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Isolation and identification of C. perfringens from field chickens suspected to be infected with Necrotic Enteritis in relation to season.

A. S. Hamouda*, M.M. Amer*, A. Abd El- F. Mohamed and R. Merati*****

* Poult. Dis. Dept., Facult. of Vet. Med. Cairo University
** Anaerobic Dept. SVRI. Abassia.
*** Ben khaldon University, Algeria.

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SUMMARY

Examined field chickens with signs and lesions of natural infection with necrotic enteritis (NE) revealed the isolation of Clostridium perfringens (C. perfringens) from positive chickens at the rate of 63.33%, 30%, 30% and 66.66% in autumn, winter, spring and summer; respectively. The overall positive samples were 57 out of examined 120 chickens with incidence of 47.5 %. Regarding chicken breed, isolation rate in autumn were 65% and 60.0%, in winter 35.0% and 20.0% , in spring 25.0% and 40.0%, in summer 85.0% and 30.0% was recorded out of broilers and layers; respectively.

C. perfringens isolates were serologically typed into 24 type A, 3 type C and 30 nontoxigenic isolates. Type A isolates were 5, 2, 6 and 11 in autumn, winter, spring and summer; respectively. While type C isolates were 2 in autumn and 1 in spring. C. perfringens Type A recovered from broiler and layer were 2 and 3, 2 and 0, 4 and 2, 10 and1 in autumn, winter, spring and summer; respectively. Only 2 isolates of type C were recovered in autumn and 1 in spring.

INTRODUCTION

Necrotic Enteritis (NE) is a worldwide poultry disease caused by the alpha toxin-producing bacterium C. perfringens that can cause both clinical and subclinical disease in poultry (Engström et al., 2003; Saif et al., 2003 and Williams, 2005).

Toxigenic strains were isolated from both diseased and healthy chickens (Timbermont et al., 2009). The disease risk factors include concurrent coccidial infection or vaccine and the dietary use of cereal

grains high in nonstarch polysaccharides (NSP), such as wheat, barley, rye, and oats (Saif et al., 2003; Pedersen et al., 2008; Jia et al .,2009 and Palliyeguru et al., 2010).

NE in chickens caused economic losses due to mortalities, low growth rate and poor feed conversion rate (FCR) (Lavland and Kaldhusdal, 2001) as well as costs associated with disease prevention. On the other hand, it is difficult to determine the prevalence of the mild infection in chickens that cause high condemnation rates in broilers due to hepatitis (Lavlandand and Kaldhusdal, 1999).

From the above mentioned finding our study was planed to investigate the recent situation of NE by isolation and identification of C. perfringens from diseased broiler and layer chickens in relation to seasons of the year as well as typing of the obtained C. perfringens isolates.

Key words: Necrotic enteritis- chickens- C. perfringens- Seasonal variation.

MATERIALS AND METHODS

Chicken flocks for samples.

A total of 120 samples were collected, representing the four year seasons as 30 samples/ season. The seasonal samples including 20 broilers and 10 layers chickens showing signs and gross lesions suspected to be NE. Examined chickens showed signs of depression, decreased appetite, decreased feed intake, low growth rate and diarrhea. Lesions were seen in the middle of small intestine that had friable wall and distended with gases. Intestinal mucosa was covered by a loose or adherent yellow to green necrotic membrane with or without serosal necrotic and hemorrhagic foci.

Sampling:

Parts from the small intestine and livers were placed in plastic bags and transferred in an ice box to the laboratory for anaerobic bacteriological examination.

Culture media:

1- Solid media.

The following solid media were used according to Cruickshank et al. (1975). Sheep blood agar medium, enriched egg yolk agar medium and Neomycin sulphate sheep blood agar medium. Semisolid agar medium was used for motility test.

2-Fluid media:

Cooked meat medium (Willis, 1977) was used for enrichment, purification and preservation of pure isolates. Robert's toxin production medium (Roberts et al., 1976) was used for culturing C. perferinges isolates to produce toxins from toxigenic types.

Reference Antisera:

Diagnostic C. perfringens antitoxin types A, B, C, D and E were obtained kindly from Dr. Abdel fatah, Chief Researcher And Head of Anaerobic Bacteriology Department in Vet. Serum and Vacc. Res. Inst. Abassia, Cairo.

Laboratory animals:

Both Guinea pigs (weight 350- 450 g) and Swiss mice (each weighing 20-25 g) were used for determination of the pathogenicity of C. perfringens isolates.

Enrichment and Isolation of C. perfringens (Willis, 1977):

Each collected sample was inoculated into tubes of cooked meat medium and incubated anaerobically for 24 hrs at 37 °C. A loopful of inoculated fluid medium was streaked onto neomycin sulphate sheep blood agar plates, the streaked plates were incubated anaerobically at 37 °C for 24 hrs. Colonies with double zone of haemolysis were subcultured onto two plates of 10% sheep blood agar and egg yolk agar plates. One plate from each inoculated solid media was incubated aerobically and the other plates were incubated anaerobically.

The colonies that grew only in anaerobic condition and lecithinase producer were picked up and tested by catalase test. Colonies that were catalase negative, lecithinase producer and showed double zone of haemolysis were purified and kept in cooked meat broth medium tubes for further identification.

Identification of isolated bacteria:

All suspected isolates obtained were identified microscopically by examination of Gram stained films to detect their stain reaction, morphological characters and spore formation. Biochemical identification was done according to Cruickshank et al. (1975) and Koneman et al. (1988).

Determination of toxigenic isolates of C. perfringens:

This was done using Naglar's test by half antitoxin plate (Smith and Holdeman, 1968), and Pathogenecity to Guinea pigs (Willis, 1964).

Typing of the toxigenic C. perfringens isolates:

Neutralization test in mice (Smith and Holdemen, 1968), and type of toxins was determined by dermonecrotic test (Bullen, 1952, Roberts et al., 1970 and Oakley and Warrack, 1953). The results were interpreted by the degree of dermonecrotic reaction and neutralization according to Stern and Batty (1975).

Table (1) Seasonal distribution of C. perfringens isolated from samples collected from dead chickens showing lesions suspected to be NE.

Season	No. of samples	Positive		Negative	
		No.	%	No.	%
Autumn	30	19	63.33	11	36.67
Winter	30	09	30	21	70
Spring	30	09	30	21	70
Summer	30	20	66.66	10	33.34
Total	120	57	47.50	63	52.5

Table (2): Seasonal distribution of C. perfringens from dead broiler and layer chickens showing lesions to NE.

Season	Chicken breed	No. of Samples	Positive		Negative	
			No	%	No	%
Autumn	Broiler	20	13	65.0	07	35.0
Winter		20	07	35.0	13	65.0
Spring		20	05	25.0	15	75.0
Summer		20	17	85.0	03	15.0
Autumn	layer	10	06	60.0	04	40.0
Winter		10	02	20.0	08	80.0
Spring		10	04	40.0	06	60.0
Summer		10	03	30.0	07	70.0
Total	broiler	80	42	52.5	38	47.5.0
	layer	40	15	37.5	25	62.5.0

Table (3): Seasonal distribution of types of C. perfringens isolates from chickens affected with NE each season.

Season	No. of isola	Type A		Type C		Non toxigenic	
		NO	%	NO.	%	NO.	%
Autumn	19	5	26.31	2	10.52	12	63.15
Winter	09	2	22.22	-	0.00	7	77.77
Spring	09	6	66.66	1	11.11	2	22.22
Summer	20	11	55.00	-	0.00	9	45.00
Total	57	24	42.10	3	0.05	30	52.63

Table (4): Seasonal distribution of C. perfringens isolate types from chickens breeds with NE .

Season	Chicken breed	No. of isolates	Type A		Type C		Non toxigenic	
			No.	%	No.	%	No.	%
Autumn	Broiler	13	02	15.38	02	15.38	09	69.23
Winter		07	02	28.57	0	0	05	71.42
Spring		05	04	80.00	0	0	01	20.00
Summer		17	10	58.82	0	0	07	41.17
Autumn	Layer	06	03	50.00	0	0	03	50.00
Winter		02	0	0.00	0	0	02	100.00
Spring		04	02	50.00	01	25	01	25.00
Summer		03	01	33.33	0	0	02	66.66

RESULTS AND DISCUSSION

It has been established that C. perfringens plays an important role in the development of NE disease in chickens (Al-Sheikhly and Truscott, 1977and Broussard et al., 1986). In poultry industry, the disease can be divided into 2 categories, clinical and subclinical NE. Clinical signs of NE include depression, decreased appetite, diarrhea, and severe necrosis of the intestinal tract (Ficken and Wages, 1997). While subclinical form lead to decreased BWG and increased FCR, will have severe consequences for the poultry industry (Kaldhusdal et al., 2001 and Hofacre et al., 2003).

There is no doubt that NE disease has caused potential losses among chickens in Egypt (El-Seedy, 1990). Understanding the disease progression of NE has been very difficult due to its complexity and because several predisposing factors such as dietary components, immunosuppression, mechanical irritation of the gut, and sudden gut microflora changes appear to contribute to this syndrome (Smith, 1965; Elwinger et al., 1992 and Ficken and Wages, 1997).

The seasonal distribution of C.perfringens (Table 1) isolated from collected samples was 63.33%, 30%, 30%, 66.66% in autumn, winter, spring and summer; respectively. The incidence was higher in autumn and summer than in winter and spring, these results agree with the findings of Berinier et al.(1974) who recorded sudden onset of NE with high mortality (75%) occurred in chicks between 2 and 4 weeks of age and with greatest incidence observed between May and November . Also, Cygan and Nawak (1974) reported NE with high mortality rate, mostly in chicks aged 4-7 weeks, with high incidence between April and October. Isolation of C. perfringens with relation to season in broilers (Table 2) was 65%, 35%, 25%, 85% in autumn ,winter, spring and summer; respectively. In layer, it was 60%, 20%, 40%, 30% in autumn, winter, spring and summer; respectively. Incidence in layer was higher in autumn and spring than in winter and summer.

In Table (3) out of 19 C. perfringens isolates in autumn ,12 strains were non toxigenic (63.15%) and the remainder 7 strains were toxigenic and serotyped as 5 strains type A (26.31%) ,2 strains type C (10.5%). In winter, out of 9 strains of C. perfringens isolates 7 were non toxigenic (77.77%) and 2 strains were toxigenic type A (22.22%). In spring, out of 9 C. perfringens isolates, 2 strains were non toxigenic (22.22%) and 7 were toxigenic and serotyped as 6 strains type A and 1 type C (11.11). In summer, out of 20 strains of C. perfringens isolated, 9 strains were non toxigenic (45%) and 11 strains were toxigenic and serotyped, all strains were type A (55%). These findings are nearly coincide with the results obtained

by Gardiner (1967) who studied the clostridial infection in poultry and found that NE caused mainly by type A and C toxin produced by C. welchii (C. perfringens) as the most significant serotype. In addition, Long et al. (1974) confirmed that NE was caused by C. perfringens and 50.0% of the isolates were belonging to type A. Also Awad et al. (1976) isolated type A of C. perfringens in higher prevalence rate than other serotypes. The results recorded by Hofshagen and Stenwing (1992) indicated isolation of C. perfringens type A from birds showing NE .furthermore, Mustafa (2000) succeeded to isolate 110 C. perfringens type A from 419 examined broiler. Nauerby et al. (2003) identified 279 C. perfringens toxigenic type A isolates from 25 chicken farms. Engstrom et al. (2003) isolated 53 C.perfringens type A isolates from 10 broiler chicken farms showing clinical and sub-clinical lesions of NE. These findings indicated that C. perfringens type A was the most prevalent as previously reported from intestinal contents of birds by Smith and Williams, (1984).

There is a variation in the prevalence of typed C.perfringens isolates according to season between broiler and layer (Table 4). In broiler the rate of C. perfringens type A was higher compared to type C, as reported by Gardiner (1967; Long et al. (1974); Monazi, (2000) and El-Refaey (2006). Our results indicated that the incidence of toxigenic C. perfringens was high in spring and summer compared to autumn and winter. These finding agreed with those of Cygan and Nawak (1974). In layers, C. perfringens type A was the most isolated compared to type C, these findings agreed with Long et al. (1974); Awad et al. (1976) ; Nauerby et al. (2003) and Engstrom et al. (2003) and the high incidence of toxigenic C.perfringens was observed in autumn and spring compared to summer and winter. The variation in the isolation rates between our results and the literature could be explained on the basis of epidemiological predisposing factors that could affect each farm leading to NE. The most important known predisposing factor is intestinal damage caused by coccidial pathogens, especially Eimeria species (Helmboldt and Bryant , 1971; Long et al .,1974 ; Al- Sheikhly and Al –Saieg, 1980; Broussard et al.,1986; Hofacre et al ., 2003 and Jackson et al ., 2003) the intestinal damage will result in release of plasma proteins into the lumen of the intestinal tract. Since the minimal requirements for growth of C. perfringens include more than 11 amino acides besides many other factors and vitamins (Boyd et al., 1948 and Petit et al., 1999), leaking of plasma to the intestinal lumen can provide a necessary growth substrate for extensive proliferation of these bacteria. It is also proven that diet strongly influences the incidence of NE, diets with high levels of indigestible, water-soluble-

non-starch polysaccharides, known to increase the viscosity of the intestinal contents, predispose to NE. This viscosity of the diets rich in rye , wheat and barely relative to diets rich in corn (Branton et al ., 1987 ; Kaldhusdal and Hofshagen , 1992 ; Riddell and kong, 1992 and Kocher , 2003) also , diet rich in fish meal predispose to NE (Kocher,2003). Poor hygienic and housing conditions such as rising on litter floor are greatly associated with NE disease (Frame and Bickford, 1986). Feed stuffs rich in zink contributes to the protection of alpha toxin from proteolysis by trypsin (Sato et al., 1978 and Baba et al., 1992a) now explained by the finding that alpha toxin is a zinc-metalloenzyme (Nayelor et al., 1998).

The C. perfringens type A and C were the only isolated with higher prevalence of the type A, regarding the season and breed. C. perfringens isolation was higher in autumn and summer than in winter and spring in broiler; while in layer, it was higher in autumn and spring than in winter and summer. The typed isolates were represented only by type A and C with higher prevalence of type A.

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عزل وتصنيف مكروب الكوليستردا بيرفيرينجزمن حلات الاصابة الحقلية للدجاج بالتهاب المعى التنكرزى وعلاقتة بفصول السنة

تم الفحص البكتيري المعملي لعدد ١٢٠ دجاجة عانت من أعراض والآفات المرضية التشريحية للإصابة الطبيعية بمرض التهاب الأمعاء التنكرزى حيث أمكن عزل, تصنيف الميكروب اللاهوائي كولسترديا برفرينجز بكتريووجيا ومصليا من الحالات الايجابية تشريحيا وعددها ٥٥ دجاجة بنسبة عزل كلية ٤٧.٥٠ %.

بالنسبة لفصول العام فكانت نسب العزل ايجابي ٦٣.٣٣ %، ٣٠% ، ٣٠ %، ٦٦.٦٦ % من العينات التي جمعت في فصل الخريف و الشتاء و الربيع و الصيف؛ على التوالي.

عند الأخذ بالاعتبار نوع الدجاج فكانت نسب العزل من التسمين و البياض ٦٥ % و ٦٠ % في الخريف – ٣٥ % ، ٢٠ % في الشتاء – ٥ % ، ٤٠ % في الربيع – ٨٥ % ، ٣٠ % في الصيف ؛ على التوالي .

تم التعريف المصلى والمقدرة على إنتاج السم للعترات المعزولة باستخدام الخنزير الهندي والفئران البيضاء حيث عرفت المعزولات الى ٢٤ من النوع المصلى(أ) و ٣ من النوع (ج) و ٣٠ معزول غير سامة. كان التوزيع الفصلي لمعزولات النوع(أ) كالتالي ٥ ، ٢ ، ٦ ، ١١ في الخريف و الشتاء و الربيع و الصيف ؛ على التوالي. بينما النوع المصلى (ج) فكان ٢ في الخريف و ١ في الربيع فقط.