LUIS ANTONIO LLANCO ALBORNOZ

CARACTERIZAÇÃO MOLECULAR DOS PRINCIPAIS FATORES DE VIRULÊNCIA E GENÓTIPOS DE *Clostridium perfringens* ISOLADOS DE FRANGOS COM ENTERITE NECRÓTICA

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Orientador: Prof. Dr. Mario Julio Avila-Campos

Co-orientadora: Profa. Dra. Viviane Nakano

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RESUMO

LLANCO, L. A. A. **Caracterização molecular dos principais fatores de virulência e genótipos de *Clostridium perfringens* isolados de frangos com enterite necrótica.** 2013. 109 f. Tese (Doutorado em Microbiologia) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2013.

Clostridium perfringens é a bactéria responsável pela enterite necrótica (EN), que afeta a produção avícola mundial. Esta bactéria se caracteriza pela produção de diversas toxinas que causam lesões no intestino provocando elevada mortalidade, e perdas econômicas pela queda na produtividade. Este estudo avaliou os principais fatores de virulência, a susceptibilidade aos antimicrobianos e a diversidade genética de *C. perfringens* isolados de frangos com EN. Foram obtidos 22 isolados de nove das 94 amostras analisadas. Todos, menos um isolado, possuíram um ou dois genes *nanI* (95%) e *nanJ* (81%) que codificam a produção de neuraminidases, e (19/22), mostraram atividade de neuraminidase em hemácias de frango. Nenhum isolado abrigou o gene *nanH*. A atividade hemaglutinante foi observada em poucos isolados (26%). Todos os isolados foram positivos para o gene *plc* (toxina α), sendo classificados como tipo A. Sete isolados (31,8%) abrigaram o gene *tpeL* que codifica a toxina TpeL, sendo este o primeiro relato da presença desta toxina no Brasil associado a quadros de EN em frangos. Isolados *tpeL*+ mostraram efeito citotóxico característico da ação da toxina TpeL. Alguns isolados mostraram capacidade de aderir e invadir as células epiteliais testadas. A maioria dos isolados foi resistente à sulfaquinoxalina (100%), cefalexina (95%), eritromicina (95%), bacitracina (50%), com valores de CIM_{90} variando entre 32 µg/mL a ≥ 512 µg/mL. Cefoxitina, amoxicilina, enrofloxacina, amoxicilina-ácido clavulânico, penicilina-estreptomicina, cloranfenicol e metronidazol se mostraram ativos contra os *C. perfringens* avaliados. Pela técnica de AP-PCR e empregando o coeficiente UN1, todos os isolados foram agrupados em sete clusters, apresentando-se como um grupo heterogêneo.

Palavras-chave: Enterite necrótica. Frangos. *Clostridium perfringens.* Fatores de virulência. Toxina TpeL. Susceptibilidade aos antimicrobianos. Diversidade genética.

ABSTRACT

LLANCO, L. A. A. **Molecular characterization of the virulence factors and genotypes of *Clostridium perfringens* isolated from chickens with necrotic enteritis.** 2013. 109 p. Ph. D. thesis (Microbiology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2013.

Clostridium perfringens cause necrotic enteritis (EN) affecting the poultry production worldwide. This bacterium produces various toxins and causes lesions in the intestine producing high mortality and economic loss due to the low productivity. In this study, the major virulence factors, antimicrobial susceptibility and genetic diversity of *C. perfringens* isolated from chickens with EN were evaluated. 22 isolates were obtained from nine out of 94 intestinal samples analyzed. All the isolates with exception of one harbored one or two genes *nanI* (95 %) and *nanJ* (81%) codifying the neuraminidase production, and 19/22 showed neuraminidase activity on chicken's erythrocytes. No isolates harbored the gene *nanH*. Haemagglutination was observed in 26% of the isolates. All isolates were positive for the gene *plc* (α toxin) being classified as type A. Seven isolates (31.8%) harbored *tpeL* gene that encodes the toxin TpeL, and this appear to be the first report of the presence of this toxin associated to chickens with EN in Brazil. Isolates *tpeL*+ showed characteristic cytotoxic effect. Some isolates showed the ability of adhere to and invade to epithelial cells. Most of the isolates were resistant to sulphaquinoxaline (100%), cephalexin (95%), erythromycin (95%), bacitracin (50%) with MIC_{90} values ranging from 32 µg /mL to ≥ 512 µg /mL. Cefoxitin, amoxicillin, enrofloxacin, amoxicillin-clavulanic acid, penicillin-streptomycin, chloramphenicol, and metronidazole showed activity against *C. perfringens*. By AP-PCR and by using the coefficient UN1, isolates were grouped into seven clusters, showing to be a heterogeneous group.

Keywords: Necrotic enteritis. Chicken. *Clostridium perfringens.* Virulence factors. Toxin TpeL. Antimicrobial susceptibility. Genetic diversity.

INTRODUÇÃO

Dentre os microrganismos anaeróbios que colonizam o trato intestinal do homem e animais, destaca-se o gênero *Clostridium,* composto por pelo menos 150 espécies (GARRITY et al., 2007; SAVAGE, 1986). Estas bactérias anaeróbias, esporuladas e fermentadoras de diversos compostos orgânicos, participam ativamente da degradação final de alguns nutrientes no ecossistema gastrointestinal, assim como, do processo de renovação da biomassa, quando habitam solos e esgotos, cumprindo um importante papel ecológico (GONG et al., 2008; SONGER, 1996).

Apesar da maioria das bactérias deste gênero *Clostridium* ser comensal, aproximadamente 10% possui elevada patogenicidade, recorrente da produção de potentes toxinas. Este gênero bacteriano abriga espécies que produzem aproximadamente 20% de todas as toxinas bacterianas conhecidas (POPPOF; VOUBET, 2013).

Dentre as espécies do gênero *Clostridium*, a mais frequentemente isolada de casos de enterites, abscessos, e toxemias, entre outros, é *C. perfringens*. Este microrganismo é o principal responsável pela gangrena gasosa, por intoxicações alimentares, e diarréias associadas ao uso de antibióticos em humanos (ROOD et al., 1997). Também, esta bactéria tem sido isolada de doenças inflamatórias crônicas, como colite ulcerativa e doença de Crohn, sendo também proposta por alguns autores a sua participação no desenvolvimento de câncer de cólon (PRUTEANU et al., 2011; PRUTEANU; SHANAHAN, 2013).

Clostridium perfringens também desenvolve processos infecciosos em animais, produzindo a enterite necrótica (EN) em aves. Esta doença causa morte súbita quando se apresenta na forma aguda, e baixo rendimento produtivo na forma subclínica, assim como também, produz grandes perdas econômicas em países exportadores de carne de aves, principalmente frangos (VAN IMMERSEEL et al., 2009).

Esta espécie bacteriana é capaz de produzir mais de quinze toxinas, que constituem seus principais fatores de virulência. Dessas toxinas somente quatro, consideradas letais: α, β, ε e ι, servem para classificar essa espécie em

cinco toxinotipos: A, B, C, D, e E, os quais estão relacionados às doenças de importância na medicina humana e veterinária (PETIT; GIBERT; POPOFF, 1999; SONGER, 1996).

A toxina α é o fator de virulência de *C. perfringens* mais estudada, devido a seu rápido efeito letal em animais de experimentação, à ampla distribuição de seus receptores e substratos na superfície de células eucarióticas, e à sua relação com doenças severas (GOÑI; MONTES; ALONSO, 2012; SONGER, 1996; TITBALL; NAYLOR; BASAK, 1999). O gene *plc* codifica a produção desta toxina, que está localizada muito próxima da origem de replicação no genoma dos *C. perfringens* sequenciados, e é considerado um fator de virulência de origem cromossomal (MYERS et al., 2006; SHIMIZU et al., 2001; SHIMIZU et al., 2002a).

Na EN se observa a degradação das células eucarióticas intestinais, e este processo é produzido pela toxina α, considerada uma fosfolipase tipo C. Estudos têm mostrado que, a inativação da toxina α diminui a severidade das lesões, sugerindo-se a participação de outros fatores no desenvolvimento deste processo infeccioso (KEYBURN et al., 2006; THOMPSON et al., 2006).

Estudos realizados por Keyburn et al. (2008), relataram a presença de uma nova toxina denominada NetB a qual é de origem plasmidial e apresenta uma atividade de formação de poros na membrana de células eucarióticas. Essa toxina NetB é a última descrita em *C. perfringens*, e também, é sugerida a sua participação nas lesões iniciais da EN. Recentemente, Lepp et al. (2010), analisando a origem plasmidial do gene *netB*, identificaram três loci de patogenicidade: locus 1, locus 2 e locus 3. Os loci 1 e 3 são de origem plasmidial, sendo que o gene *netB* se encontra localizado no locus 1. O locus 2 é considerado de origem cromossomal que ao lado do locus 3 (plasmidial) carregam genes relacionados ao metabolismo bacteriano e produção de adesinas, entre outros. Também, estes autores relataram que esses três loci eram observados somente em cepas patogênicas e não em cepas comensais.

Estudos têm mostrado que a prevalência do gene *netB* em *C. perfringens* de diferentes origens, é baixa, e frequentemente observado em isolados bacterianos de animais sadios do que em doentes (ABILDGAARD et al., 2010; CHALMERS et al., 2008a; MARTIN; SMYTH, 2009). Isto sugere que, a presença da toxina NetB não seja suficiente para iniciar o processo

infeccioso. Por outro lado, Coursodon et al. (2010), avaliando a produção da toxina α no intestino de frangos inoculados com *C. perfringens* (*plc* mutantes) questionou o experimento realizado por Keyburn et al. (2008), uma vez que estes autores não utilizaram animais livres de germes, e esta toxina poderia ser produzida por *C. perfringens* residentes no intestino aviário.

Amimoto et al. (2007) relataram a presença da toxina TpeL a qual foi classificada como pertencente a Família das Grandes Citotoxinas Clostridiais (LCT): junto às toxinas A e B de *C. difficile*, toxina letal de *C. sporogenes,* e toxina alfa de *C. novyi*. Esta família de toxinas se constitui importante fator de virulência envolvida em doenças entéricas de interesse médico veterinário (AKTORIES et al., 2012; BUSCH; AKTORIES, 2000).

Também, sabe se que, a atividade desta toxina TpeL tem atividade glicosilante nas proteínas Rho-Ras GTPases, modifica a estrutura da actina e afeta a fisiologia das células epiteliais, particularmente, das células Vero (rim de macaco verde) (CARTER; ROOD; LYRAS, 2012; DJOUDER et al., 2000; NAGAHAMA et al., 2011). Assim, Chalmers et al. (2008a) e Coursodon et al. (2012), ressaltaram a importância dessa toxina no agravamento das lesões, aumentando a mortalidade por EN em animais de experimentação.

O processo de adesão ao epitélio intestinal se constitui a etapa mais importante na colonização bacteriana, a qual pode ser mediada por estruturas fimbriais e não fimbriais (PARKER; SPERANDIO, 2009). Em *C. perfringens* o processo de adesão tem sido demonstrado pela capacidade de produzir biofilmes, e essa capacidade é favorecida pela presença de pili tipo IV e pela produção de sialidases (BORASTON; FICKO-BLEAN; HEALEY, 2007; VARGA; THERIT; MELVILLE, 2008; WALTERS; STIREWALT; MELVILLE, 1999). Vidal et al. (2009b) e McClane (2010), relataram que o processo de adesão realizado por *C. perfringens* serviria como um regulador positivo para a produção de neuraminidases e toxinas.

A EN é considerada um processo infeccioso que causa aproximadamente U$ 2 bilhões de dólares por ano, no mundo todo, e é causada pelas toxinas produzidas por *C. perfringens*, adicionado da ausência de vacinas efetivas para o controle da EN; assim, maiores estudos são necessários para minimizar os fatores que participam dessa doença. Alguns antimicrobianos vêm sendo muito utilizados como promotores de crescimento

de aves, trazendo como consequência o surgimento de bactérias resistentes a múltiplas drogas antimicrobianas, dificultando o tratamento de vários processos infecciosos de interesse na medicina veterinária (VAN IMMERSEEL et al., 2009). A presença de cepas bacterianas multirresistentes constitui-se um sério risco para saúde humana, uma vez que esses animais são utilizados na alimentação do homem (KATHER; MARKS; FOLEY, 2006; SCHENTAG; GUILLILAND; PALADINO, 2001; SONGER, 1996).

Estudos de susceptibilidade aos antimicrobianos de *C. perfringens* são pouco observados na literatura, principalmente, avaliando-se comparativamente os perfis de resistência entre localidades e granjas de criação avícola (MARTEL et al., 2004; VAN IMMERSEEL et al., 2004). Drogas como penicilinas, bacitracina e ionóforos vêm sendo utilizados como promotores de crescimento em aves, assim como para o tratamento e prevenção da EN em vários países como Índia, Argentina e Brasil. Entretanto, os Estados Unidos, Canadá e países Europeus, o uso de antimicrobianos como promotores de crescimento está totalmente proibido.

CONCLUSÕES

Os resultados obtidos neste estudo permitem concluir que:

1. Os *C. perfringens* isolados foram classificados como tipo A (*plc*+), sendo que alguns deles abrigaram também o gene *tpeL*;
2. A maioria dos isolados apresentou característica citotóxica, adesiva e invasiva;
3. Os isolados mostraram perfil similar de resistência para alguns antimicrobianos; e
4. Os *C. perfringens* avaliados neste estudo foram agrupados em clusters estreitamente relacionados apesar de apresentarem características fenotípicas e genotípicas heterogêneas.

REFERÊNCIAS*

AARESTRUP, F. M. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. **Int. J. Antimicrob. Agents**, v. 12, p. 279-285, 1999.

AARESTRUP, F. M.; OLIVER, DURAN, C.; BURCH, D. G. Antimicrobial resistance in swine production. **Anim. Health Res. Rev.,** v. 9, p. 135-148, 2008.

ABILDGAARD, L.; ENGBERG, R. M.; PEDERSEN, K.; SCHRAMM, A.; HOJBERG, O. Sequence variation in the α-toxin encoding *plc* gene of *Clostridium perfringens* strains isolated from diseased and healthy chickens. **Vet. Microbiol.,** v. 136, p. 293-299, 2009a.

ABILDGAARD, L.; SCHRAMM, A.; RUDI, K.; HOJBERG, O. Dynamics of plc gene transcription and α-toxin production during growth of *Clostridium perfringens* strains with contrasting α-toxin production. **Vet. Microbiol.,** v. 139, p. 202–206, 2009b.

ABILDGAARD, L.; SONDERGAARD, T. E.; ENGBERG, R. M.; SCHRAMM, A.; HØJBERG, O. In vitro production of necrotic enteritis toxin B, NetB, by *netB*-positive and *netB*-negative *Clostridium perfringens* originating from healthy and diseased broiler chickens. **Vet. Microbiol.,** v. 144, p. 231-235, 2010.

ACAR, J.; CASEWELL, M.; FREEMAN, J.; FRIIS, C.; GOOSSENS, H. Avoparcin and virginiamycin as animal growth promoters: a plea for science in decision-making. **Clin. Microbiol. Infect.,** v. 6, p. 477-482, 2000.

ACINAS, S. G.; MARCELINO, L. A.; KLEPAC-CERAJ, V.; POLZ, M. F. Divergence and Redundancy of 16S rRNA Sequences in Genomes with Multiple rrn Operons. **J. Bacteriol.,** v. 186, p. 2629–2635, 2004.

ADAMS, C. A. Nutrition-based health in animal production. **Nut. Res. Rev.,** v. 19, p. 79–89, 2006.

AGUNOS, A.; LÉGER, D.; CARSON, C. Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada. **Can. Vet. J.,** v. 53, p. 1289-1300, 2012.

AKTORIES, K.; SCHWAN, C.; PAPATHEODOROU, P.; LANG, A. E. Bidirectional attack on the actin cytoskeleton. Bacterial protein toxins causing polymerization or depolymerization of actin. **Toxicon,** v. 60, p.572–581, 2012.

ALLAART, J. G.; VAN ASTEN A. J.; GRÖNE, A. Predisposing factors and prevention of *Clostridium perfringens* associated enteritis. **Comp. Immunol. Microbiol. Infect. Dis.,** v. 36, p. 449-64, 2013.

AL-SHEIKHLY, F.; AL-SAIEG, A. Role of coccidia in the occurrence of necrotic enteritis of chickens. **Avian Dis.,** v. 24, p. 324-333, 1980.

AL-SHEIKHLY, F.; TRUSCOTT, R. B. The pathology of necrotic enteritis of chickens following infusion of broth cultures of *Clostridium perfringens* into duodenum. **Avian Dis.,** v. 21, p. 230-240, 1977a.

AL-SHEIKHLY, F.; TRUSCOTT, R. B. The interaction of *Clostridium perfringens* and its toxins in the production of necrotic enteritis of chickens. **Avian Dis.,** v. 21, p. 256-263, 1977c.

AMIMOTO, K.; NORO, T.; OISHI, E.; SHIMIZU M. A novel toxin homologous to large clostridial cytotoxins found in culture supernatant of *Clostridium perfringens* type C. **Microbiology,** v. 153, p. 1198-1206, 2007.

ANGELAKIS, E.; RAOULT, D. The Increase of *Lactobacillus* Species in the Gut Flora of Newborn Broiler Chicks and Ducks Is Associated with Weight Gain. **PLoS ONE,** v. 5, p. e10463. 2010.

ARVANS, D. L.; VAVRICKA, S. R.; REN, H.; MUSCH, M. W.; KANG, L.; ROCHA, F. G.; LUCIONI, A.; TURNER, J. R.; ALVERDY, J.; CHANG, E. B. Luminal bacterial flora determines physiological expression of intestinal epithelial cytoprotective heat shock proteins 25 and 72. **Am. J. Physiol. Gastrointest. Liver. Physiol.,** v. 288, p. G696-G704. 2005.

ASAOKA, Y., YANAI, T., HIRAYAMA, H., UNE, Y., SAITO, E., SAKAI, H., GORYO, M., FUKUSHI, H., MASEGI, T. Fatal necrotic enteritis associated with *Clostridium perfringens* in wild crows (*Corvus macrorhynchos*). **Avian Pathol.,** v. 33, p. 19-24, 2004.

AWAD, M. M.; BRYANT, A. E.; STEVENS, D. L.; ROOD, J. L. Virulence studies on chromosomal α-toxin and θ-toxin mutants constructed by allelic exchange provide genetic evidence for the essential role of α-toxin in *Clostridium perfringens* -mediated gas gangrene. **Mol. Microbiol.,** v. 15, p. 191-202, 1995.

BABA, E.; IKEMOTO, T.; FUKATA, T.; SASAI, K.; ARAKAWA, A.; MCDOUGALD, L. R. Clostridial population and the intestinal lesions in chickens infected with *Clostridium perfringens* and *Eimeria necatrix*. **Vet. Microbiol.,** v. 54, p. 301-308, 1997.

BAKER, A. A.; DAVIS, E.; REHBERGER, T.; ROSENER, D. Prevalence and diversity of toxigenic *Clostridium perfringens* and *Clostridium difficile* among swine herds in the midwest. **Appl. Environ. Microbiol**., v. 76, p. 2961-2967, 2010.

BALDASSI, L.; CASTRO, A. G. M.; GUERRA, J. L.; PORTUGAL, M. A. S. C.; CALIL, E. M. B.; MACRUZ, R. Necrotic enteritis in broilers in São Paulo state. **Arq. Inst. Bio., São Paulo,** v. 62, p. 37-43, 1995.

BANU, S.; OHTANI, K.; YAGUCHI, H.; SWE, T.; COLE, S. T.; HAYASHI, H.; SHIMIZU, T. Identification of novel VirR/VirS-regulated genes in *Clostridium perfringens*. **Mol. Microbiol.,** v. 35, p. 854-864, 2000.

BARBARA, A. J.; TRINH, H. T.; GLOCK, R. D.; SONGER, J. G. Necrotic enteritis-producing strains of *Clostridium perfringens* displace non-necrotic enteritis strains from the gut of chicks. **Vet. Microbiol.,** v.126, p. 377–382, 2008.

BARD, R.; GUNSALUS, I. Glucose metabolism of *Clostridium perfringens*: Existence of a metallo-aldolase. **J. Bacteriol.,** v. 59, p. 387-400, 1950.

BARE, L. N.; WISEMAN, R. F. Delayed Appearance of Lactobacilli in the Intestines of Chicks Reared in a "New" Environment. **Appl. Microbiol.,** v. 12, p. 457-459, 1964.

BARNES, E. M.; MEAD, G. C.; BARNUM, D. A.; HARRY, E. G. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. **Br. Poult. Sci.**, v. 13, p. 311-326, 1972.

BARE, L. N.; WISEMAN, R. F. Delayed Appearance of Lactobacilli in the Intestines of Chicks Reared in a "New" Environment. **Appl. Microbiol.,** v. 12, p. 457-459, 1964.

BARNES, E. M.; MEAD, G. C.; BARNUM, D. A.; HARRY, E. G. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. **Br. Poult. Sci.**, v. 13, p. 311-326, 1972.

BARROW, P. A.; TUCKER, J. F.; SIMPSON, J. M. Inhibition of colonization of the chicken alimentary tract with *Salmonella typhimurium* gram-negative facultatively anaerobic bacteria. **Epidemiol. Infect.,** v. 98, p. 311-322,1987.

BA-THEIN, W.; LYRISTIS, M.; OHTANI, K.; NISBET, I. T.; HAYASHI, H.; ROOD, J. I.; SHIMIZU, T. The virR/virS locus regulates the transcription of genes encoding extracellular toxin production in *Clostridium perfringens*. **J. Bacteriol.**, v. 178, p. 2514-2520, 1996.

BAUER, E.; WILLIAMS, B. A.; SMIDT, H.; MOSENTHIN, R.; VERSTEGEN, M. W. A. Influence of dietary components on development of the microbiota in single-stomached species. **Nutr. Res. Rev.,** v. 19, p. 63-78, 2006.

BAUMS, C. G.; SCHOTTE, U.; AMTSBERG, G.; GOETHE, R. Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. **Vet. Microbiol.,** v. 100, p. 11-16. 2004.

BELYI, Y.; AKTORIES, K. Bacterial toxin and effector glycosyltransferases. **Biochim. Biophys. Acta,** v. 1800, p. 134-143, 2010.

BERNIER, G.; FILION, R. Necrotic enteritis in broiler chickens. **J. Am. Vet. Med. Assoc.,** v. 158, p.1896-1897, 1971.

BEZIRTZOGLOU, E. The intestinal Microflora during the first weeks of life. **Anaerobe**, v. 3, p. 173-177, 1997.

BLAUT, M.; COLLINS, M. D.; WELLING, G. W.; DORE, J.; van LOO, J.; de VOS, W. Molecular biological methods for studying the gut microbiota: the EU human gut flora project. **Br. J. Nutr.,** v. 87, p. S203–S211, 2002.

BORASTON, A. B.; FICKO-BLEAN, E.; HEALEY, M. Carbohydrate Recognition by a Large Sialidase Toxin from *Clostridium perfringens*. **Biochemistry,** v. 46, p. 11352-11360, 2007.

BORRIELLO, S. P.; LARSON, H. E.; WELCH, A. R.; BARCLAY, F.; STRINGER, M. F.; BARTHOLOMEW, B. A. Enterotoxigenic *Clostridium perfringens*: a possible cause of antibiotic-associated diarrhoea. **Lancet**, v. 1, p. 305-307, 1984.

BORRIELLO, S. P. Pathogenesis of *Clostridium difficile* infection. **J. Antimicrob. Chemother.**, v. 41, p. 13-19, 1998.

BRADY, J.; HERNANDEZ-DORIA, J. D.; BENNETT, C.; GUENTER. W.; HOUSE, J. D.; RODRIGUEZ-LECOMPTE, J. C. Toxinotyping necrotic enteritis producing and commensal isolates of *Clostridium perfringens* from chickens fed organic diets. **Avian Pathol.,** v. 39, p. 475-481, 2010.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). **Instrução Normativa N.42. Define os requisitos e critérios específicos para o funcionamento dos laboratórios de Análises de resíduos e Contaminantes em**

Alimentos integrantes da Rede Nacional de Laboratórios Agropecuários. Brasília, 1999.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). **Portaria do Ministério da Agricultura, Pecuária e Abastecimento, nº 808.** Brasília, 2003.

BRYANT, A. E.; BERGSTROM, R.; ZIMMERMAN, G. A.; SALYER, J. L.; HILL, H. R.; TWETEN, R. K.; SATO, H.; STEVENS, D. L. *Clostridium perfringens* invasiveness is enhanced by effects of theta toxin upon PMNL structure and function: the roles of leukocytotoxicity and expression of CD11/CD18 adherence glycoprotein. **FEMS Immunol. Med. Microbiol.**, v. 7, p. 321-336, 1993.

BRYANT, A. E.; STEVENS, D. L. Phospholipase C and Perfringolysin O from *Clostridium perfringens* Upregulate Endothelial Cell-Leukocyte Adherence Molecule 1 and Intercellular Leukocyte Adherence Molecule 1 Expression and Induce Interleukin-8 Synthesis in Cultured Human Umbilical Vein Endothelial Cells. **Infect. Immun.,** v. 64, p. 358–362, 1996.

BRYNESTAD, S.; SYNSTAD, B.; GRANUM, P. The *Clostridium perfringens* enterotoxin gene is on transposable element in type A human food poisoning strains. **Microbiology,** v. 143, p. 2109-2115, 1997.

BRYNESTAD, S.; GRANUM, P. E. *Clostridium perfringens* and foodborne infections. **Int. J. Food Microbiol.**, v. 74, p. 195-202, 2002.

BUSCH, C.; AKTORIES, K. Microbial toxins and the glycosylation of rho family GTPases. **Curr. Opin. Struct. Biol.**, v. 10, p. 528-535, 2000.

BUSCH, C.; HOFMANN, F.; GERHARD, R.; AKTORIES, K. Involvement of a conserved tryptophan residue in the UDP-glucose binding of large clostridial cytotoxin glycosyltransferases. **J. Biol. Chem.,** v. 275, p. 13228-13234, 2000.

CABEZAS, J. A. Some questions and suggestions on the type references of the official nomenclature (IUB) for sialidase(s) and endosialidase. **Biochem. J**., v. 278, p. 311-312, 1991.

CARON, E.; HALL, A. Identification of Two Distinct Mechanisms of Phagocytosis Controlled by Different Rho GTPases. **Science,** v. 282, p. 1717-1721, 1998.

CARR, D.; SHAW, D. D.; HALVORSON, A.; RINGS, B.; ROEPKE, D. Excessive mortality in market-age turkeys associated with cellulitis. **Avian Dis.,** v. 40, p. 736-741, 1996.

CARTER, G. P.; ROOD, J. I.; LYRAS, D. The role of toxin A and toxin B in the virulence of *Clostridium difficile*. **Trends Microbiol.,** v. 20, p. 21-29, 2012.

CHAKRABORTY, G. C.; CHAKRABORTY, D.; BHATTACHARYYA, D.; BHATTACHARYYA, S.; GOSWAMI, U. N.; BHATTACHARYYA, H. M. Necrotic enteritis in poultry in West Bengal. **Indian J. Comp. Microbiol. Immunol. Infect. Dis.,** v. 5, p. 54-57, 1984.

CHALMERS, G.; BRUCE, H. L.; TOOLE, D.L.; BARNUM, B. A.; P. BOERLIN. Necrotic Enteritis Potential in a Model System Using *Clostridium perfringens* Isolated from Field Outbreaks. **Avian Dis.,** v. 51, p. 834-839, 2007.

CHALMERS, G.; BRUCE, H. L.; HUNTER, D. B.; PARREIRA, V. R.; KULKARNI, R. R.; JIANG, Y. F.; PRESCOTT, J. F.; BOERLIN, P. Multilocus Sequence Typing Analysis of *Clostridium perfringens* Isolates from Necrotic Enteritis Outbreaks in Broiler Chicken Populations. **J. Clin. Microbiol.,** v. 46, p. 3957-3964, 2008a.

CHALMERS, G.; MARTIN, S.; HUNTER, D.; PRESCOTT, J.; WEBER, L.; BOERLIN, P. Genetic diversity of *Clostridium perfringens* isolated from healthy broiler chickens at a commercial farm. **Vet. Microbiol.,** v. 127, p. 116-127, 2008b.

CHEUNG, J. K.; AWAD, M. M.; MCGOWAN, S.; ROOD, J. I. Functional Analysis of the VirSR Phosphorelay from *Clostridium perfringens*. **PLoS ONE,** v. 4, p. e5849, 2009.

CHEUNG, J. K.; KEYBURN, A. L.; CARTER, G. P.; LANCKRIET, A. L.; VAN IMMERSEEL, F.; MOORE, R. J.; ROOD, J. I. The VirSR Two-Component Signal Transduction System Regulates NetB Toxin Production in *Clostridium perfringens*. **Infect. Immun.,** v. 78, p. 3064-3072, 2010.

CHIAREZZA, M.; LYRAS, D.; PIDOT, S. J.; FLORES-DÍAZ, M.; AWAD, M. M.; KENNEDY, C. L.; CORDNER, L. M.; PHUMOONNA, T.; POON, R.; HUGHES, M. L.; EMMINS, J. J.; ALAPE-GIRÓN, A.; ROOD, J. I. The NanI and NanJ Sialidases of *Clostridium perfringens* are not essential for virulence. **Infect. Immun.,** v. 77, p. 4421–4428, 2009.

CLSI. Clinical and Laboratory Standards Institute. **Approved standard M100-S17. Performance standards for antimicrobial susceptibility testing, seventeenth informational supplement**. Clinical and Laboratory Standards Institute, Wayne, PA: Clinical and Laboratory Standards Institute, 2007.

COLLIER, C. T.; van der KLIS, J. D.; DEPLANCKE, B.; ANDERSON, D. B.; GASKINS, H. R. Effects of tylosin on bacterial mucolysis, colonization, and intestinal barrier function in a chick model of necrotic enteritis. **Antimicrob. Agents Chemother.,** v. 47, p. 3311-3317, 2003.

COLLIER, C. T.; HOFACRE, C. L.; PAYNE, A. M.; ANDERSON, D. B.; KAISER, P.; MACKIE, R. I.; GASKINS, H. R. Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting growth. **Vet. Immunol. Immunopathol.,** v. 122, p.104-115, 2008.

COLLINS, M. D.; LAWSON, P. A.; WILLEMS, A.; CORDOBA, J. J.; FERNANDEZ-GARAYZABAL, J.; GARCIA, P.; CAI, J.; HIPPE, H.; FARROW, J. A. E. The Phylogeny of the Genus Clostridium: Proposal of Five New Genera and Eleven New Species Combinations. **Int. J. Syst. Bacteriol.,** v. 44, p. 812-826, 1994.

COOPER, K. K.; TRINH, H. T.; SONGER, J. G. Immunization with recombinant alpha toxin partially protects broiler chicks against experimental challenge with *Clostridium perfringens*. **Vet. Microbiol.,** v. 133, p. 92–97, 2009.

COOPER, K. K.; SONGER, J. G. Necrotic enteritis in chickens: A paradigm of enteric infection by *Clostridium perfringens* type A. **Anaerobe,** v. 15, p. 55-56, 2009.

COOPER, K. K.; SONGER, J. G. Virulence of *Clostridium perfringens* in an experimental model of poultry necrotic enteritis. **Vet. Microbiol.,** v. 142, p. 323-328, 2010.

CORFIELD, T. Bacterial sialidases roles in pathogenicity and nutrition. **Glycobiology**, v. 2, p. 509-521, 1992.

COURSODON, C. F.; TRINH, H. T.; MALLOZZI, M.; VEDANTAM, G.; GLOCK, R. D.; SONGER, J. G. *Clostridium perfringens* alpha toxin is produced in the intestines of broiler chicks inoculated with an alpha toxin mutant. **Anaerobe,** v. 16, p. 614-617, 2010.

COURSODON, C. F.; GLOCK, R. D.; MOORE, K. L.; COOPER, K. K.; SONGER, J. G. TpeL-producing strains of *Clostridium perfringens* type A are highly virulent for broiler chicks. **Anaerobe,** v. 18, p. 117-121, 2012.

CRAVEN, S. E.; COX, N. A.; BAILEY, J. S.; COSBY, D. E. Incidence and tracking of *Clostridium perfringens* through an integrated broiler chicken operation. **Avian Dis.,** v. 47, p. 707-711, 2003.

CZÁRÁN, T. L.; HOEKSTRA, R. F.; PAGIE, L. Chemical warfare between microbes promotes biodiversity. **PNAS,** v. 99, p. 786 -790, 2002.

DAHIYA, J. P.; HOEHLER, D.; WILKIE, D. C.; VAN KESSEL, A. G.; DREW, M. D. Dietary Glycine Concentration Affects Intestinal *Clostridium perfringens* and *Lactobacilli* Populations in Broiler Chickens. **Poult. Sci.,** v. 84, p. 1875-1885, 2005.

DAFAALLA, E. N.; SOLTYS, M. Agglutination of red cells by *Clostridium welchii*. **Nature,** v. 172, p. 38-39, 1953.

DE CESARE, A.; BORILOVA, G.; SVOBODOVA, I.; BONDIOLI, V.; MANFREDA, G. *Clostridium perfringens* occurrence and ribotypes in healthy broilers reared in different European countries. **Poult. Sci.,** v. 88, p. 1850-1857, 2009.

DEPLANCKE, B.; GASKINS, H. R. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. **Am. J. Clin. Nutr,** v. 73, p. 1131S–1141S, 2001.

DEPLANCKE, B.; VIDAL, O.; GANESSUNKER, D.; DONOVAN, S.M.; MACKIE, R.I.; GASKINS, H. R. Selective growth of mucolytic bacteria including *Clostridium perfringens* in a neonatal piglet model of total parenteral nutrition. **Am. J. Clin. Nutr.,** v. 76, p. 1117-1125, 2002.

DIBNER, J. J.; RICHARDS, J. D. Antibiotic growth promoters in agriculture: history and mode of action. **Poult. Sci.,** v. 84, p. 634-643, 2005.

DJOUDER, N.; PREPENS, U.; AKTORIES, K.; CAVALIE, A. Inhibition of Calcium Release-activated Calcium Current by Rac/Cdc42-inactivating Clostridial Cytotoxins in RBL Cells. **J. Biol. Chem.,** v. 275, p. 18732-18738, 2000.

DUBOS, R. J.; SCHAEDLER, R. W. The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. **J. Exp. Med.**, v. 1, p. 407-417, 1960.

DUBOS, R.; SCHAEDLER, R.; COSTELLO, R.; HOET, P. Indigenous, Normal, and Autochthonous Flora of the Gastrointestinal Tract. **J. Exp. Med.,** v. 122, p. 67-76, 1965.

DUERDEN, B. I.; DRASAR, B. B. **Anaerobes and Human Disease.** London: Ed. Hodder Arnold, 1991.

DUNCAN, C. L.; STRONG, D. H. Improved medium for sporulation of *Clostridium perfringens*. **Appl. Microbiol**, v. 16, p. 82-89, 1968.

DUTTA, G. N.; DEVRIESE, L. Susceptibility of *Clostridium perfringens* of animal origin to fifteen antimicrobial agents. **J. Vet. Pharmacol. Therap.,** v. 3, p. 227-237, 1980.

DWORKIN, M. M.; FALKOW, S.; ROSENBERG, E.; SCHLEIFER, K. H.; STACKEBRANDT, E. **The Prokaryotes. A Handbook on the Biology of Bacteria.** USA. Third Edition. Ed. Springer, 2006.

ENGBERG, R. M.; HEDEMANN, M. S.; LESER, T. D.; JENSEN, B. B. Effect of Zinc Bacitracin and Salinomycin on Intestinal Microflora and Performance of Broilers. **Poult. Sci.,** v. 79, p. 1311-1319, 2000.

ENGBERG, R. M.; HEDEMANN, M. S.; STEENFELDT, S.; JENSEN, B. B. Influence of Whole Wheat and Xylanase on Broiler Performance and Microbial Composition and Activity in the Digestive Tract. **Poult. Sci.,** v. 83, p. 925-938, 2004.

ENGSTRÖM, B. E.; FERMÉR, C.; LINDBERG, A.; SAARINEN, E.; BAVERUD, V.; GUNNARSSON, A. Molecular typing of isolates of *Clostridium perfringens* from healthy and diseased poultry. **Vet. Microbiol.,** v. 94, p. 225-235, 2003.

ENGSTRÖM, B. E.; JOHANSSON, A.; ASPAN, A.; KALDHUSDAL, M. Genetic relatedness and *netB* prevalence among environmental *Clostridium perfringens* strains associated with a broiler flock affected by mild necrotic enteritis. **Vet. Microbiol.,** v. 159, p. 260-264, 2012.

FENG, Y.; GONG, J.; YU, H.; JIN, Y.; ZHU, J.; HAN, J. Identification of changes in the composition of ileal bacterial microbiota of broiler chickens infected with *Clostridium perfringens*. **Vet. Microbiol.,** v. 140, p. 116-121, 2010.

FICKEN, M. D., WAGES, D. P. Necrotic enteritis. In: CALNEK, B. W. (Ed.). **Diseases of poultry**. 10. ed. Iowa: Mosby-Wolfe, 1997.

FISHER, D. J.; FERNANDEZ-MIYAKAWA, M. E.; SAYEED, S.; POON, R.; ADAMS, V.; ROOD, J. I.; UZAL, F. A.; McCLANE, B. A. Dissecting the contributions of *Clostridium perfringens* type C toxins to lethality in the mouse intravenous injection model. **Infect. Immun.**, v. 74, p. 5200-5210, 2006.

FLORES-DÍAZ, M.; ALAPE-GIRÓN, A.; TITBALL, R.; MOOS, M.; GUILLOUARD, I.; COLE, S.; HOWELLS, A. M.; VON EICHEL-STREIBER, C.; FLORIN, I.; THELESTAM, M. UDP-glucose Deficiency Causes Hypersensitivity to the Cytotoxic Effect of *Clostridium perfringens* Phospholipase C. **J. Biol. Chem.,** v. 273, p. 24433-24438, 1998.

FLORES-DÍAZ, M.; ALAPE-GIRÓN, A.; CLARK, G.; CATIMEL, B.;HIRABAYASHI, Y.; NICE, E.; GUTIÉRREZ, J. M.; TITBALL, R.;THELESTAM, M. A Cellular Deficiency of Gangliosides Causes Hypersensitivity to *Clostridium perfringens* Phospholipase C. **J. Biol. Chem.,** v. 280, p. 26680-26689, 2005.

FOOD AND DRUG ADMINISTRATION. US FDA takes steps to reduce use of antibiotic growth promoters. **Vet. Rec.**, v. 170, p. 404**,** 2012.

FOOD AND DRUG ADMINISTRATION. **Guidance for Industry. The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals**. USA. 2012

FOSSUM, O.; SANDSTEDT, K.; ENGSTRÖM, B. E. Gizzard erosions as a cause of mortality in White Leghorn chickens. **Avian Pathol**., v. 17, p. 519-525, 1988.

FRASER A. G. Neuraminidase production by *Clostridia*. **J. Med. Microbiol.,** v. 11, p. 269-280, 1978.

GADBOIS, P.; ,BRENNAN, J. J.; BRUCE, L.; WILSON, J. B.; ARAMINI, J. J. The role of penicillin G potassium in managing *Clostridium perfringens* in broiler chickens. **Avian Dis.**, v. 52, p. 407-411, 2008.

GARRITY, G. M.; LILBURN, T. G.; COLE, J. R.; HARRISON, S. H.; EUZÉBY, J.; TITDALL, B. J. **Taxonomic outline of the bacteria and Archaea.** Michigan State University Board of Trustees, 2007.

GAUDIER, E.; JARRY, A.; BLOTTIÈRE, H. M.; DE COPPET, P.; BUISINE, M. P.; AUBERT, J. P.; LABOISSE, C.; CHERBUT, C.; HOEBLER, C. Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. **Am. J. Physiol. Gastrointest. Liver Physiol.**, v. 287, p. G1168-1174, 2004.

GAZDZINSKI, P.; JULIAN, R. J. Necrotic enteritis in turkeys. **Avian Dis.,** v. 36, p. 792-798, 1992.

GHARAIBEH, S.; AL RIFAI, R.; AL-MAJALI, A. Molecular typing and antimicrobial susceptibility of *Clostridium perfringens* from broiler chickens. **Anaerobe,** v. 16, p. 586-589, 2010.

GHOLAMIANDEKHORDI, A. R.; DUCATELLE, R.; HEYNDRICKX, M.; HAESEBROUCK, F.; VAN IMMERSEEL, F. Molecular and phenotypical characterization of *Clostridium perfringens* isolates from poultry flocks with different disease status. **Vet Microbiol.,** v. 113, p. 143-152, 2006.

GHOLAMIANDEKHORDI, A.; EECKHAUT, V.; LANCKRIET, A.; TIMBERMONT, L.; BJERRUM, L.; DUCATELLE, R.; HAESEBROUCK, F.; VAN IMMERSEEL, F. Antimicrobial resistance in *Clostridium perfringens* isolates from broilers in Belgium. **Vet. Res. Commun.,** v. 33, p. 1031-1037, 2009.

GIBERT, M.; JOLIVET-RENAUD, C.; POPOFF, M. R. Beta2 toxin, a novel toxin produced by *Clostridium perfringens*. **Gene,** v. 203, p. 65-73, 1997.

GILCHRIST, M. J.;GREKO, C.; WALLINGA, D. B.; BERAN, G. W.; RILEY, D. G.; THORNE, P. S. The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. **Environ. Health Perspect.**, v. 115, p. 313-316, 2007.

GILLOR, O.; ETZION, A.; RILEY, M. A. The dual role of bacteriocins as anti- and probiotics. **Appl. Microbiol. Biotechnol.,** v. 8, p. 591-606, 2008.

GODDARD, P.; FERNANDEZ, F.; WEST, B.; HILL, M. J.; BARNES, P. The nuclear dehydrogenation of steroids by intestinal bacteria. **J. Med. Microbiol.**, v. 8, p. 429-435, 1975.

GOLDER, H. M.; GEIER, M. S.; FORDER, R. E. A.; HYND, P. I.; HUGHES, R. J. Effects of necrotic enteritis challenge on intestinal micro-architecture and mucin profile. **Br. Poult. Sci.,** v. 52, p. 500-506, 2011.

GONG, J.; SI, W.; FORSTER, R.; HUANG, R.; YU, H.; YIN, Y.; YANG, C.; HAN, Y. 16S rRNAgene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. **FEMS Microbiol. Ecol.,** v. 59, p. 147-157, 2007.

GONG, J.; YU, H.; LIU, T.; GILL, J. J.; CHAMBERS, J. R.; WHEATCROFT, R.; SABOUR, P. M. Effects of zinc bacitracin, bird age and access to range on bacterial microbiota in the ileum and caeca of broiler chickens. **J. Appl. Microbiol.,** v. 104, p. 1372-1382, 2008.

GOÑI, F. M.; MONTES, L. R.; ALONSO, A. Phospholipases C and sphingomyelinases:Lipids as substrates and modulators of enzyme activity. **Prog. Lipid. Res.**, v. 51, p. 238-266. 2012.

GRAVE, K.; KALDHUSDAL, M. C.; KRUSE, H.; HARR, L. M.; FLATLANDSMO, K. What has happened inNorway after the ban of avoparcin? Consumption of antimicrobials by poultry. **Prev. Vet. Med.**, v. 62, p. 59-72, 2004.

GRAVE, K.; JENSEN, V. F.; ODENSVIK, K.; WIERUP, M.; BANGEN, M. Usage of veterinary therapeutic antimicrobials in Denmark, Norway and Sweden following termination of antimicrobial growth promoter use. **Prev. Vet. Med.**, v. 75, p. 123-132, 2006.

GREGG, K.; FINN, R.; ABBOTT, D.; BORASTON, A. Divergent of glycan recognition by a new family of carbohydrate-binding modules. **J. Biol. Chem.,** v. 283, p. 12604-12613, 2008.

GUTTENBERG, G.; HORNEI, S.; JANK, T.; SCHWAN, C.; LÜ, W.; EINSLE, O.; PAPATHEODOROU, P.; AKTORIES, K. Molecular Characteristics of *Clostridium perfringens* TpeL Toxin and Consequences of Mono-O-GlcNAcylation of Ras in Living Cells. **J. Biol. Chem.,** v. 287, p. 24929–24940, 2012.

HATHEWAY, C. Toxigenic Clostridia. **Clin. Microb. Rev.,** v. 3, p. 66-98, 1990.

HEIKINHEIMO, A.; KORKEALA, H. Multiplex PCR assay for toxinotyping *Clostridium perfringens* isolates obtained from Finnish broiler chickens. **Lett. Appl. Microbiol.**, v. 40, p. 407-411, 2005.

HERHOLZ, C.; MISEREZ, R.; NICOLET, J.; FREY, J.; POPOFF, M.; GIBERT, M.; GERBER, H.; STRAUB, R. Prevalence of β2-Toxigenic *Clostridium perfringens* in Horses with Intestinal Disorders. **J. Clin. Microbiol.**, v. 37, p. 358-361, 1999.

HIBBERD, M. C.; NEUMANN, A. P.; REHBERGER, T. G.; SIRAGUSA, G. R. Multilocus sequence typing subtypes of poultry *Clostridium perfringens* isolates demonstrate disease niche partitioning. **J. Clin. Microbiol**., v. 49, p. 1556-1567, 2011.

HOLDEMAN, L. V.; CATO, E. P.; MOORE, W. E. **Anaerobic laboratory manual.** 4. ed. Blackburg, VA: Virginia Polytechnic Institute, 1977.

JEPSON, M.; BULLIFENT, H. L.; CRANE, D.; FLORES-DIAZ, M.; ALAPE-GIRON, A.; JAYASEKEERA, P.; LINGARD, B.; MOSS, D.; TITBALL, R. W. Tyrosine 331 and

phenylalanine 334 in *Clostridium perfringens* α-toxin are essential for cytotoxic activity. **FEBS Lett.,** v. 495, p. 172-177, 2001.

JIANG, Y.; KULKARNI, R. R.; PARREIRA, V. R.; PRESCOTT, J. F. Immunization of broiler chickens against *Clostridium perfringens* induced necrotic enteritis using purified recombinant immunogenic proteins. **Avian Dis.**, v. 53, p. 409-415, 2009.

JOHANSSON, A.; GREKO, C.; ENGSTRÖM, B. E.; KARLSSON, M. Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of *Clostridium perfringens* from poultry, and distribution of tetracycline resistance genes. **Vet. Microbiol.,** v. 99, p. 251–257, 2004.

JOHNSON, C.; PINEDO, C. Gizzard erosion and ulceration in Peru broilers. **Avian Dis.,** v. 15, p. 835-837, 1971.

JOHNSON, J. L.; FRANCIS, B. Taxonomy of the Clostridia: Ribosomal Ribonucleic Acid Homologies among the Species. **J. Gen. Microbiol.**, v. 88, p. 229-244, 1975.

JORDAN, F. T. W. Clostridia. In: JORDAN, F. T. W.; PATTISON, M. (Ed.). **Poultry diseases.** 4th ed. London: W. B. Saunders Co, 1996.

JOST, B. H.; TRINH, H. T.; SONGER, J. G. Clonal relationships among *Clostridium perfringens* of porcine origin as determined by multilocus sequence typing. **Vet. Microbiol.,** v. 116, p. 158-165, 2006.

KALDHUSDAL, M.; HOFSHAGEN, M.; LØVLAND, A.; LANGSTRAND, H.; REDHEAD, K. Necrotic enteritis challenge models with broiler chickens raised on litter: evaluation of preconditions, *Clostridium perfringens* strains and outcome variables. **FEMS Immunol. Med. Microbiol.**, v. 24, p. 337-343, 1999.

KALDHUSDAL, M. I.; LOVLAND, A. Necrotic enteritis (4): the economical impact of *Clostridium perfringens* is greater than anticiped. **World Poult.,** v. 16, p. 50-51, 2000.

KALDHUSDAL, M.; SCHNEITZ, C.; HOFSHAGEN, M.; SKJERVE, E. Reduced incidence of *Clostridium perfringens* -associated lesions and improved performance in broiler chickens treated with normal intestinal bacteria from adult fowl. **Avian Dis.,** v. 45, p. 149-156, 2001.

KATHER, E. J.; MARKS, S. L.; FOLEY, J. E. Determination of the prevalence of antimicrobial resistance genes in canine *Clostridium perfringens* isolates. **Vet. Microbiol.,** v. 113, p. 97-101, 2006.

KEYBURN, A. L.; SHEEDY, S. A.; FORD, M. E.; WILLIAMSON, M. M.; AWAD, M. M.; ROOD, J. I.; MOORE, R. J. Alpha-toxin of *Clostridium perfringens* is not essential virulence factor in necrotic enteritis in chickens. **Infect. Immun.,** v. 74, p. 6496-6500, 2006.

KEYBURN, A. L.; BOYCE, J. D.; VAZ, P.; BANNAM, T. L.; FORD, M. E.; PARKER, D.; DI RUBBO, A.; ROOD, J. I.; MOORE, R. J. NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens*. **PLoS Pathogens,** v. 4, p. 1-11, 2008.

KOHLER, B., MARX, G.; KOLBACH, S.; BOTTCHER, E. Untersuchungen zur nekrotischen enteritis der huhner 1. Mitt.: Diagnostik und bekampfung. **Monatsh Veterinaermed,** v. 29, p. 380-384, 1974.

KONEMAN, E. W.; ALLEN, S. D.; JANDA, W. M.; SCHRECKENBERGER, P. C.; WINN, W. C. **Diagnóstico microbiológico.** 5. ed. Argentina: Editorial Médica Panamericana, 2004.

KULKARNI, R. R.; PARREIRA, V. R.; SHARIF, S.; PRESCOTT, J. F. Immunization of broiler chickens against *Clostridium perfringens* induced necrotic enteritis. **Clin. Vaccine Immunol.,** v. 14, p. 1070-1077, 2007.

LANCKRIET, A.; TIMBERMONT, L.; EECKHAUT, V.; HAESEBROUCK, F.; DUCATELLE, R.; VAN IMMERSEEL, F. Variable protection after vaccination of broiler chickens against necrotic enteritis using supernatants of different *Clostridium perfringens* strains. **Vaccine**, v. 28, p. 5920-5923, 2010.

LAWHON, S. D.; TAYLOR, A.; FAJT, V. R. Frequency of resistance in obligate anaerobic bacteria isolated from dogs, cats, and horses to antimicrobial agents. **J. Clin. Microbiol**., v. 51, p. 3804-3810, 2013.

LEE, K.; LILLEHOJ, H. S.; LI, G.; PARK, M. S.; JANG, S. I.; JEONG, W.; JEOUNG, H. Y.; AN, D. J.; LILLEHOJ, E. P. Identification and cloning of two immunogenic *Clostridium perfringens* proteins, elongation factor Tu (EF-Tu) and pyruvate:ferredoxin oxidoreductase (PFO) of *C. perfringens.* **Res. Vet. Sci.**, v. 91, p. e80-86, 2011.

LEPP, D.; ROXAS, B.; PARREIRA, V. R.; MARRI, P. R.; ROSEY, E. L.; GONG, J.; SONGER, J. G.; VEDANTAM, G.; PRESCOTT, J. F. Identification of novel pathogenicity loci in *Clostridium perfringens* strains that cause avian necrotic enteritis. **PLoS One**, v. 5, p. e10795, 2010.

LEPP, D.; GONG, J.; SONGER, J. G.; BOERLIN, P.; PARREIRA, V. R.; PRESCOTT, J. F. Identification of Accessory Genome Regions in Poultry *Clostridium perfringens* Isolates Carrying the *netB* Plasmid. **J. Bacteriol**., v. 195, p. 1152-1166, 2013.

LEV, M.; BRIGGS, C. A. E. The gut flora of the chick.I. The flora of newly hatched chicks. **J. Appl. Bacteriol.,** v. 19, p. 36-38, 1956.

LI, J.; SAYEED, S.; ROBERTSON, S.; CHEN, J.; McCLANE, B. A. Sialidases Affect the Host Cell Adherence and Epsilon Toxin-Induced Cytotoxicity of *Clostridium perfringens* Type D Strain CN3718. **PLoS Pathog.,** v. 7, p. e1002429, 2011.

LONG, J. R.; Necrotic enteritis in broiler chickens. I. A review of the literature and the prevalence of the disease in Ontario. **Can. J. Comp. Med.**, v. 37, p. 302-308, 1973.

LONG, J. R.; PETTIT, J. R.; BARNUM, D. A. Necrotic enteritis chickens. II. Pathology and proposed pathogenesis. **Can. J. Comp. Med.,** v. 38, p. 467-474, 1974.

LONG, J. R.; TRUSCOTT, R. Necrotic enteritis in broiler chickens. III. Reproduction of the disease. **Can. J. Comp. Med.,** v. 40, p. 53-59. 1976.

LOVLAND, A.; KALDHUSDAL, M. Severely impaired production performance in broiler flocks with high incidence of *Clostridium perfringens* -associated hepatitis. **Avian Pathol.,** v. 30, p. 73-81, 2001.

LOVLAND, A.; KALDHUSDAL, M. Liver lesions seen at slaughter as the indicator of necrotic enteritis in broilers flocks. **FEMs Immunol. Med. Microbiol.,** v. 24, p. 345-351, 2001b.

MARKS, S. L.; RANKIN, S. C.; BYRNE, B. A.; WEESE, J. S. Enteropathogenic bacteria in dogs and cats: diagnosis, epidemiology, treatment, and control. **J. Vet. Intern. Med**., v. 25, p. 1195-1208, 2011.

MARTEL , A.; DEVRIESE, L. A.; CAUWERTS, K.; DE GUSSEN, K.; DECOSTERE, A.; HAESEBROUCK, F. Susceptibility of *Clostridium perfringens* to antibiotic and anticoccidials. **Avian Pathol.,** v. 33, p. 3-7, 2004.

MARTIN, T. G.; SMYTH, J. A. Prevalence of *netB* among some clinical isolates of *Clostridium perfringens* from animals in the United States. **Vet. Microbiol.,** v. 136, p. 202-205, 2009.

MARTINEZ, F. A.; BALCIUNAS, E. M.; CONVERTI, A.; COTTER, P. D.; de SOUZA OLIVEIRA, R. P. Bacteriocin production by *Bifidobacterium* spp. A review. **Biotechnol. Adv.** v. 4, p. 482-488, 2013.

MATSUSHITA, O.; YOSHIHARA, K.; KATAYAMA, S. I. ; MINAMI, J.; OKABE, A. Purification and Characterization of a *Clostridium perfringens* 120-Kilodalton Collagenase and Nucleotide Sequence of the Corresponding Gene. **J. Bacteriol.,** v. 176, p. 149-156, 1994.

MATSUSHITA, C.; MATSUSHITA, O.; KATAYAMA, S.; MINAMI, J.; TAKAI, K.; OKABE, A. An upstream activating sequence containing curved DNA involved in activation of the *Clostridium perfringens plc* promoter. **Microbiology**, v. 142, p. 2561-2566, 1996.

MATSUSHITA, O.; JUNG, C. M.; KATAYAMA, S. I.; MINAMI, J.; TAKAHASHI, Y.; OKABE, A. Gene Duplication and Multiplicity of Collagenases in *Clostridium histolyticum***. J. Bacteriol.,** v. 181, p. 923-933, 1999.

McCLANE, B. A.; McDONEL, J. L. The effects of *Clostridium perfringens* enterotoxin on morphology, viability, and macromolecular synthesis in Vero cells. **J. Cell Physiol.**, v. 99, p. 191-200, 1979.

McCLANE, B. A. *Clostridium perfringens* type C isolates rapidly upregulate their toxin production upon contact with host cells. New insights into virulence?. **Virulence,** v. 1-2, p. 97-100, 2010.

McCOURT, M. T.; FINLAY, D. A.; LAIRD, C.; SMYTH, J. A.; BELL, C.; BALL, H. J. Sandwich ELISA detection of *Clostridium perfringens* cells and α-toxin from field cases of necrotic enteritis of poultry. **Vet. Microbiol.,** v. 106, p. 259-264, 2005.

McGUCKIN, M. A.; LINDÉN, S. K.; SUTTON, P.; FLORIN, T. H. Mucin dynamics and enteric pathogens. **Nat. Rev. Microbiol.,** v. 9, p. 265-278, 2011.

MEAD, G. C.; ADAMS, B. W. Some observations on the caecal microflora of the chick during the first two weeks of life. **Br. Poult. Sci**., v. 16, p. 169-176, 1975.

MEAD, G. C. Microbes of the avian cecum: types present and substrates utilized. **J. Exp. Zool**., v. 3, p. 48-54, 1989.

MORCH, J. Necrotic enteritis in broilers in Denmark. **Proc. XV World's Poult. Congr. Expos.**, p. 290-292, 1974.

MYERS, G. S.; RASKO, D. A.; CHEUNG, J. K.; RAVEL, J.; SESHADRI, R.; DEBOY, R. T.; REN, Q.; VARGA, J.; AWAD, M. M.; BRINKAC, L. M.; DAUGHERTY, S. C.; HAFT, D. H.; DODSON, R. J.; MADUPU, R.; NELSON, W. C.; ROSOVITZ, M. J.; SULLIVAN, S. A.; KHOURI, H.; DIMITROV, G. I.; WATKINS, K. L.; MULLIGAN, S.; BENTON, J.; RADUNE, D.; FISHER, D. J.; ATKINS, H. S.; HISCOX, T.; JOST, B. H.; BILLINGTON, S. J.; SONGER, J. G.; MCCLANE, B. A.; TITBALL, R. W.; ROOD, J. I.; MELVILLE, S. B.; PAULSEN, I. T. Skewed genomic variability in strains of the toxigenic bacterial pathogen, *Clostridium perfringens*. **Genome Res.** v. 16, p. 1031-1040, 2006.

NAGAHAMA, M.; OHKUBO, A.; ODA, M.; KOBAYASHI, K.; AMIMOTO, K.; MIYAMOTO, K.; SAKURAI, J. *Clostridium perfringens* TpeL Glycosylates the Rac and Ras Subfamily Proteins. **Infect. Immun.,** v. 79, p. 905-910, 2011.

NAIRN, M. E.; BAMFORD, V. W. Necrotic enteritis of broiler chickens in western Australia. **Aust. Vet. J.**, v. 43, p. 49-54, 1967.

NAKANO, V.; FONTES PIAZZA, R. M.; AVILA-CAMPOS, M. J. A rapid assay of the sialidase activity in species of the *Bacteroides fragilis* group by using peanut lectin hemagglutination. **Anaerobe,** v. 12, p. 238-241, 2006.

NAKANO, V.; PIAZZA, R. M.; CIANCIARULLO, A. M.; BUERIS, V.; SANTOS, M. F.; MENEZES, M. A.; MENDES-LEDESMA, M.R.; SZULCZEWSKI, V.; ELIAS, W. P.; PUMBWE, L.; WEXLER, H.; AVILA-CAMPOS, M. J. Adherence and invasion of *Bacteroidales* isolated from the human intestinal tract. **Clin. Microbiol. Infect.**, v. 14, p. 955-963, 2008.

NAUERBY, B.; PEDERSEN, K.; MADSEN, M. Analysis by pulsed-field gel electrophoresis of the genetic diversity among *Clostridium perfringens* isolates from chickens. **Vet. Microbiol.,** v. 94, p. 257-266, 2003.

NAVANEETHAN, U.; GIANNELLA; R. A. Mechanisms of infectious diarrhea. **Nat. Clin. Pract. Gastroenterol. Hepatol.**, v. 5, p. 637-647, 2008.
NELSON, D.; COX, M. **Lehninger principles of biochemistry**. 4. ed, USA. Ed. W. H. FREEMAN, 2005.

NIEWOLD, T. A. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. **Poultry Sci.,** v. 86, p. 605-609, 2007.

NIILO, L. *Clostridium perfringens* in animal disease: a review of current knowledge. **Can**. **Vet. J.,** v. 21, p. 141-148, 1980.

NOEL, K.; HOLT, J. **Bergey's manual of systematic bacteriology**. Baltimore: Williams e Wilkins, 2001.

O'BRIEN, D. K.; MELVILLE, S. B. The anaerobic pathogen *Clostridium perfringens* can escape the phagosome of macrophages under aerobic conditions. **Cell Microbiol**., v. 2, p. 505-519, 2000.

ODOU, M. F.; MULLER C, CALVET, L.; DUBREUIL, L. In vitro activity against anaerobes of retapamulin, a new topical antibiotic for treatment of skin infections. **J. Antimicrob. Chemother.**, v. 59, p. 646-651, 2007.

OHTANI, K.; HAYASHI, H.; SHIMIZU, T. The *luxS* gene is involved in cell-cell signaling for toxin production in *Clostridium perfringens*. **Mol. Microbiol.,** v. 44, p. 171-179, 2002.

OHTANI, K.; KAWSAR, H. I.; OKUMURA, K.; HAYASHI, H.; SHIMIZU, T. The VirR/VirS regulatory cascade affects transcription of plasmid-encoded putative virulence genes in *Clostridium perfringens* strain 13. **FEMS Microbiol. Lett.,** v. 222, p. 137-141, 2003.

OLKOWSKI, A. A.; WOJNAROWICZ, C.; CHIRINO-TREJO, M., DREW, M. D. Responses of broiler chickens orally challenged with *Clostridium perfringens* isolated from Field cases of necrotic enteritis. **Res. Vet. Sci.**, v. 81, p. 99-108, 2006.

PAREDES-SABJA, D.; SARKER, N.; SARKER, M. R. *Clostridium perfringens tpeL* is expressed during sporulation. **Microb. Pathog.**, v. 51, p. 384-388, 2011.

PARKER, C. T.; SPERANDIO, V. Cell-to-cell signalling during pathogenesis. **Cell. Microbiol.**, v. 11, p. 363–369, 2009.

PEDERSEN, K.; BJERRUM, L.; NAUERBY, B.; MADSEN, M. Experimental infections with rifampicin-resistant *Clostridium perfringens* strains in broiler chickens using isolator facilities. **Avian Pathol.,** v. 32, p. 403-411, 2003.

PERELLE, S.; GIBERT, M.; BOQUET, P.; POPOFF, M. R. Characterization of *Clostridium perfringens* iota-toxin genes and expression in *Escherichia coli*. **Infect. Immun.**, v. 61, p. 5147-5156, 1993.

PETIT, L.; GIBERT, M.; POPOFF, M. *Clostridium perfringens*: toxinotype and genotype. **Trends Microbiol.**, v. 7, p. 104-110, 1999.

PLÜDDEMANN, A.; MUKHOPADHYAY, S.; GORDON, S. The interaction of macrophage receptors with bacterial ligands. **Expert. Rev. Mol. Med.,** v. 8, p. 1-25, 2006.

POPOFF, M. R.; DODIN, A. Survey of Neuraminidase Production by *Clostridium butyricum*, *Clostridium Beijerinckii*, and *Clostridium difficile* Strains from Clinical and Nonclinical Sources. **J. Clin. Microbiol.,** v. 22, p. 873-876, 1985.

POPOFF, M. R.; BOUVET, P. Genetic characteristics of Toxigenic Clostridia and toxin gene evolution. **Toxicon**, v. 75, p. 63-89, 2013.

PRUTEANU, M.; HYLAND, N. P.; CLARKE, D. J.; KIELY, B.; SHANAHAN, F. Degradation of the Extracellular Matrix Components by Bacterial-derived Metalloproteases: Implications for Inflammatory Bowel Diseases. **Inflamm. Bowel. Dis.**, v. 17, p. 1189-1200. 2011.

PRUTEANU, M.; SHANAHAN, F. Digestion of epithelial tight junction proteins by the commensal *Clostridium perfringens*. **Am. J. Physiol. Gastrointest. Liver Physiol.**, v. 305, p. G740-748.2013

RAY, P. K.; SIMMONS, R. Comparative effect of viral and bacterial neuraminidase on the complement sensitivity of lymphoid cells. **Clin. Exp. Immunol**., v. 10, p. 139-150, 1972.

RHEE, S. J.; WALKER, W. A.; CHERAYIL, B. J. Developmentally regulated intestinal expression of IFN-gamma and its target genes and the age-specific response to enteric *Salmonella* infection. **J. Immunol.**, v. 175, p. 1127-1136, 2005.

RICHARD, J. F.; PETIT, L.; GIBERT, M.; MARVAUD, J. C.; BOUCHAUD, C.; POPOFF, M. R. Bacterial toxins modifying the actin cytoskeleton. **Int. Microbiol.**, v. 2, p. 185-194, 1999.

RILEY, M. A. Molecular mechanisms of Bacteriocin evolution. **Annu. Rev. Genet.,** v. 32, p. 255-278, 1998.

RINKINEN, M.; JALAVA, K.; WESTERMARCK, E.; SALMINEN, S.; OUWEHAND, A. C. Interaction between probiotic lactic acid bacteria and canine enteric pathogens: a risk factor for intestinal *Enterococcus faecium* colonization?. **Vet. Microbiol.,** v. 92, p. 111-119, 2003.

ROBERTS, S. A.; SHORE, K. P.; PAVIOUR, S. D.; HOLLAND, D.; MORRIS, A. J. Antimicrobial susceptibility of anaerobic bacteria in New Zealand:1999-2003. **J. Antimicrob. Chemother**., v. 57, p. 992-998, 2006.

ROGGENTIN, P.; KLEINEIDAM, R. G.; SCHAUER, R. Diversity in the properties of two sialidase isoenzymes produced by *Clostridium perfringens* spp. **Biol. Chem. Hoppe Seyler**, v. 376, p. 569-575, 1995.

ROOD, J.; COLE, S. Molecular genetics and pathogenesis of *Clostridium perfringens*. **Microbiol. Rev.,** v. 55, p. 621-648, 1991.

ROOD, J. I.; McCLANE, B. A.; SONGER, J. G.; TITBALL, R. W. **The clostridia:** molecular biology and pathogenesis. San Diego, California: Academic Press, 1997.

ROOD, J. I. Virulence Genes of *Clostridium perfringens*. **Annu. Rev. Microbiol.,** v. 52, p. 333-360, 1998.

ROONEY, A. P.; SWEZEY, J. L.; FRIEDMAN, R.; HECHT, D. W.; MADDOX, C. W. Analysis of core housekeeping and virulence genes reveals cryptic lineages of *Clostridium perfringens* that are associated with distinct disease presentations. **Genetics**, v. 172, p. 2081-2092, 2006.

ROSADIO, R.; LONDOÑE, P.; PÉREZ, D.; CASTILLO, H.; VÉLIZ, A.; LLANCO, L.; YAYA, K.; MATURRANO, L. *Eimeria macusaniensis* associated lesions in neonate alpacas dying from enterotoxemia. **Vet. Parasitol.,** v. 168, p. 116-120, 2010.

SAKURAI, J.; NAGAHAMA, M.; ODA, M. *Clostridium perfringens* Alpha-Toxin: Characterization and Mode of Action. **J. Bio. Chem.,** v. 136, p. 569–574, 2004.

SALANITRO, J. P.; BLAKE, I. G.; MUIRHEAD, P. A.; MAGLIO, M.; GOODMAN, J. R. Bacteria Isolated from the Duodenum, Ileum, and Cecum of Young Chicks. **Appl. Environ. Microbiol.,** v. 35, p. 782-790, 1978.

SALYERS, A. A.; VERCELLOTTI, J. R.; WEST, S. E. H.; WILKINS, T. D. Fermentation of mucin and plant polysac-charides by strains of *Bacteroides* from human colon. **Appl. Environ. Microbiol.,** v. 33, p. 319-322, 1977a.

SALYERS, A. A.; WEST, S. E. H.; VERCEILOTTI, J. P.; WLKINS, T. D. Fermentation of mucins and plant polysac-charides by anaerobic bacteria from the human colon. **Appl. Environ. Microbiol.,** v. 34, p. 529-533, 1977b.

SALYERS, A. A. Energy sources of major intestinal fermentative anaerobes. **Am. J. Clin. Nutr.,** v. 32, p.158-163, 1979.

SAMBROOK, J.; FRITSCH, E.; MANIATIS, T. **Molecular Cloning:** a laboratory manual. **Book** 1-3. 2nd ed. USA: Cold Spring Harbor Laboratory Press, 1989.

SASAKI, J.; GORYO, M.; OKADA, K. Cholangiohepatitis in chickens induced by bile duct ligations and inoculation of *Clostridium perfringens*. **Avian Pathol**., v. 29, p. 405-410, 2000.

SAVAGE, D. C. Gastrointestinal microflora in mammalian nutrition. **Ann. Rev. Nutr.,** v. 6, p. 155-178, 1986.

SAWIRES, Y. S.; SONGER, J. G. Multiple-locus variable-number tandem repeat analysis for strain typing of *Clostridium perfringens*. **Anaerobe**, v. 11, p. 262-272, 2005.

SAWIRES, S. Y.; SONGER, J. G. *Clostridium perfringens*: Insight into virulence evolution and population structure. **Anaerobe,** v. 12, p. 23-43, 2006.

SAYEED, S.; FERNANDEZ-MIYAKAWA, M. E.; FISHER, D. J.; ADAMS, V.; POON, R.; ROOD, J. I.; UZAL, F. A.; McCLANE, B. A. Epsilon-toxin is required for most *Clostridium perfringens* type D vegetative culture supernatans to cause lethality in the mouse intravenous injection model. **Infect. Immun.,** v. 73, p. 7413-7421, 2005.

SCHAEDLER, R.; DUBOS, R.; COSTELLO, R. Association of germfree mice with bacteria isolated from normal mice. **J. Exp. Med.,** v. 122, p. 77-82, 1965.

SCHAUER, R. Sialic acids and their role as biological masks. **Trends Biochem. Sci.,** v. 10, p. 357-360, 1985.

SCHAUER, R. Achievements and challenges of sialic acid research. **Glycoconj. J.**, v. 17, p. 485-499, 2000.

SCHENTAG, J. J.; GILLILAND, K. K.; PALADINO, J. A. What have we learned from pharmacokinetic and pharmacodynamic theories? **Clin. Infect. Dis.**, v. 32, p. S39-46, 2001.

SCHOEPE, H.; NEUBAUER, A.; SCHLAPP, T.; WIELER, L. H.; BALJER, G. Immunization with an alpha toxin variant 121A/91-R212H protects mice against *Clostridium perfringens* alpha toxin. **Anaerobe,** v. 12, p. 44-48, 2006.

SCHWARTZMAN, J. D.; RELLER, L. B.; WANG, W. L. Susceptibility of *Clostridium perfringens* isolated from human infections to twenty antibiotics. **Antimicrob. Agents Chemother.**, v. 11, p. 695-697, 1977.

SENGUPTA, N.; ALAM, S. I.; KUMAR, R. B.; SINGH, L. Diversity and antibiotic susceptibility pattern of cultivable anaerobic bacteria from soil and sewage samples of India. **Infect. Genet. Evol.**, v. 11, p. 64-77, 2011.

SEVERI, E.; HOOD, D. W.; THOMAS, G. H. Sialic acid utilization by bacterial pathogens. **Microbiology,** v. 153, p. 2817-2822, 2007.

SHANE, S. M.; KOETTING, D. G.; HARRINGTON, K. S. The occurrence of *Clostridium perfringens* in the intestine of chicks. **Avian Dis.,** v. 28, p. 1120-1124, 1984.

SHEEDY, S. A.; INGHAM, A. B.; ROOD, J. I.; MOORE. R. J. Highly conserved alpha-toxin sequences of avian isolates of *Clostridium perfringens*. **J. Clin. Microbiol.,** v. 42, p. 1345-1347, 2004.

SHEU, S. Y.; TSENG, H. J.; HUANG, S. P.; CHIEN, C. H. Cloning, expression, and deletion analysis of large *nanH* of *Clostridium perfringens* ATCC 10543. **Enzyme Microb. Technol.,** v. 31, p. 794-803, 2002.

SHIMIZU, T.; OHSHIMA, S.; OHTANI, K.; HOSHINO, K.; HONJO, K.; HAYASHI, H.; SHIMIZU, T. Sequence heterogeneity of the ten rRNA operons in *Clostridium perfringens*. **Syst. Appl. Microbiol.,** v. 24, p. 149-156, 2001.

SHIMIZU, T.; OHTANI, K.; HIRAKAWA, H.; OHSHIMA, K.; YAMASHITA, A.; SHIBA, T.; OGASAWARA, N.; HATTORI, M.; KUHARA, S.; HAYASHI, H. Complete genome sequence of *Clostridium perfringens*, an anaerobic flesh-eater. **Proc. Natl. Acad. Sci. USA.,** v. 99, p. 996-1001, 2002a.

SHIMIZU, T.; SHIMA, K.; YOSHINO, K. I.; YONEZAWA, K.; SHIMIZU, T.; HAYASHI, H. Proteome and Transcriptome Analysis of the Virulence Genes Regulated by the VirR/VirS System in *Clostridium perfringens.* **J. Bacteriol.***,* v. 184, p. 2587-2594, 2002b.

SHOJADOOST, B.; VINCE, A. R.; PRESCOTT, J. F. The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: a critical review. **Vet. Res.,** v. 43, p. 74, 2012.

SI, W.; GONG, J.; HAN, Y.; YU, H.; BRENNAN, J.; ZHOU, H.; CHEN, S. Quantification of Cell Proliferation and Alpha-Toxin Gene Expression Of *Clostridium perfringens* in the Development of Necrotic Enteritis in Broiler Chickens. **Appl. Environ. Microbiol.,** v. 73, p. 7110-7113, 2007.

SILVA, R. O. S.; SALVARANI, F. M.; ASSIS, R. A.; MARTINS, N. R. S.; PIRES, P. S.; LOBATO, F. C. Antimicrobial susceptibility of *Clostridium perfringens* strains isolated from broiler chickens. **Braz. J. Microbiol.,** v. 40, p. 262-264, 2009.

SINGER, R.; HOFACRE, C. Potential Impacts of Antibiotic Use in Poultry Production. **Avian Dis.,** v. 50, p. 161-172, 2006.

SKLAN, D.; SHACHAF, B.; BARON, J.; HURWITZ, S. Retrograde Movement of Digesta in the Duodenum of the Chick: Extent, Frequency, and Nutritional Implications. **J. Nutr**., v. 108, p. 1485-1490, 1978.

SLAVIĆ, D.; BOERLIN, P.; FABRI, M.; KLOTINS, K.C.; ZOETHOUT, J. K.; WEIR, P. E.; BATEMAN, D. Antimicrobial susceptibility of Clostridium perfringens isolates of bovine, chicken, porcine, and turkey origin from Ontario. **Can. J. Vet. Res.,** v. 75, p. 89-97, 2011.

SMEDLEY, J. G.; FISHER, D. J.; SAYEED, S.; CHAKRABARTI, G.; McCLANE, B. A. The enteric toxins of *Clostridium perfringens*. **Rev. Physiol. Biochem. Pharmacol.,** v. 152, p. 183-204, 2004.

SONGER, J. G. Clostridial Enteric Diseases of Domestic Animals. **Clin. Microbiol. Rev.,** v. 9, p. 216-234, 1996.

STACKEBRANDT, E.; KRAMER, I.; SWIDERSKI, J.; HIPPE, H. Phylogenetic basis for a taxonomic dissection of the genus *Clostridium*. **FEMS Immunol. Med. Microbiol.**, v. 24, p. 253-258, 1999.

STANLEY, D.; KEYBURN, A. L.; DENMAN, S. E.; MOORE, R. J. Changes in the caecal Microflora of chickens following *Clostridium perfringens* challenge to induce necrotic enteritis. **Vet. Microbiol.,** v. 159, p. 155-162, 2012.

STARR, M.; REYNOLDS, D. Streptomycin Resistance of coliform Bacteria from Turkeys Fed Streptomycin. **Am. J. Public Health,** v. 41, p. 1375-1380, 1951.

STEVENS, D. L.; MITTEN, J. E; HENRY, C. Effects of α and θ toxins from *Clostridium perfringens* on human polymorphonuclear leukocytes. **J. Infect. Dis.,** v. 156, p. 324-333, 1987.

STEVENS, D. L.; TROYER, B. E.; MERRICK, D. T.; MITTEN, J. E.; OLSON, R. D. Lethal effects and cardiovascular effects of purified α and θ toxins from *Clostridium perfringens*. **J. Infect. Dis.,** v. 157, p. 272-279, 1988.

STEVENS, D. L.; BRYANT, A. E. Pathogenesis of *Clostridium perfringens* infection: mechanisms and mediators of shock. **Clin. Infect. Dis.**, v. 25, p. S160-164, 1997.

STRONG, D. H.; DUNCAN, C. L.; PERNA, G. *Clostridium perfringens* Type A Food Poisoning II. Response of the Rabbit Ileum as na Indication of Enteropathogenicity of Strains of *Clostridium perfringens* in Human Beings. **Infect. Immun.**, v. 3, p. 171-178, 1971.

STUTZ, M. W.; LAWTON, G. C. Effects of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens*, and ileal weight of broiler chicks. **Poult. Sci.**, v. 63, p. 2036-2042, 1984.

SUERBAUM, S.; JOSENHANS, C. Helicobacter pylori evolution and phenotypic diversification in a changing host. **Nat. Rev. Microbiol.**, v. 5, p. 441-452, 2007.

TANSUPHASIRI, U.; MATRA, W.; SANGSUK, L. Antimicrobial resistance among *Clostridium perfringens* isolated from various sources in Thailand. **Southeast Asian J. Trop. Med. Public Health**, v. 36, p. 954-961, 2005.

THACHIL, A. J.; MCCOMB, B.; ANDERSEN, M.; SHAW, D.; HALVORSON, D.; NAGARAJA, K. Role of *Clostridium perfringens* and *Clostridium septicum* in Causing Turkey Cellulitis. **Avian Dis.,** v. 54, p. 795-801, 2010.

TIMBERMONT, L.; LANCKRIET, A.; PASMANS, F.; HAESEBROUCK, F.; DUCATELLE, R.; VAN IMMERSEEL, F. Intra-species growth-inhibition by *Clostridium perfringens* is a possible virulence trait in necrotic enteritis in broilers. **Vet. Microbiol.**, v. 137, p. 388-391, 2009.

TIMBERMONT, L.; HAESEBROUCK, F.; DUCATELLE, R.; VAN IMMERSEEL, F. Necrotic enteritis in broilers: an updated review on the pathogenesis. **Avian Pathol**., v. 40, p. 341-347, 2011.

THOMPSON, D. R.; PARREIRA, V. R.; KULKARNI, R. R.; PRESCOTT, J. F. Live attenuated vaccine-based control of necrotic enteritis of broiler chickens. **Vet. Microbiol**., v. 113, p. 25-34, 2006.

TITBALL, R. W. Bacterial phospholipases C. **Microbiol. Rev.**, v. 57, p. 347-366, 1993.

TITBALL, R. W.; NAYLOR, C. E.; BASAK, A. K. The *Clostridium perfringens* α-toxin. **Anaerobe,** v. 5, p. 51-64, 1999.

TSAI, S. S.; TUNG, M. C. An outbreak of necrotic enteritis in broiler chickens. **J. Chin. Soc. Vet. Sci***.,* v. 7, p. 13-17, 1981.

TSURUMURA, T.; TSUMORI, Y.; QIU, H.; ODA, M.; SAKURAI, J.; NAGAHAMA, M.; TSUGE, H. Arginine ADP-ribosylation mechanism based on structural snapshots of iota-toxin and actin complex. **Proc. Natl. Acad. Sci. U S A**., v. 110, p. 4267-4272, 2013.

TYRRELL, K. L.; CITRON, D. M.; WARREN, Y. A.; FERNANDEZ, H. T.; MERRIAM, C. V.; GOLDSTEIN, E. J. In vitro activities of daptomycin, vancomycin, and penicillin against *Clostridium difficile*, *C. perfringens*, *Finegoldia magna*, and *Propionibacterium acnes*. **Antimicrob. Agents Chemother.**, v. 50, p. 2728-2731, 2006.

VAN IMMERSEEL, F.; DE BUCK, J.; PASMANS, F.; HUYGHEBAERT, G.; HAESEBROUCK, F.; DUCATELLE, R. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. **Avian Pathol.,** v. 33, p. 537-549, 2004.

VAN IMMERSEEL, F.; ROOD, J. I.; MOORE, R. J.; TITBALL, R. W. Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. **Trends Microbiol.,** v. 17, p. 32-36, 2009.

VARGA, J. J.; THERIT, B.; MELVILLE, S. B. Type IV pili and the CcpA protein are needed for maximal biofilm formation by the Gram-positive anaerobic pathogen *Clostridium perfringens*. **Infect. Immun.**, v. 76, p. 4944-4951, 2008.

VIDAL, J.; CHEN, J.; LI, J.; McCLANE, B. Use of an EZ-Tn 5 -Based Random Mutagenesis System to Identify a Novel Toxin Regulatory Locus in *Clostridium perfringens* Strain 13. **PLoS ONE,** v. 4, p. e 6232, 2009a.

VIDAL, J. E.; OHTANI, K.; SHIMIZU, T.; McCLANE, B. A. Contact with enterocyte-like Caco-2 cells induces rapid upregulation of toxin production by *Clostridium perfringens* type C isolates. **Cell Microbiol.,** v. 11, p. 1306-1328, 2009b.

VIDAL, J. E.; MA, M.; SAPUTO, J.; GARCIA, J.; UZAL, F. A.; McCLANE, B. A. Evidence that the Agr-like quorum sensing system regulates the toxin production, cytotoxicity and pathogenicity of *Clostridium perfringens* type C isolate CN3685. **Mol. Microbiol.**, v. 83, p. 179-194, 2012.

VIMR, E. R.; KALIVODA, K. A.; DESZO, E. L.; STEENBERGEN, S. M. Diversity of Microbial Sialic Acid Metabolism. **Microbiol. Mol. Biol. Rev.,** v. 68, p. 132-153, 2004.

WALTERS, D. M.; STIREWALT, V. L.; MELVILLE, S. B. Cloning, Sequence, and Transcriptional Regulation of the Operon Encoding a Putative N-Acetylmannosamine-6-Phosphate Epimerase (*nanE*) and Sialic Acid Lyase (*nanA*) in *Clostridium perfringens*. **J. Bacteriol.,** v. 181, p. 4526-4532, 1999.

WATKINS, K. L.; SHRYOCK, T.; DEARTH, R.; SAIF, Y. In vitro antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. **Vet. Microbiol.,** v. 54, p. 195-200, 1997.

WEN, Q.; McCLANE, B. A. Detection of enterotoxigenic *Clostridium perfringens* type A isolates in American retail foods. **Appl. Environ. Microbiol**., v. 70, p. 2685-2691, 2004.

WICKHAM, N. The production and inhibition of the haemagglutinin of *Clostridium welchii*. **J. Comp. Path.,** v. 66, p. 62-70, 1956.

WILLIAMS, J. G. K.; KUBELIK, A. R.; LIVAK, K. J.; RAFALSKI, J. A.; TINGEY, V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Res**., v. 18, p. 6531-6535, 1990.

WILLIAMS, R. B. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. **Avian Pathol.,** v. 34, p. 159-180, 2005.

WILLIAMSON, R. Resistance of *Clostridium perfringens* to β-Lactam Antibiotics Mediated by a Decreased Aflinity of a Single Essential Penicillin-binding Protein. **J. Gen. Microbiol.,** v. 129, p. 2339-2342, 1983.

WILSON, J.; TICE, G.; BRASH, M.; HILAIRE, S. Manifestations of *Clostridium perfringens* and related bacterial enteritides in broiler chickens. **World's Poult. Sci. J.**, v. 61, p. 435-448, 2005.

WITTE, W. Selective pressure by antibiotic use in livestock. **Int. J. Antimicrob. Agents.,** v. 16, p. S19-24, 2000.

WRIGLEY, D. M. Inhibition of *Clostridium perfringens* sporulation by *Bacteroides fragilis* and short-chain fatty acids. **Anaerobe,** v. 10, p. 295-300, 2004.

YOO, H. S.; LEE, S. U.; PARK, K. Y.; PARK, Y. H. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR. **J. Clin. Microbiol.**, v. 35, p. 228-232, 1997.