (cpe gene), which are responsible for food poisoning disease, while type G produces NetB toxin (netB gene) which causes NE in brolier chickens (Anju *et al*., 2021).

NetB, a pore-forming toxin, is considered to be the major virulence agent (Profeta *et al.*, 2020). It is in charge of the new strain (type G) synthesis which was previously belonged to *Clostridium. perfringens* type A (AKM *et al*., 2021).

For years, studies have focused only on the function of toxins, Phospholipase C, which induces hydrolysis of the phospholipids and causes destruction of the enterocyte wall and leads to cell death. The function of CPA in the generation of NE was, however, returned when CPA-deleted mutants maintained full virulence in vivo as a result the NetB toxin was discovered (Keyburn *et al*., 2006 and Paiva and McElroy, 2014).

NetB-positive strains have the capability to enhance lesions of NE, while negative strains unable to induce NE lesions (Keyburn *et al*., 2008).

The NetB toxin causes pores to creates bilayers in human and animal cell phospholipid membrane, permitting ions (such as Na+, Cl-, Ca2+, and others) to get through and leads to osmotic cell lysis (Datta *et al*., 2014). So, this study was designed to determine the prevalence of *C.perfringens* in broiler chickens from different farms in Assiut Governorate.

MATERIALS AND METHODS

Sampling:

From February to August 2021, 100 intestinal specimens were collected aseptically from newly sacrificed 3-6 weeks aged broiler chickens from different 18 poultry farms in Assiut governorate. After a postmortem inspection, the samples were taken from sections of the intestine that had macroscopic lesions which were thought to be NE. As quickly as possible, samples were sent in an ice box to the laboratory of faculty of Veterinary Medicine-Assiut University.

Bacteriological and biochemical examination:

Inoculating intestinal samples into a cooked meat broth medium (Oxoid,UK) and incubation for 48 hours at 37°C in an anaerobic gas pack jar was used to isolate *Clostridia*. In an anaerobic environment, a loop of broth culture was streaked onto blood agar plates containing 7% sheep blood agar then incubated anaerobically for 48 hours at 37°C. For purification, colonies with double hemolytic zones were chosen and subcultured in Reinforced *Clostridial* agar. (LAB M 23) (Willis, 1977). Microscopic examination and biochemical tests were used for identification of isolates according to Macfaddin, (2000).

Genotyping of the Toxigenic *Clostridium perfringens* Isolates:

According to the manufacturer's recommendations, DNA was extracted from *C. perfringens* isolates using the QIAamp DNA Mini Kit (QIAGEN). Specific oligonucleotide primer sequences for the α, β and ε genes of C. perfringens and the NetB toxin gene, according to Yoo *et al*. (1997) and Bailey *et al*. (2013) respectively were purchased

from Midland Certified Reagent Company, (Oligos). The details of primers and amplification cycling condition were listed in table (1 and 2).

For DNA amplification 50 µL of the following reaction mixture (For α, β and ε toxins): 6 µL of DNA template, 25 µl Emerald Amp GT PCR master mix (2x premix), 1 µL from each primer (20 pmol µL), and 13 µl of DNase-RNase-free water. Specific preparation of the reaction mixture For NetB toxin was done as the following: 5 µl of DNA template, 12.5 µL EmeraldAmp GT PCR master mix, 1 µL from each NetB primers (20 pmol µL), 5.5 µL of DNase-RNase-free water, to a total volume of 25 µL.

Table 1: *Clostridium perfringens* specific genes and primer sequences used for molecular identification and typing

Toxin	Primer	Sequence	Amplified product	Reference
Alpha Toxin	F	GTTGATAGCGCAGGACATGTTAAG	402 bp	Yoo *et al*., 1997
	R	CATGTAGTCATCTGTTCCAGCATC		
Beta Toxin	F	ACTATACAGACAGATCATTCAACC	236 bp	
	R	TTAGGAGCAGTTAGAACTACAGAC		
Epsilon Toxin	F	ACTGCAACTACTACTCATACTGTG	541 bp	
	R	CTGGTGCCTTAATAGAAAGACTCC		
Net B Toxin	F	CGCTTCACATAAAGGTTGGAAGGC	316 bp	Bailey *et al*., 2013
	R	TCCAGCACCAGCAGTTTTTCCT		

Table 2: Cycling conditions of the different primers during PCR

Toxin	**Primary denaturation**	**Secondary denaturation**	**Annealing**	**Extension**	**No. of cycles**	**Final extension**
Alpha, Beta and Epsilon	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
NetB toxin	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.

Finally, using 1.5 percent agarose gel in the presence of a 100-bp DNA ladder provided by QIAGEN 20 µL of the amplified product of the NetB toxin gene and 30 µL of the amplified product of the α, β and ε toxin genes were electrophoresed. To observe under UV light, the agarose gel was treated with ethidium bromide. A gel documentation system was used to photograph the gel, and computer software was used to evaluate the data.

RESULTS

Bacteriological and Biochemical:

Among 100 intestinal samples 52 were identified. The colony on reinforced *clostridial* agar were large, regular, round, slightly opaque but shiny colonies, as shown in Fig.1. On sheep blood agar colonies were surrounded by double zone of haemolysis, as shown in Fig.2. Gram's-stained smears showed that the organism was Gram positive large bacilli, and often coccobacillus or short

rod forms with blunt end, as shown in Fig.3.

Biochemically, all isolates produced milk digestion on litmus milk medium as shown in Fig .4 and lecithinase activity on egg yolk agar as shown in Fig.5.

Genotypic Detection of *C. perfringens* Toxins:

Multiplex PCR was used to genotype toxigenic *C. perfringens* isolates. The 10 toxigenic isolates all contained the alpha gene (402 bp) and none of them had the genes beta (236 bp) or epsilon (541 bp), as shown in Fig. 6. All isolates were proven to be positive for the netB gene, which generates the NetB toxin, using a Uniplex PCR for the identification of this gene, as shown in Fig.7. This explain that all isolates were *C. perfringens* type G (10/10), as justified by the presence of the alpha and Net B genes (table.3)

Table 3: New revised classification of *C.perfringens* typing scheme toxin-based (Rood *et al.*, 2018)

Toxin produced	α-toxin	β-toxin	ε-toxin	ι-toxin	CPE	Netβ
A	+	-	-	-	-	-
B	+	+	+	-	-	-
C	+	+	-	-	+/-	-
D	+	-	+	-	+/-	-
E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

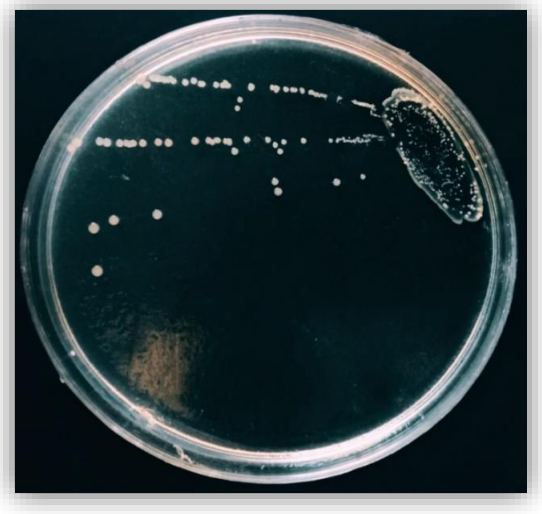

Fig.1: Typical colonies presumed to be *C. perfringens* on reinforced *clostridial* agar medium

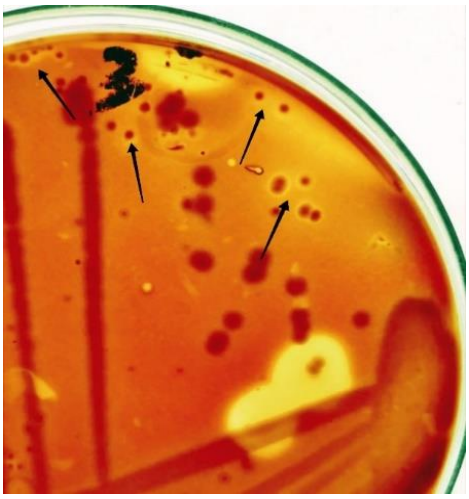

Fig.2: *C. perfringens* colonies surrounded by double zone of haemolysis on sheep blood agar medium

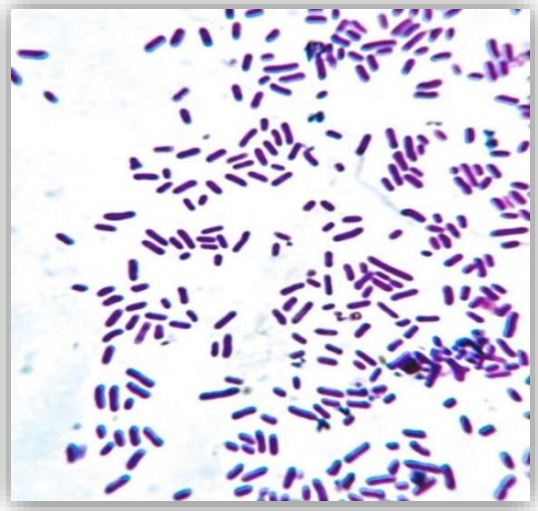

Fig.3: Showing the gram positive bacilli of *C. perfringens.*

Fig.4: Stormy reaction and clotting of milk of *C. perfringens* on litmus milk media

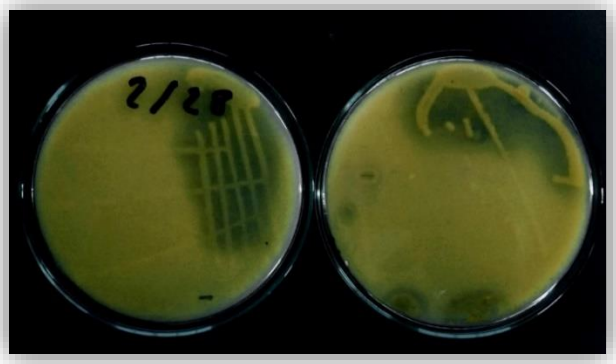

Fig.5: Showing lecithinase activity on egg yolk agar medium

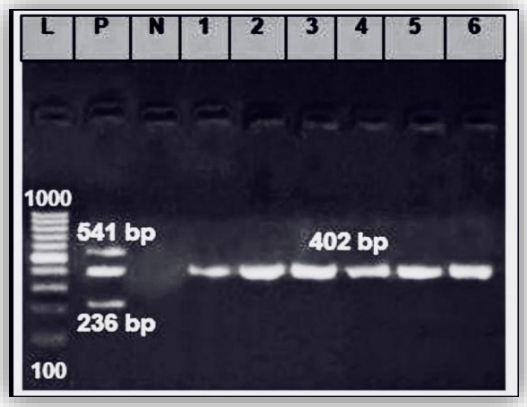

Fig.6: Typing of *C. perfringens* toxin genes by Multiplex PCR. Pos: positive control; Neg: negative control; L: DNA ladder (molecular weight marker 100-bp); lane 1-6: cpa positives *C. perfringens* toxigenic isolate

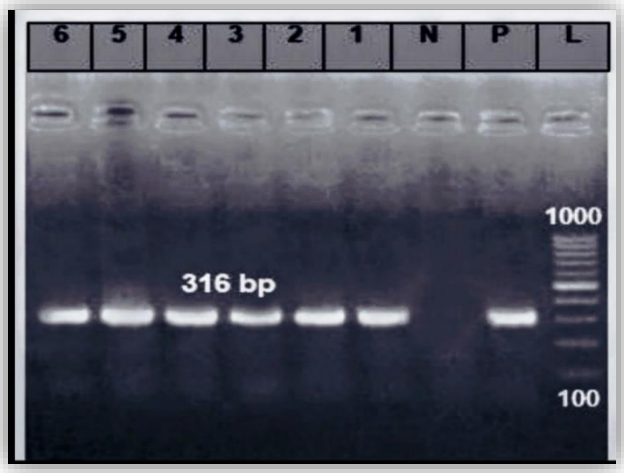

Fig. 7: Uniplex PCR identification of netB gene. Pos: positive control; Neg: negative control; L: DNA ladder (molecular weight marker 100-bp); lane 1-6: netB positive

DISCUSSION

Necrotic enteritis (NE) is a serious avian disease, with global losses estimated at $5 to $6 billion (Moore, 2016). The most common encountered signs observed in the examined birds were depression, decreased appetite, lowered growth rates and diarrhoea. These findings were recorded by several authors as Elwinger *et al. (*1992); Kaldhusdal *et al*. (2001) and Ibrahim *et al.* (2017).

The most significant postmortem lesions include swelling in small intestines with an unpleasant-smelling brown fluid, the pseudomembrane formation which is a brownish membrane lining the inner coat of the gut and necrotic hepatitis which characterized by small, white, pinpoint-like foci in the liver (Timbermont *et al*., 2011; Rizk *et al*., 2017 and Yadav and Sandeep, 2021).

Different broiler chicken flocks in Assiut Province were examined for *Clostridium perfringens* and the results recorded that 52 of the 100 suspected samples were positive to *Clostridium perfringens*. This explains that other infections may have had a role in the development of these lesions.

It is worth mentioning that in the present study that *C. perfringens* type G was isolated for the first time in Assiut Province (Table 3).

In the current investigation, the prevalence that rate of C. *perfringens* was 52% (52/100) in the examined flocks. This result is nearly similar to that of Abd El-wahab (2002), Mahmoud *et al.* (2008) and Shaaban *et al.* (2017) who recorded that the infection rate of *C. perfringens* was 54.4% and 54.1% and 55.9% respectively. However, lower prevalence rates of *C.perfringens* were detected by Abd-El Gwad and El-Kader (2001); Mostafa *et al. (*2016) and Merati *et al*. (2017), who detected a prevalence rates of *C.perfringens* was 44,4% , 16% and 34.44% respectively. Changes in prevalence rates across studies might be due to differences in sample methods, condition of the birds (healthy or diseased), and managemental practices inside the farm associated with antibiotic usage policies.

NetB has been identified as one of the primary virulence factors in NE outbreaks in birds (Keyburn *et al.,* 2008). Type G of *C. perfringens* has been associated with cpa and NetB gene for the generation of α and NetB toxin and it is believed to be significant in the pathogenesis of NE in poultry. In the present study, all isolates (10/10) were positive for the CPA and NetB gene and grouped as toxin types G. These results are in line with the findings of AKM *et al.* (2021) and Khan *et al*. (2021) who found types G (α-toxin, NetB positive) of *C. perfringens* from fecal samples of commercial broilers from Pakistan and Japan respectively.

The existence of the netB gene in isolates from various countries was reported by several studies.in a study in Canada out of 41 isolates from broilers with NE 39 (95%) were positive to netB, while just 35 % of healthy boilers infected with *C. perfringens* was identified positive to netB gene (Chalmers *et al*., 2008). However, in the United States, the prevalence rate of netB gene was 58 % in birds with necrotic enteritis, otherwise, the netB gene was found in only 8.75 percent of normal microbiota strains (Martin and Smyth, 2009).

In fact, the netB gene is significantly linked to necrotic enteritis-produced strains, although in some research a small percentage of diseased birds was recorded as negative to netB and a small percentage of healthy birds were reported as positive to netB gene. As NE is a complex disease, the lack of disease in positive netB strains might be contributed to absence of predisposing factors.

Not only netB gene is responsible for NE in birds but also other virulence factor might be produced from *C.perfringens* and induce NE. Despite, in vitro avian models, netB-negative strains do not produce disease. Thus, It's essential to detect the capacity of both netB-positive strains isolated from healthy birds and netB-negative strains isolated from diseased birds to produce NE to identify if netB is necessary for virulence. Induction of broilers by NetB-positive and NetB-negative strains in a large number produced an excellent association between NetB and the capacity to produce NE. (Keyburn *et al.*, 2010 and Martin and Smyth, 2010).

Based on this finding, the highest prevalence rate of *C. perfringens* was detected in 3-6 weeks old broiler chicks. This might be explained by the risk of NE increases by insufficient levels of maternal antibodies in the circulatory system of birds up to 3 weeks of age. Stress could be one of the major causes produced by intestinal flora changes as a result of alternation of diet from starter to growth which facilities the environment spreading of *C. perfringenes (*Moore, 2016).

CONCLUSION

The decreased age (3-6 weeks) is considered as risk factor which cause high occurrence of *C. perfringens* in broiler chicks and *Clostridium perfringens* type G is the most prevalent in Assiut province. The NetB is a key factor in the pathogenesis of NE.

REFERENCE

Abd-El Gwad, A.M. and El-Kader, H.A.A. (2001): The occurrence of *Clostridium perfringens* in the intestine of broiler chickens in Assiut Governorate. The occurrence of clostridium perfringens in the intestine of broiler chickens in assiut governorate. The Assiut University Bulletin for Environ. Res. 4(2): Pages are missing.

Abd El-Wahab, H.S. (2002): Studies on *Clostridium* microorganisms in newly hatched poultry. M.V.Sc. Thesis, Microbiology, Fac. Vet. Med., Cairo University.

AKM, A.; Nakatani, M.; Nakajima, T.; Kohda, T. and Mukamoto, M. (2021): The cytotoxicity and molecular mechanisms of the *Clostridium perfringens* NetB toxin. Journal of Veterinary Medical Science, 83(2):187-194.

Ali, M.Z. and Islam, M.M. (2021): Characterization of β-lactamase and quinolone resistant *Clostridium perfringens* recovered from broiler chickens with necrotic enteritis in Bangladesh. Iranian journal of veterinary research, 22(1), 48.

Ali, M.Z.; Islam, M.M. and Zaman, S. (2020): Effects of Turmeric Powder on *Clostridium Perfringens* Load in Broiler Chickens. *SAARC Journal of Agriculture*, *18*(1): 209-218.

Anju, K.; Karthik, K.; Divya, V.; Priyadharshini, M.L.M.; Sharma, R.K. and Manoharan, S. (2021): Toxinotyping and molecular characterization of antimicrobial resistance in *Clostridium perfringens* isolated from different sources of livestock and poultry. Anaerobe, 67, 102298.

Bailey, M.A.; Macklin, K.S. and Krehling, J.T. (2013): Use of a Multiplex PCR for the Detection of Toxin-Encoding Genes netB and tpeL in Strains of *Clostridium perfringens*. *International Scholarly Research Notices, 2013*.

Chalmers, G.; Bruce, H.L.; Hunter, D.B.; Parreira, V.R.; Kulkarni, R.R. and Jiang, Y.-F. (2008): Multilocus sequence typing analysis of *Clostridium perfringens* isolates from necrotic enteritis outbreaks in broiler chicken populations. Journal of Clinical Microbiology, (46): 3957-3964.

Datta, S.; Rakha, N.K.; Narang, G.; Arora, D. and Mahajan, N.K. (2014): Prevalence of α, β and netb toxin producing strains of *Clostridium perfringens* in broiler chickens in Haryana. Haryana Vet, 53(1): 39-42.

Elwinger, K.; Schneitz, C.; Berndtson, E.; Fossum, O.; Teglo¨F, B. and Engstro¨m, B. (1992): Factors affecting the incidence of necrotic enteritis, caecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. Acta Veterinaria Scandinavica. (33):369-378.

Ibrahim, G.A.; Mahmoud, B.S.; Ammar, A.M. and Youssef, F.M. (2017): Toxin genotyping of *C. perfringens* isolated from broiler cases of necrotic enteritis. Animal and Veterinary Sciences., 5(6): 108.

Kaldhusdal, M.; Schneitz, C.; Hofshagen, M. and Skjerve, E. (2001): Reduced incidence of *Clostridium perfringens*-associated lesions and improved performance in broiler chickens treated with normal intestinal bacteria from adult fowl. Avian Diseases, (45): 149 -156.

Keyburn, A.L.; Sheedy, S.A.; Ford, M.E.; Williamson, M.M.; Awad, M.M.; Rood, J.I. and Moore, R.J. (2006): Alpha-toxin of *Clostridium perfringens* is not an essential virulence factor in necrotic enteritis in chickens. Infection and immunity, 74(11): 6496-6500.

Keyburn, A.L.; Boyce, J.D.; Vaz, P.; Bannam, T.L.; Ford, M.E.; Parker, D. and Moore, R.J. (2008): NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens*. PLoS pathogens, 4(2): e26.

Keyburn, A.L.; Yan, X.-X.; Bannam, T.L.; Van Immerseel, F.; Rood, J.I. and Moore, R.J. (2010): Association between avian necrotic enteritis and *Clostridium perfringens* strains expressing NetB toxin. Veterinary Research, 41: 21

Khan, M.U.Z.; Liu, B.; Yang, S.; Xu, X.; Wang, Y. and Cai, J. (2021): Genetic Diversity of *Clostridium perfringens* Strains Isolated from Broiler Chickens Revealed by PFGE Analysis in China and Pakistan. Pakistan Veterinary Journal, 41(1).

Macfaddin, J.F. (2000): Biochemical test for identification of medical bacteria 3rd edn., LippinCott Willians and wilkins, Philadelphia, Pa.

Mahmoud, A.A.; Abdel-Elah, M. and Mohamed, A.I. (2008): Study the high Mortalities of necrotic enteritis in Layer Flocks at North Sinai

Governorate. Egypt J. Comp. Pathol. Clin. Pathol., 21 (1): 360–371.

Martin, T.G. and Smyth, J.A. (2009): Prevalence of netB among some clinical isolates of *Clostridium perfringens* from animals in the United States. Veterinary Microbiology, (136): 202-205.

Martin, T.G. and Smyth, J.A. (2010): The ability of disease and nondisease producing strains of *Clostridium perfringens* fradhere to extracellular matrix molecules and Caco-2 cells. Anaerobe. (16): 533-539.

McMullin, P.F. (2020): Diseases of poultry 14th edition: David E. Swayne, Martine Boulianne, Catherine M. Logue, Larry R. McDougald, Venugopal Nair, David L. Suarez, Sjaak de Wit, Tom Grimes, Deirdre Johnson, Michelle Kromm, Teguh Yodiantara Prajitno, Ian Rubinoff and Guillermo Zavala (Eds.), Hoboken, NJ, John Wiley and Sons, 2020, 1451 pp., £ 190 (hardcover)/£ 171.99 (e-book), ISBN 9781119371168.

Merati, R.; Temim, S. and Mohamed, A.A.A.F. (2017): Identification and Characterization of *Clostridium perfringens* Isolated from necrotic Enteritis in Broiler Chickens in Tiaret, Western Algeria.

Moore, R.J. (2016): Necrotic enteritis predisposing factors in broiler chickens. Avian Pathology., 45(3): 275-281.

Mostafa, A.H.; Abdeen, E.E. and Abou-Hadeed, M.G. (2016): Multiplex PCR and detection of Net B gene of *Clostridium Perfringens* from broiler with necrotic enteritis. Asian Journal of Animal Veterinary Advances. (11): 248- 252.

Paiva, D. and McElroy, A. (2014): Necrotic enteritis: applications for the poultry industry. Journal of Applied Poultry Research., 23(3): 557-566.

Profeta, F.; Di Francesco, C.E.; Di Provvido, A.; Scacchia, M.; Alessiani, A.; Di Giannatale, E. and Marsilio, F. (2020): Prevalence of netB-positive *Clostridium perfringens* in Italian poultry flocks by environmental sampling. Journal of Veterinary Diagnostic Investigation, 32(2): 252-258.

Rizk, A.; Umar, S.; Munir, M.T. and Tariq, M. (2017): Replacements of antibiotics in the control of necrotic enteritis: a review. Sci Lett., 5(3): 208-216.

Rood, J.I.; Adams, V.; Lacey, J.; Lyras, D.; McClane, B.A.; Melville, S.B. and Van Immerseel, F. (2018): Expansion of the *Clostridium perfringens* toxin-based typing scheme. Anaerobe. (53): 5-10.

Shaaban, A.; A Zoulfakar, S.; I Youssef, Y. and Shalaby, B. (2017): The incidence of *C. perfringens* in chickens in different seasons and Governorates in Egypt. Journal of Veterinary Medical Research., 24(1): 12-20.

Timbermont, L.; Haesebrouck, F.; Ducatelle, R. and Van Immerseel, F. (2011): Necrotic enteritis in broilers: an updated review on the pathogenesis. Avian Pathology., 40(4): 341-347.

Willis, A.T. (1977): Anaerobic Bacteriology: Clinical and Laboratory Practice, 3 rd edn, Butterworths, London.

Yadav, J.V. and Sandeep, G.S. (2021): Diagnosis of *Clostridium perfringens* infection (Necrotic enteritis) in a flock of Giriraja birds.

Yoo, H.S.; Lee, S.U.; Park, K.Y. and Park, Y.H. (1997): Molecular Typing and Epidemiological Survey of Prevalence of *Clostridium perfringens* Types by Multiplex PCR. Journal Of Clinical Microbiology.Vol. 35, No. 1. P: 228-232.

توكسين نت بي الجديد المرتبط بالكلوستريديوم بيرفرينجينز والمسبب لمرض النخر المعوي في دجاج التسمين

رجب فاروق ، عمر امين ، احمد حسن ، رجب سيد ابراهيم

E-mail: ragabfarouk@vet.aun.edu.eg Assiut University web-site: www.aun.edu.eg

اجريت هذه الدراسة بهدف معرفة مدى انتشار الكلوستريديوم بيرفرينجينز في كتاكيت التسمين في محافظة اسيوط وكذلك لعزل وتحديد العامل المسبب للتنكرز المعوي والجينات المسئولة عن السمية في المعزولات. تم جمع مئة (١٠٠) عينة من الأمعاء من الكتاكيت المصابة أو نافقة حديثا يتراوح أعمارها من ٣ الي ٦ أسابيع. تم تسجيل الأعراض المرضية الظاهرة علي الكتاكيت المصابة علي شكل انخفاض في حيويتها، وامتناعها عن الاكل والشراب مع وجود اسهالات ، ولوحظ ان بعضها مصابة باضطرابات حركية. وتم تسجيل الصفة التشريحية التي بينت تضخم في جدران الامعاء مع وجود مناطق بها نخر شديد، ووجود تشققات عميقة ومناطق ذات اسطح محببة ، كما ان الامعاء تحتوي علي مخلفات دموية متساقطة من جدرانها ذات رائحة كريهة. اسفرت نتائج الاختبارات البكترولوجية المستحدمة لعزل الميكروب والتعرف عليه (مورفولوجيا الخلايا والمستعمرات البكترية والاختبارات البيوكيمائية) عن عزل اثنتين وخمسين (٥٢) عترة محتملة للميكروب قيد الدراسة وذلك بنسبة ٥٢% . وللتعرف الجزئيي تم اجراء تقنية تفاعل البلمرة المتسلسل علي بعض العترات المعزولة عشرة (١٠) والذي اظهر أن جميع العترات المختبرة ايجابية لبكتريا الكلوستريديوم بيرفرينجينز (لكلا من الفا والنت بي جين) مما يجدر الاشارة اليه من نتائج الاختبار الاخيره (تفاعل البلمرة المتسلسل) ان الكلوستريديوم بيرفرينجينز من النوع جي وتم تسجيله لاول مرة في محافظه اسيوط.