NECROTIC ENTERITIS IN MEAT CHICKEN RAISED AT THE GROUND IN PERMANENT BEDDING

Cristina HORHOGEA, Oana BUSUIOC, Cristina RÎMBU, Mircea LAZĂR, Carmen CREȚU, Cătălin CARP-CĂRARE, Eleonora GUGUIANU
University of Agricultural Sciences and Veterinary Medicine Ion Ionescu de la Brad Iași
rebegeacristina@yahoo.com

Abstract
Poultry necrotic enteritis is an acute clostridial infection characterized by severe necroses of intestinal mucosa. The disease begins suddenly, with a sharp increase in death rate and dehydration. Clostridium perfringens, a sporulated, anaerobic, Gram-positive bacterium is commonly found in the environment and in the gastrointestinal tract as part of the normal intestinal flora. Frequent presence in the digestive tract of healthy birds is associated with necrotic enteritis in broilers. The research was conducted on 323 samples (120 live chickens, 89 corpses, 104 feed samples and 10 water samples) collected from a farm with 32 253 hybrid Ross 308 broilers (21 days), raised at the ground on permanent bedding, where there was a significant increase in mortality above the permissible limit. The necropsy performed on 980 chicken corps revealed a different prevalence of intestinal tract lesions: bleeding wall (28.37%), mucosal necrosis (23.22%), gas content (18.57%), mucosal inflammation (15.73%) and red orange mucus in the intestines (14.10%). Bacteriological examination identified Clostridium perfringens in 11.66% of live broilers, 10.11% of chicken corps, 61.53% of feed samples and 3.09% of water samples. Increased percentage this species isolation suggests that feed taken from the hall was an important source of infection for broilers reared on the ground.
***Key words**: Clostridium, broilers, necrotic enteritis, feed*

Introduction

Necrotic enteritis (NE) is the most common anaerobic enteric disease in poultry which typically occurs in broiler chickens [5, 15, 18]. The causative agent is *Clostridium perfringens*, a Gram-positive, sporulated, anaerobic bacteria, which is commonly found in the environment [11, 17] and in the gastrointestinal tract, part of the normal intestinal flora [3]. NE is characterized by inflammation and necrosis in the gastrointestinal tract, with a major decrease of the growth performance and a high level of mortality [14, 20].

Clostridium perfringens is able to produce several toxins, A type (α-toxin) and C type (α and β toxins) being associated with NE in poultry [2, 10]. Toxins produced by the bacteria cause damage to the small intestine, liver lesions and mortality [13].

The disease mostly occurs in the intensive breeding system in broiler from the age of 15 days. Because of the clinical disease and the significantly increasing of the mortality percentage (20%), research followed identification of the etiology and the factors that favored the emergence and evolution of the necrotic enteritis episode.

Material and method

Research was conducted on samples taken from a flock of 32 253 hybrid Ross 308 broilers, raised at the ground on permanent litter, aged 21 days, where a significant mortality level, above the permissible limit, was observed.

To establish the etiology, bacteriological examination was performed on 323 samples harvested from 120 live broilers (minus variants, clinically healthy), 89 corpses (whole organs or fragments, tissues: liver, portions of intestine, bone long), 104 feed samples and 10 water samples.

Microbiological examination was performed according to protocol. Direct microscopic examination was performed on Gram stained smears obtained by fingerprinting the damaged intestinal mucosa. Bacteria were isolated from fresh organs (heart, unopened long bone, liver, spleen) and intestinal contents, on usual and selective anaerobic nutrient media (VL – viande /levure with 10% blood and sodium azide, Veillon agar, TSC agar - Tryptose Sulphite Cycloserine and SPS agar - Sulfite Polymyxin Sulfadiazine) incubated for 24 hours at 37°C in an atmosphere with 5-7% CO2 [3]. In order to promote the production of toxins, media were supplemented with

1% glucose and 3% normal horse serum. Serological confirmation of *Clostridium perfringens* was made with API galleries 20A, Biomerieux.

Toxin producing strains were identified using seroneutralisation reaction. In order to detect the toxin, the antigen prepared by cultures centrifugation was mixed with polyclonal (A, B, C, D, E biotypes) and monoclonal antitoxin antibodies, left in contact for 30 minutes and inoculated in white mice [4]. Positive reaction (biotype identification) was revealed by mice survival.

Results and disscutions

In the farm with 32215 hybrid Ross 308 broiler, aged 21 days, raised at ground on permanent litter, a significant increase in clinical disease and mortality rate was observed.

Necropsy on 980 broiler corpses showed lesions with diagnostic value: highlighting the intestinal wall vascular profile (fig. 1), hemorrhagic lesions visible crossed the intestinal wall (fig. 2), air presence within the intestinal lumen with transparent intestinal mucosa, almost non-existent (fig. 3), gas presence in the intestinal content with inflammatory and bleeding intestinal mucosa (fig. 4), intestinal content with orange mucus consistency (fig. 5), the presence of gas and incompletely digested feed in the intestinal lumen and inflamed intestinal mucosa, with hemorrhagic and necrotic appearance (fig. 6), similar with the ones cited in literature [16].

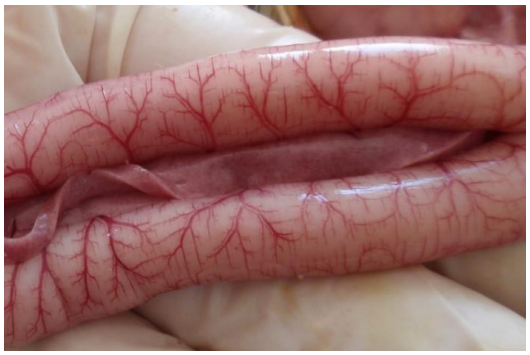


Fig. 1 - Intestinal wall with evidenced vascular profile

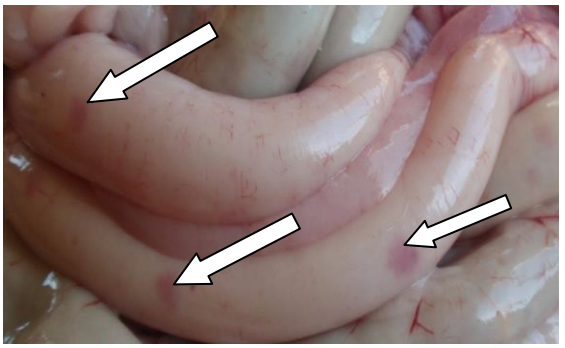


Fig. 2 - Hemorrhagic lesions visisble cross the intestinal wall

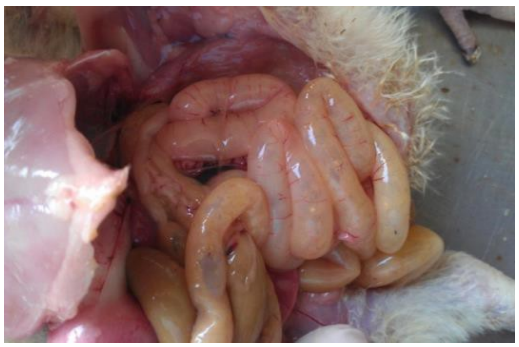


Fig. 3 - Intestinal mucoasa transparency presence of intestinal gas

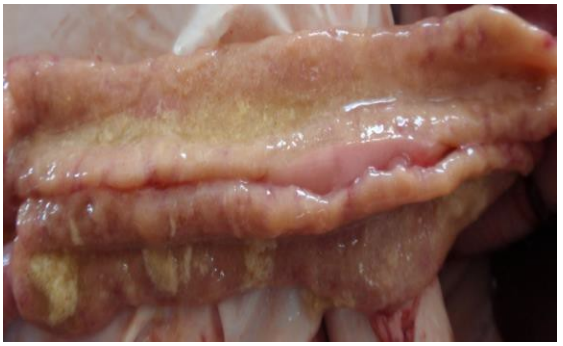


Fig. 4 - Inflamed intestinal mucosa and with hemorrhagic areas

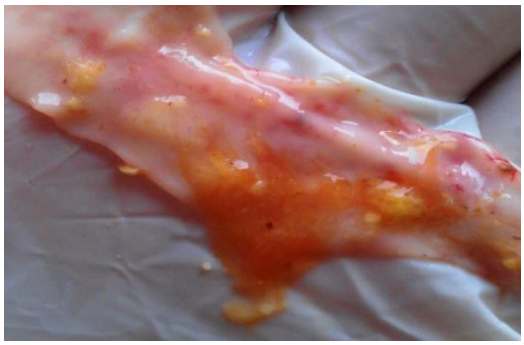


Fig. 5 - Intestinal orange mucus

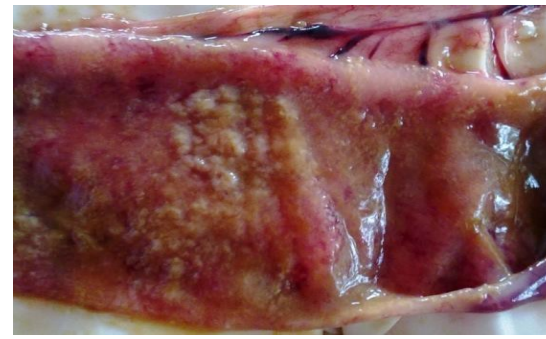
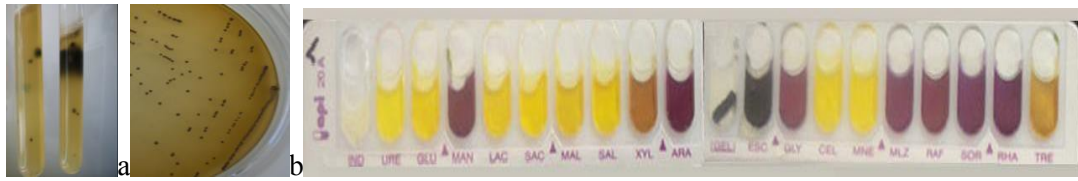


Fig. 6 - Inflamed intestinal mucosa with hemorrhagic and necrotic appearance

The prevalence of the intestinal lesions was different: bleeding from the wall (28.37%), mucosal necrosis (23.22%), gas content (18.57%), mucosal inflammation (15.73%) and orange mucus in the content (14.10%).

As it is mentioned in the literature, gross lesions are usually restricted to the duodenum, jejunum and ileum [19] but can be observed also in the caeca [21].

Bacteriological and serological examination isolated and identified *Clostridium perfringens* (fig. 7 a, b, c), with different incidence depending on the sample type (Table 1).



a b c

Fig. 7 - Clostridium perfringens. a-SPS medium, b-TSC medium, c-API gallerie

From the total number of 323, *Clostridium perfringens* was isolated and identificated in 87 (26,93%) samples, and the rest of 236 (73,07%) consisted of mix bacteria microflora (*Escherichia coli, Pseudomonas aeruginosa, Proteus spp*. etc.).

From the 87 positive strains of *Clostridium perfringens,* 10 of them tested for toxin showed the presence of A biotype.

Table 1.

Clostridium perfringens incidence

No	Sample type	Total samples number	Positive samples number	%
1	Broilers with clinical signs	120	14	11,66
2	Broiler corpses	89	9	10,11
3	Feed	104	64	61,53
4	Water	10	0	3.09
	Total	**323**	**87**	**26.93**

The presence of *Clostridium perfringens* was different in function of the sample type: 11,66% in broilers with some clinical signs, 10,11% in corpses, 61,53% in feed and 3,09% in water samples. Because of the highest percent of *Clostridium perfringens* isolation and identification, we considered that the feed samples represented an important sourse of infection for the broilers bred on the ground.

Tabel 2.

Feed microbial load in the broilers hall

Collecting aria	CFU	Sulphite-reducing bacteria	*Clostridium perfringens*
Proximal	120000	50	12
Middle	500	0	0
Distal	12000	26	0

Bacteriological examination of the feed samples harvested from the proximal area (not consumed by chicken) showed a value of 120 000 CFU (Colony Forming Units), 50 colonies being produsedby sulphite-reducing bacteria and 12 of these corresponded to the species *Clostridium perfringens* (Table 2). These results suggest that the source of primary contamination for chicken (aged 4-5 days) was predominantly represented by the forage (food source). In areas where chicken consume feed, this is contaminated with feces, but aeration performed with claw makes the number of sulphite-reducing bacteria be very low and without any strain of *Clostridium perfringens*.

The alteration of the intestinal environment plays a key role in this diseases because creates favorable conditions for *C. perfringens* growth [14].

At the same time, we appreciate that the main cause of *Clostridium perfringens* multiplying in broilers after the age of 14 days was the changing of feed recipe (protein content) from 21-22% to 20-20.5% and the basic raw material (from maize to wheat). According to some authors, protein content and the physical form of diets is able to influence the physiological and morphological characteristics of the gut, finely ground feed increasing the occurrence of NE [8, 9, 14].

Those changes produced body resistance fall and allowed the development of the potentially pathogenic epiphytic flora, including *Clostridium perfringens*. So, diet is a recognized factor with a strong impact on the incidence of NE in broiler chickens [1, 7].

The nutritional and health status of broilers are related with intestinal tract health, including immune system, microbial balance and structural integrity of the gut. The disturbances of these processes affects digestion, absorption and metabolism of nutrients, disease resistance and immune response [12, 22] and can cause the enteric diseases [6].

Maintaining balance between Gram positive (90%) and Gram negative microflora in the avian gastrointestinal tract has a special importance. At this level, germs multiply intensively, modify the intestinal biocenosis and synthesize toxic factors in quantities beyond tolerable limits of the unimmunized body. These processes occur only in a slightly alkaline medium with a decreased intestinal peristalsis (hypo motility), thus favoring the development of germs and toxins that act brutally on the intestinal mucosa, with hemolytic, necrotic and lethal effect.

Intestinal lesions caused by *Clostridium perfringens* leads to production loss, due to decreased digestion and absorption, reduce development rate and increase feed conversion. It is known that although clinical necrotic enteritis outbreaks can cause high levels of mortality, however subclinical disease is more important because it can persist in broilers farms without visible clinical signs visible but with significant losses.

Conclusions

Research conducted in an episode of necrotic enteritis produced by *Clostridium perfringens* in broilers raised at the ground on permanent litter, revealed the following conclusions:

1. Necropsy showed a different prevalence of the intestinal lesions: bleeding from the wall (28.37%), mucosal necrosis (23.22%), gas content (18.57%), mucosal inflammation (15.73%) and orange mucus in the content (14.10%).

2. *Clostridium perfringens* strains were isolated and identificated in almost 27% from the samples, A toxin biotype.
3. Because of the highest percent of *Clostridium perfringens* identification in the feed samples (61,53%), it suggest that was an important sourse of infection for the broilers bred on the ground.

Bibliography

1. Annett C.B., Viste J.R., Chirino-Trejo M., Classen H.L., Middleton D.M., Simko E., 2002, Necrotic enteritis: effect of barley, wheat and corn diets on proliferation of *Clostridium perfringens* type A, Avian Pathol, 31, pp. 598–601
2. Brynestad S., Granum P.E., 2002, Clostridium perfringens and foodborne infections. Int J Food Microbiol 74:195–202
3. Carp-Carare M., Guguianu E., Timofte D., 1977 – Lucrări practice de microbiologie veterinară. Litografia UAMV Iași
4. Carp-Cărare M., Timofte D., 1998, Imunologie şi Imunopatologie, manual practic., Ed. Vasiliana, Iaşi
5. Cooper K.K., Bueschel D.M., Songer J.G., 2013, Presence of *Clostridium perfringens* in retail chicken livers. Anaerobe. ;21:67–68.
6. Dekich M.A., 1998, Broiler industry strategies for control of respiratory and enteric diseases, Poult Sci, 77, pp. 1176–1180
7. Drew M.D., Syed N.A., Goldade B.G., Laarveld B., Van Kessel A.G., 2004, Effects of dietary protein source and level on intestinal populations of *Clostridium perfringens*in broiler chickens, Poult Sci, 83, pp. 414–420
8. Engberg R.M., Hedemann M.S., Jensen B.B., 2002, The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens, Br Poult Sci, 43, pp. 569–579
9. Engberg R.M., Hedemann M.S., Jensen B.B., 2004, Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract, Poult Sci, 83, pp. 925–938
10. Engstro¨m B.E., Ferme´r C., Lindberg A., et al., 2003, Molecular typing of isolates of Clostridium perfringens from healthy and diseased poultry. Vet Microbiol 94:225–235
11. Hatheway C.L., 1990, Toxigenic clostridia, Clin Microbiol Rev. Jan ; 3(1):66-98.
12. Kelly D., Conway S., 2001, Genomics at work: the global gene response to enteric bacteria, Gut, 49, pp. 612–613
13. Merck Manual
14. M'Sadeq S.A., Wu S., Swick R.A., Choc M., 2015, Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide, Animal Nutrition, Volume 1, Issue 1, March, Pages 1–11
15. Nauerby, B., Pedersen K., Madsen M., 2003, Analysis by pulsedfield gel electrophoresis of the genetic diversity among Clostridium perfringens isolates from chickens. Vet. Microbiol. 94:257–266.
16. Olkowski A.A., Wojnarowicz C., Chirino-Trejo M., Drew M.D., 2006, Responses of broiler chickens orally challenged with *Clostridium perfringens* isolated from field cases of necrotic enteritis, Res Vet Sci, 81, pp. 99–108
17. Songe J.G., 1996, Clostridial enteric diseases of domestic animals, Clin Microbiol Rev. Apr ; 9(2): 216–234.
18. Svobodová I., Steinhauserová I., Nebola M., 2007, Incidence of Clostridium perfringens in Broiler Chickens in the Czech Republic, Acta Vet. Brno 76: S25–S30;
19. Timbermont L., Lanckriet A., Gholamiandehkordi A.R., Pasmans F., Martel A., Haesebrouck F., 2009, Origin of *Clostridium perfringens* isolates determines the ability to induce necrotic enteritis in broilers, Comp Immunol Microbiol Infect Dis, 32, pp. 503–512
20. Van der Sluis, W., 2000, Clostridial enteritis is an often underestimated problem. World Poultry 16, 42–43.
21. Van Immerseel F., De Buck J., Pasmans F., Huyghebaert G., Haesebrouck F., Cucatelle R., 2004, *Clostridium perfringens* in poultry: an emerging threat for animal and public health, Avian Pathol, 33, pp. 537–549
22. Yegani M., Korver D.R., 2008, Factors affecting intestinal health in poultry, Poult Sci, 87, pp. 2052–2063