## Enterotoxaemia In Calves Due To Clostridium Perfringens Types A And C With Synergism Between Propolis And Antibiotics For Its Treatment

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#### ABSTRACT

Clostridium perfringens is an important agent of enteritis and fatal enterotoxaemia in domestic animals and humans. The goal of the present work was to throw a spot light on the clinical and laboratory diagnosis, with trail of field treatment and control strategies of enterotoxaemia in calves. This study was carried out on 507 calves aged from 7 up to 69 days old distributed in 3 private farms of Sharkia, Ismailia and Al-Bhera Governorates in which the problem of sudden death was recorded in 85 (16.7%) calves of 9 to 28 days old. The clinical findings were observed on 120 (23.7%) calves aged from 7 up to 69 days. The majority (81) of these calves developed pasty yellow and bloody diarrhoea, and the abdomen became distended and painful. Some (25) of them showed nervous signs including, animals running, tetany and opisthotonus. Few (14) of them suffered from fever (39.9 - 41.3°C) and dyspnea. Post-mortem findings of the 27 recently dead calves showed haemorrhagic necrotic enteritis with ulceration of the mucosa. Blood stained contents were present in the intestine. Excessive serous fluids in pericardial sac with subendocardial and subepicardial haemorrhages were observed. Bacteriological examination revealed that the typical colonies of Cl. Perfringens were observed in anaerobic culture. The toxins of Cl. Perfringens type A and C were determined in 12/27 and 15/27 of intestinal filtrates respectively by toxin-anti-toxin neutralization test. Virus isolation results were negative. Parasitological examination indicated the presence of toxocara vitolrurm eggs in 3 faecal samples. The results of treatment revealed that the calves treated with intramuscular administration of Penicillin associated with Propolis reduced significantly the overall severity of enterotoxaemia better than those treated with Penicillin alone or Cefotaxime associated with Propolis or alone. In conclusion, our findings showed the severe and highly fatal effect of Cl. Perfringens type A and C infection in calves. Moreover, the synergism between Propolis and Penicillin gave high curative rate in clinical enterotoxaemia in calves.

#### INTRODUCTION

Bovine enterotoxaemia is a highly fatal disease of newly born calves and characterised by sudden death and haemorrhagic enteritis of small intestine (1). It is caused by Clostridium (Cl.) perfringens, Gram-positive, forming, anaerobe, that considered important human and veterinary pathogen. This bacterium can produce at least 15 different toxins, five of which (the alpha, beta, epsilon, iota and enterotoxin) are responsible for the enteric histotoxic infections and the death of the host (2-5). The beta2 toxin (CPB2) is recently postulated to play an important role in Cl. perfringens neonatal enteritis in cattle enterotoxaemia (6) and gastrointestinal disease in man (7). Cl.

perfringens isolates are classified into one of five types (A–E) on the basis of differential production of their toxins and according to specific animal hosts. Infections by Cl. perfringens type A and C are perhaps the most common causes of clostridial hemorrhagic enteritis in neonatal ruminants (8).

Propolis (Bee-glue) is a resinous substance collected by honey bees from tree buds and active against most anaerobic strains of different genera with property of immunity improvement (9).

In Egypt, the Egyptian General Organization for Vet. Service recorded that 75% of total mortalities among calves were due to enteric affections that lead to highly economic losses. So, the purpose of this study

was to describe the problem of enterotoxaemia in calves from clinical aspect of view and throw a spot light on its confirmatory diagnosis as well as recent trials for treatment and control strategies in 3 private farms of Sharkia, Ismailia and Al-Bhera Governorates.

#### MATERIAL AND METHODS

#### I. Animals and clinical examination

The present study was carried out on 3 private farms of Sharkia (Belbes city), Ismailia (Eltal-Elkeber city) and Al-Bhera (El-Nobaria) governorates between February 2007 and November 2007 in a total number of 507 calves aged from 7 up to 69 days old (Table 1). Sudden death was recorded in 85 calves of 9 to 28 days old while the clinical findings were observed on 120 calves which were isolated and subjected to further investigations. All calves were closely monitored for one month to assess day to day changes. Detailed past and immediate history and management practices were recorded. The calves were subjected to thorough clinical examination in the morning and evening. Daily rectal temperature was recorded.

## II. Samples

### 1. Samples for bacterial isolation

Faecal samples, rectal swabs and blood on EDTA were collected aseptically for bacterial isolation from the 120 clinically diseased animals. In addition; the samples were taken from lungs, heart, liver, lymph nodes and small intestine loop with its contents from the 27 recently dead calves. Handling, transporting and storing of collected samples were carried out at refrigeration temperature (4°C).

#### 2. Samples for virus isolation

Blood samples on Alsever solution from the 14 feverish animals and small intestine loop with its contents from the 27 recently dead calves were taken for virus isolation.

#### 3. Samples for parasitological examination

Faecal samples were also collected from the 120 clinically diseased animals for parasitological examination.

#### III. Post-mortem examination

Post-mortem examination (PM) was conducted on the 27 recently dead calves.

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#### IV. Bacteriological investigation

The Bacteriological examination was made for aerobic and anaerobic bacteria to eliminate the Enterobacteriaceae infections. The detection of Enterobacteriaceae and the isolation of Cl. Perfringens were performed (10). Briefly, the Cl. Perfringens were isolated on cooked meat media using anaerobic jar and gas generating kits at 37°C for 24-48 hours. then a loopful was streaked onto the surface of 10% sheep blood agar with neomycin sulphate (200µg/ml) at 37°C for 24-48 hours. In addition to direct smears made from intestinal mucosa of dead calves and stained with Gram's stain. The isolated Cl. Perfringens identified morphologically, biochemically and by determination of their toxins in the intestinal contents (11) using diagnostic antisera types A, B, C, D and E which obtained from Burrnghs, Wellcome Research Laboratories, Backanham, London, England.

#### V. Enteric virus isolation

Trails for enteric virus isolation as Rota and Corona viruses were carried out (12) for elimination of the viral causes of enteritis.

#### VI. Parasitic investigation

The faecal samples were examined for elimination of the parasitic causes of enteritis at this age.

# VII. Antimicrobial susceptibility test: It was carried out (13).

## VIII. Field treatment trials and control strategies of the problem

For field treatment, the affected calves were divided into 4 groups (Table 3), each group contained 30 animals. Group I was intramuscularly injected with Penicillin alone (CID, Egypt); 22.0000 I.U/ kg B.W twice daily for 5 successive days (14). Group II was intramuscularly administrated with Penicillin; 22.0000 I.U/ kg B.W twice daily for 5 successive days associated with BioPropolis (Sigma); 400mg/day, per Os for 5 successive days. Group III was intramuscularly injected

with Cefotaxime alone (PRIMAVET, Egypt); 13 mg/kg B.W twice daily (15) for 5 successive days. Group IV was intramuscularly administrated with Cefotaxime; 13 mg/kg B.W twice daily for 5 successive days associated with BioPropolis; 400mg/day, per Os for 5 successive days. All treated calves were observed daily for monitoring any clinical improvement and rectal swabs were collected for bacteriological examination for one month at weekly interval to ensure the complete recovery. Oral and intravenous fluid therapy was given for all treated calves in addition to the removing of all predisposing factors concerning calves, their dams and environment particularly the high concentrated ration.

#### IX. Statistical analysis

Analysis by using computer program SPSS/PCT. Levels of significance of the differences between groups of treated animals were determined by Chi-square ( $\chi$ 2) test.

#### RESULTS

Clinical findings: The clinical findings were observed on 120 calves aged from 7 up to 69 days (Table 1). The majority of these calves (81) developed pasty yellow and bloody diarrhoea, and the abdomen became distended and painful. Some (25) of them showed nervous signs including, animals running, tetany and opisthotonus. Few (14) of them suffered from fever (39.9 – 41.3) and dyspnea. Sudden death was recorded in 85 calves of 9 to 28 days old. Death may be peracute, or following a clinical course of several days.

Post-mortem findings: Post-mortem findings of the recently dead calves showed haemorrhagic (Fig. 1) and necrotic enteritis with ulceration of the mucosa (Fig. 2). Blood stained contents were present in the intestine (Fig. 3). Excessive serous fluid was observed

in pericardial sac with subendocardial and subepicardial haemorrhages (Fig. 4).

Bacteriological examination: The preliminary step in bacteriological examination was the examination of stained smears from intestinal mucosa that revealed single or several Gram positive bacilli. The results of aerobic culture on sheep blood agar and MacConky agar plates together with Selenite F, both were negative for Salmonella organisms. While the results of anaerobic culture on cooked meat media proved the typical colonies of Cl. Perfringens. The isolated Cl. Perfringens were identified by determination of their toxins in the intestinal contents using diagnostic antisera types A, B, C, D and E and the results showed that the toxins of Cl. Perfringens type A and C were determined in 12/27 and 15/27 filtrates respectively (Table 2).

Enteric virus isolation: Enteric virus isolation results were negative.

Parasitic investigation: The results of faecal examination indicated the presence of toxocara vitolrurm eggs in 3 faecal samples.

In vitro, antimicrobial susceptibility test: The field isolates exhibited, in vitro, high susceptibility to penicillin- G, amoxicillin, cephalosporins, Clindamycin, chloramphenicol, trimethoprim-sulfadoxine; intermediary susceptibility to erythromycin and tetracycline, and low susceptibility to enrofloxacin.

Field treatment and control strategies of the problem: The field treatment revealed that the calves injected intramuscularly with Penicillin associated with BioPropolis reduced significantly the overall severity of enterotoxaemia better than those treated with Penicillin alone or Cefotaxime alone or associated with BioPropolis. (Table 3).

Table 1. Epidemiological data and clinical presentation of calf enterotoxaemia.

| Locality                | No. of diseased/<br>No. of animals<br>(7-69 days old) | Clinical suspect   | No. of sudden<br>deaths<br>(9-28 days old) |
|-------------------------|---|--|--|
| Sharkia<br>governorate  | 39/172  | The majority (81) of calves<br>developed pasty yellow and                                  | 16   |
| Ismailia<br>governorate | 27/118  | and painful abdomen. Some (25)<br>of them showed nervous signs as                          | 19   |
| Al-Bhera<br>governorate | 54/217  | tetany and opisthotonus. Few (14) of them suffered from fever (39.9  – 41.3°C) and dyspnea | 50   |
| Total                   | 120/507   | - 1  | 85   |

Table 2. Results of neutralization test for Cl. Perfringens toxins.

| Toxin anti-toxin neutralization test in intestinal contents filtrates |       |  |  |  |  |
|---|-------|--|--|--|--|
| Cl. Perfringens type A  | 12/27 |  |  |  |  |
| Cl. Perfringens type B  | 0/27  |  |  |  |  |
| Cl. Perfringens type C  | 15/27 |  |  |  |  |
| Cl. Perfringens type D  | 0/27  |  |  |  |  |
| Cl. Perfringens type E  | 0/27  |  |  |  |  |

Table 3. Results of field treatment.

| Treatment                   | Group I                        | Group II                            | Group III                      | Group IV                               |
|-----------------------------|--------------------------------|-------------------------------------|--------------------------------|--|
| No. of animals              | 30                             | 30                                  | 30                             | 30                                     |
| Therapeutic regime          | Penicillin alone<br>for 5 days | Penicillin with Propolis for 5 days | Cefotaxime alone<br>for 5 days | Cefotaxime with<br>Propolis for 5 days |
| Clinical<br>improvement     | 18/30                          | 30/30**                             | 16/30                          | 21/30                                  |
| Bacteriological examination | 12/30 positive                 | 3/30 positive**                     | 16/30 positive                 | 9/30 positive                          |

<sup>\*\*</sup> P<0.01; highly significant differences between groups for clinical improvement and bacteriological examination.



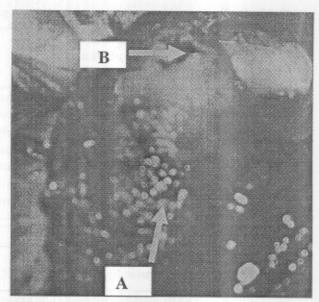


Fig. 1. Haemorrhagic enteritis in the intestine of a calf died from enterotoxaemia.

Fig. 2. Necrotic enteritis (A) with ulceration (B) of the intestinal mucosa of a calf died from enterotoxaemia.

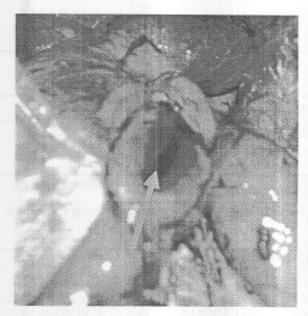


Fig. 3. Blood stained contents in the intestine of a calf died from enterotoxaemia.

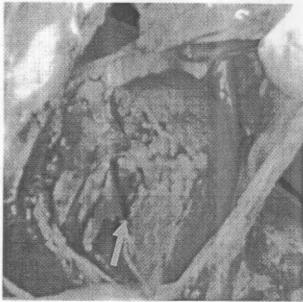


Fig. 4. Excessive serous fluid in pericardial sac with subendocardial and subepicardial haemorrhages of a calf died from enterotoxaemia.

#### DISCUSSION

Clostridial infections of the gastrointestinal tract of calves remain a common problem, in spite of the widespread availability of effective immunoprophylactic products (1, 16). So, our study was firstly planned to describe the problem of enterotoxaemia in calves and throw a spot light on its confirmatory diagnosis as well as recent trials for treatment and control strategies.

Concerning the clinical findings, the majority (67.5%) of affected calves developed pasty yellow and bloody diarrhoea, tympany and the abdomen became distended and painful while some (20.8%) of them showed nervous signs including, animals running, tetany and opisthotonus These findings are recorded in several reports (17, 8). Few (11.7%) of affected calves suffered from fever (39.9 - 41.3°C) and dyspnea which nearly similar to that previously reported (14). Sudden death was recorded in 85 calves of 9 to 28 days old. Death may be peracute, or following a clinical course of several days. This observation is the characteristic finding of calf enterotoxaemia (1).

Post-mortem findings of the recently dead calves showed haemorrhagic and necrotic enteritis with ulceration of the mucosa. This observation was previously reported (18). Other study (8) added that the abomasal and omasal walls were thickened and hemorrhagic. Blood stained contents were present in the intestine. Excessive serous fluid in pericardial sac with subendocardial and subepicardial haemorrhages were observed. Such observation has been recorded (14). The beta2 toxin (CPB2), encoded by cpb2, is suspected to be the prime player in the pathogenesis of Cl. perfringens infections (6). Death is due, ultimately, to beta toxaemia. The disease cannot be reproduced with toxin alone (19), but protection is primarily antitoxic.

The results of anaerobic culture and the determination of toxins in the filtrate of intestinal contents using diagnostic antisera types A, B, C, D and E confirmed the clinical

diagnosis of this problem and proved that Cl. Perfringens type A and C and their toxins were responsible for calf enterotoxaemia in the farms of this study. Infections by Cl. perfringens type A and C are perhaps the most common causes of clostridial hemorrhagic enteritis in neonatal ruminants (8). Other studies (20-22) revealed that that Cl. perfringens type A is the most common bacterial finding in cases of clostridial enteritis in neonatal calves, while the occurrence of type A organisms as normal flora in virtually all warm-blooded animals has complicated its assignment as an etiologic agent of enteric disease (23, 24). On the other hand neonatal infection by Cl. perfringens type C is widely recognized, and, like other infections by toxin types of Cl. perfringens type C may colonize rapidly in the absence of established normal flora (25). Another study (14) declared that Cl. Perfringens type A and D and their toxins were responsible for calf enterotoxaemia while the enterotoxaemia in newborn calves due to Cl. perfringens types A, C and D was reported (26). Aspects of aetiology beyond the infecting organism are little understood, but probably included dietary issues, perhaps relating to overfeeding, feeding of barely thawed or contaminated colostrum, or conditions which effect decreased gut motility.

Concerning the results of field treatment and control strategies, the calves treated with intramuscular administration of Penicillin associated with Propolis reduced significantly the overall severity of enterotoxaemia better than those treated with Penicillin alone or Cefotaxime with Propolis or alone. Propolis was active against most anaerobic strains of different genera (9). It also exhibits antimicrobial. anti-inflammatory, healing and other properties (27, 28). Propolis is bacteriostatic and can be bactericidal in high concentrations (29, 30). Synergism between the propolis and some antibacterial agents has been observed (31, 32). In conclusion, our findings showed the severe and highly fatal effect of Cl. Perfringens type A and C infection in calves. Moreover, the synergism between Propolis and Penicillin gave high curative rate in clinical enterotoxaemia in

calves. The most prominent needs for further understanding of this problem are the molecular epidemiology of the disease with the development of molecular vaccine that gives longer reasonable period of protection.

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### الملخص العربي

مرض التسمم المعوي في العجول نتيجة الإصابة بالكولستريديم بيرفرنجز نوع (A) و(C) مرض التسمم المعوية في العجول نتيجة الإصابة بالكولستريديم بيرفرنجز نوع (A)

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تعتبر الكولستريديم بير فرنجز من أهم مسببات الإلتهاب والتسمم المعوي القاتل في الحيوان والإنسان. لذا كان الهدف من هذه الدراسة هو إلقاء الضوء على التشخيص الإكلينيكي والمعملي وخاصة البكتريولوجي لهذا المرض بالإضافة إلى محاولات حديثة لعلاجه ووضع استيراتيجية عامة للسيطرة علية.

وقد أجريت هذه الدراسة على عدد ٥٠٧ عجل بقري من عمر ٧ إلى ٦٩ يوم بثلاث مزارع بمحافظات الشرقية والإسماعيلية والبحيرة والتي تم الإبلاغ فيها عن حالات موت فجائي وصل عددها إلى ٨٥ حالة من إجمالي العدد الكلي لهذه الدراسة والتي تراوحت أعمارهم ما بين ٩ إلى ٢٨ يوم.

وبإجراء الفحوصات الإكلينيكية تبين ظهور أعراض مختلفة لمرض التسمم المعوي على عدد 120 حالة تراوحت أعمارهم ما بين ٧ إلى ٦٩ يوم ، الغالبية منهم ظهر عليهم أعراض إسهال أصفر ومدمم مع ألم وزيادة في حجم البطن والبعض منهم ظهرت عليه أعراض عصبية وقلة عانوا من ارتفاع في درجة الحرارة وصعوبة في التنفس.

وباجراء الصفة التشريحية على ٢٧ حالة حديثة النفوق لوحظ وجود التهاب معوي مدمم مع تنكرز وتقرح في جدار الأمعاء بالإضافة إلى تدمم لمحتويات الأمعاء. كما لوحظ تواجد أنزفه دموية بأغشية القلب مع كمية كبيرة من السوائل.

أسفرت نتائج العزل البكتيري عن عزل الكولستريديم بيرفرنجز من جميع الحالات (27) التي أجريت عليها الصفة التشريحية والتي تم تصنيفها باستخدام اختبار التعادل السمي في الفنران والذي أسفرت نتائجه عن تواجد الكولستريديم بيرفرنجز نوع (A) في ٢٧/١2 والنوع (C) في ٢٧/١5 من عينات محتويات الأمعاء، في حين كانت نتائج العزل للبكتريا الهوائية والفيروسات سلبية. وقد تبين إصابة ٣ حالات بالأسكارس.

أظهرت نتائج العلاج الحقلي أفضلية العلاج بالبنسلين والبروبولس لمدة ٥ أيام متتالية وذلك اعتمادا على التحسن الإكلينيكي والفحص البكتريولوجي بعد العلاج والذي أدى إلى شفاء جميع الحالات . هذا بالإضافة إلى تحصين جميع الأمهات قبل الولادة باللقاح المتعدد العترات وعدم تقديم علائق عالية التركيز للعجول عند تحويلها للفطام.

الخلاصة: يبرز هذا البحث مدي خطورة التسمم المعوي بالكولستريديم بيرفرنجز نوع (A) والنوع (C) وتأثير ها على الإقتصاد القومي ويوصي باستخدام البروبولس في نفس الوقت مع البنسلين لرفع الكفاءه العلاجية وللحفاظ على الثروة الإقتصادية والصحة العامة.