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# FIRST REPORT ON INFECTIOUS NECROTIC ENTERITIS IN PIGLETS IN IRAN

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**Introduction:**

Infectious Necrotic Enteritis in piglets caused by Clostridium perfringens, Type C, was first described in England by Field and Gibson, 1955 (1), and then in Hungary, USA, and Denmark respectively by Szent-Ivanyi, 1955,(2), Szabo, 1957, (3) Barnes and Moon, 1964, (4), and Hogh, 1967, (5). A similar disease has been reported from USSR by Bakhtin, 1956, (6), in which Cl. perfringens, Type B, was isolated.

The object of this communication is to report an outbreak of necrotic enteritis caused by Cl. perfringens, Type C, in piglets in Iran.

**HISTORY:**

A severe outbreak of necrotic enteritis occurred in neonatal piglets of a herd in September 1971.

The herd was located in Gazvin area 140 Km. far-west of Tehran. The herd contained more than 200 sows and 1300 piglets, that maintained under good nutritional, but, kept in a poor sanitary conditions.

The infected animals were very young and they were approximately 2-6 days old. The older animals were looking healthy and they did not show any sign of the disease. Most of the sick piglets showed hemorrhagic diarrhea at the time of visiting the farm. The stool of some of them was soft, bloody and contained shreds of necrotic debris. The rear quarters of the animals were soiled with bloody fluid. Piglets were anemic and showing severe weakness with sunken eyes. The visible mucous membranes were pale. Most of them lying down and some were moving with recumbency. The clinical course was very short, the sick animals died within 12-24 hours after the onset of the disease. In some cases the animals died very rapidly without showing any clinical sign.

Morbidity was very high approximately 100 % in young animals and mortality rate ranged about 70% 900 of 1300 piglets died during the outbreak.

## MATERIALS AND METHODS:

Sick and recent died piglets were submitted to the Pathology Department of Razi State Institute for diagnostic procedure. The sick piglets were sacrificed and necropsy performed immediately. Tissues from lesioned areas of different parts of small intestine and mesenteric lymphnodes were collected in 10 % formaline saline solution. Moreover the tissues from other organs such as lungs, spleen, liver, and bone-marrow also were preserved in formaline saline solution for histopathological slides preparation. The tissues processed by paraffin embeding methods. Sections were cut in 5 microns in thickness and stained by routine Hematoxyline-Eosine (HE). A bunch of prepared slides were stained by Gram's method.

Culture was made aero-anaerobically from various organs for bacteriological studies.

## NECROPSY FINDINGS :

The pathological aspects were similar in all piglets which were posted, but, individual variation was seen in the extent and severity of the lesions which

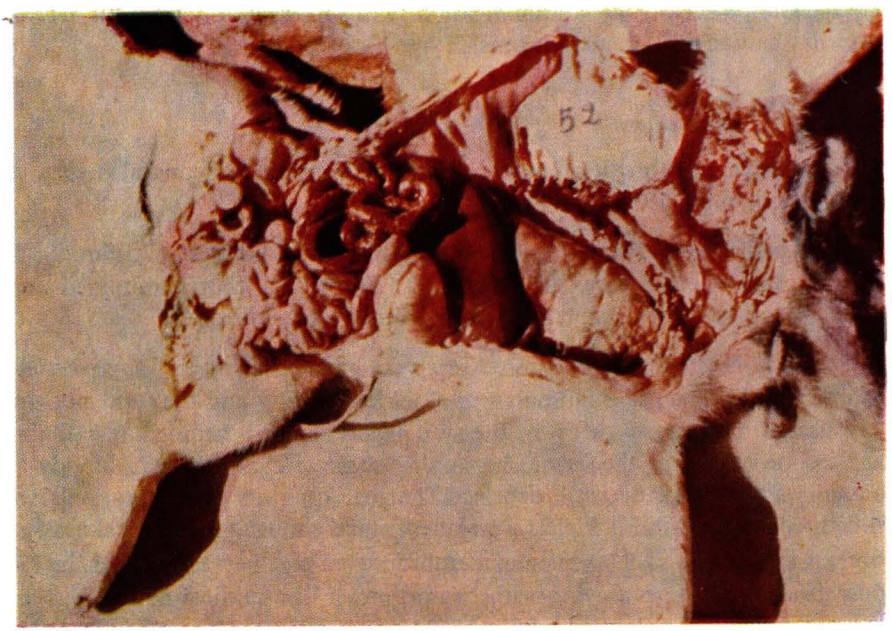


Fig. 1 – Note the lesioned part of the small intestine.

might have been dependent on the clinical course. The main lesions were confined to the small intestine excitingly in the upper portion of jujenum, but, in some cases the lesion extended up to the ileum (Fig. 1). While in some animals the lesions were located only in the ileum. No sign of the lesion was noted in the duodenum (Fig. 2). Lesioned areas were dark-red in color on serosal surface. The infected part of the intestine was swollen, hemorrhagic, thickened and friable. Buble formation in the intestinal wall of the infected part, specially at the upper portion of the jujenum was noted. The emphysematous segments were approximately 40 cm in lenght, and were loosely adhered to the adjecent intestinal segments and peritoneum. The intestinal lumen was filled with bloody materials which extended backwards throughout the digestive tract. The mucosal surface was rough and yellow in color which covered by blood tinged necrotic materials and could be detached very easily from mucosal surface.

Mesenteric lymphnodes were enlarged and moderately congested with some petecchial or ecchymotic hemorrhages.

Small amount of blood tinged clear watery fluid was collected from abdominal cavity. Acute fibrinous peritonitis was noted in the site of the lesioned part of the intestine.

In some cases petecchial or ecchymotic hemorrhages were noted on the

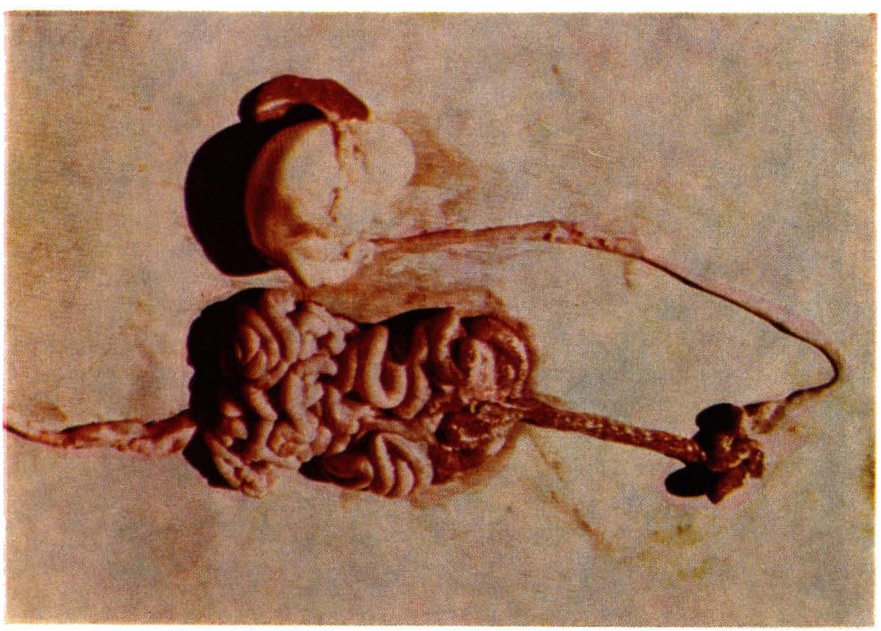

Fig. 2 – Note the duodenum is looking normal while the most exciting lesioned part of the small intestine is confirmed to the upper part of jujenum.

pericardial sac and myocardium.

## HISTOPATHOLOGICAL FINDINGS :

The most prominent changes were presented in small intestine. The histopathological changes varied from catarrhal enteritis, hyperemia and hemorrhages in the mucosa and lamina propria up to necrotic enteritis. The intestine was severely congested with dilated vesseles that were filled with red blood cells, severe hemorrhages were presented between the vili and glandular tissues, which extended deeply into the submucosa, tunica muscularis and serosa. Most of the vili underwent necrosis and partially into the intestinal lumen (Fig. 3).

In some cases severe edema in the submucosa in wide area accompanied with inflammatory cell infiltrations predominantly neutrophiles with fibrinous deposits were developed. Numerous round vacuoles were presented in the submucosa region. They were in various size and surrounded partially with a single tissue fiber.

In some cases there were ruptures in septal walls of neighbouring vacuoles which resulted a large cavity. The vacuoles were empty and formed by distension of connective tissue and intestinal muscle fibers due to the gas formation.

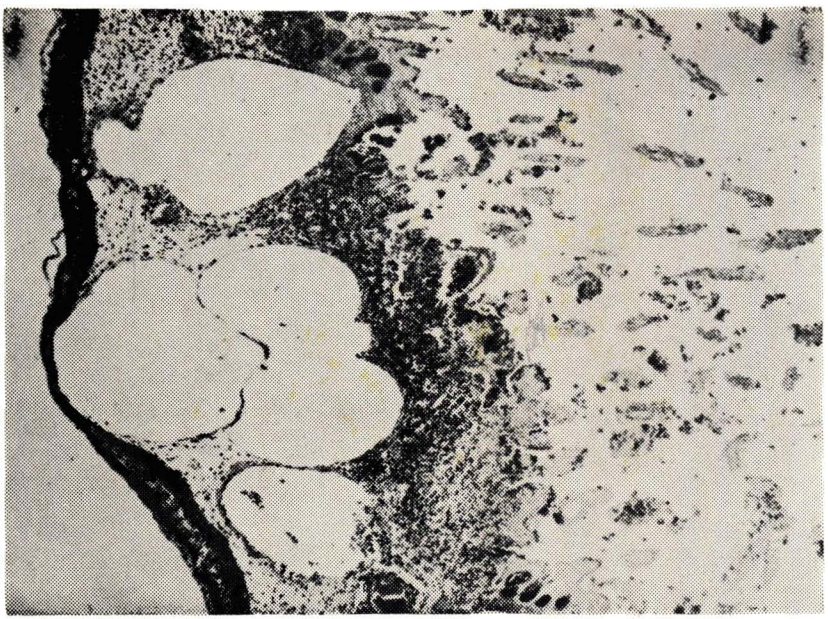

Fig. 3 – Section from the mid jujenum. Note already all of the necrotized vili slaughed into the lumen X 25

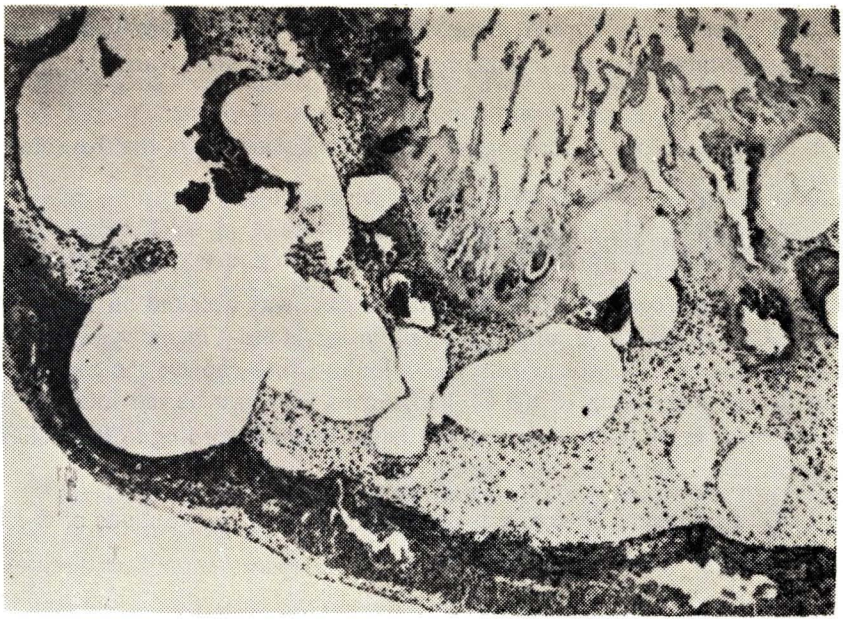

Fig. 4- Section from the upper jujenum. Note the vacuoles in the differnt part of the intestinal wall. X 25

In some sections prepared from the jujenum these emphysematous features extended up to the tunica muscularis and resulted the projection of intestinal serosa (Fig. 4).

Some of the vesseles showed thrombosis and filled with embolic materials.

The lumen content composed of necrotic epithelial cells, degenerated inflammatory and red blood cells with clump of bacilli. The Gram's staining of intestinal section revealed that these bacilli were Gram positive.

The mesenteric lymphnodes were severely hyperemia and hemorrhagic. Large number of erythrocytes were seen in the lymph sinuses. Edema spread the sinuses and the surrounding tissue apart. Severe inflammatory cells infiltration predominantly neutrophiles were scattered through.

## BACTERIOLOGICAL STUDIES :

Smears were taken from the necrotic enteritis in small intestine of piglets immediately after death. The organisms were Gram positive and bacilli morphologically resembled Cl. perfringens. Intestinal contents were diluted with saline and centrifuged at 3000 r.p.m. for 30 minutes and Sietz filtered of the supernatant was used for necrotising skin test into depilated albino guinea-pigs and intravenously inoculation in mice for lethal toxin.

Cl. perfringens Type A, C and D diagnostic sera (*) were used for typing of the intestinal contents, according to the Oakley and Warrack, 1953 (7), methods.

Beta toxin of Cl. perfringens Type C was detected in the five intestinal contents.

## ISOLATION OF ORGANISMS :

Intestinal contents from each died piglet was streaked on Bacto Difco Blood Agar plates containing 5 per cent sheep blood. Plates were incubated anaerobically at 37°C overnight. The colonies which showed the zones of hemolysis and resembling to the Cl. perfringens were transferred into the fresh cooked meat broth for testing of major lethal toxins according to the technique described by Oakley and Warrack (7).

Cl. perfringens Type C was isolated from intestinal contents of the submitted piglets.

## EXAMINATION OF FAECES OF SOWS AND LITTER :

Samples of the rectal swabs of the healthy sows were taken from the affected litters for isolation of micro-organisms.

Samples were also taken from the litter of affected piglets. Each swab or sample was suspended into the tubes of cooked meat broth and incubated anaerobically overnight at 37°C. The smears prepared from these showed Gram positive rods bacilli. The cultures were streaked on the fresh blood agar plates. The isolated colonies were typed as the mentioned technique. Four strains of Cl. perfringens Type C were also isolated from these healthy sows, and affected litters.

The strain isolated from the cases of necrotizing enteritis of piglets in Iran produced the major lethal Alpha and Beta toxins and must be classified as Type C (Glennys et al., 1933, (8), Dalling and Rosse, 1938, (9), Bosworth, 1940–43, (10), but, these strains did not produce Delta toxin which is produced by classical Type C. Brooks et al., 1957, (11), Sterne and Warrack, 1964, (12), in the survey of type differentiation of the large numbers of Cl. perfringens suggested those strains of Cl. perfringens Type C which failed to produce Delta antigen must be classified as subtypes of Type C.

## PREVENTION AND CONTROL OF THE DISEASE :

For prevention of the disease, injection of antiserum to the piglets and

(*) = Wellcome Research Laboratories, Kent, England.

vaccination of the pregnant sows was done.

The piglets were injected shortly after birth with two ml. of the Cl. perfringens Type C Beta antitoxin, and this treatment was effective in reducing the death and mortality in piglets.

The alum precipitated toxoid was prepared with the toxigenic strain of Cl. perfringens Type C isolated from affected piglets. This prepared vaccine was injected 3 ml. per pregnant sows first, then a second injection of the same dose was given 15 days after to the same group of sows.

The procedure was followed for two years in this farm and no death was observed during this time.

## DISCUSSION :

Mortality in piglets has been a big problem in pig industries. The general diseases of newly born piglets have been mostly, chillling, crushing, acute hypoglycemia, bad management, etc....

In the present study no such cases could be detected. The piglets were born quite strong and healthy, but, within 12–24 hours some of them showed disinclination to move and became rapidly weaker. On post-mortem examination characteristic lesions of infectious necrotizing enteritis was noted. Subsequently histopathological and microbiological findings proved that the cause of the disease was Cl. perfringens Type C. Fortunately the death in piglets was prevented by antiserum into the newly born piglets and vaccination of the pregnant sows. Isolation of Cl. perfringens from the faeces of the sows indicated that the possible source of infection for the piglets was their mothers. These sows were the healthy carrier of the causal organisms. The piglets get the infection at the time of forrowing or may get infection from the contaminated teats, skin of their mothers, and or litter.

## SUMMARY :

The infectious necrotic enteritis in piglets caused by Cl. perfringens Type C was diagnosed for the first time in Iran.

Authors confirmed their diagnosis with: clinical, histopathological, bacteriological, treatment with specific antiserum and vaccination.

## ACKNOWLEDGEMENT :

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## References


1- FIELD, H.I., & GIBSON, E.A. (1955)
Vet. Rec., **67**, 31

2- SZENT-IVANYI, T.H., & SZABO, S.T. (1955)
Magy. Allatorv. Lapj., **10**, 403

3- SZABO, S.T. SZENT-IVANYI, T.H. (1957)
Acta Vet. Hung., 7, 413

4- BARNES, D.M., & MOON, H.W. (1964)
J. Amer. Vet. Med. Assn., Vol. **144**, 1391

5- HOGH, P. (1967)
Acta Vet. Scand. **8**, 301

6- BAKHTIN, A.G. (1956)
Vet. Moscow., **33**, 30

7- OAKLY, C.L., & WARRACK, G.H. (1953)
J. Hyg., Vol. **51**, No 1, 102

8- GLENNY, A.T., et al.,
J. Hyg., Vol. **37**, 53

9- DALLING, T. & ROSS, H.E. (1938)
J. Com. Path. & Therap., **57**, 235

10- BOSWORTH, T.J. (1943)
J. Com. Path. & Therap., **53**, 245

11- BROOKS, M., STERNE, M., & WARRACK, G.H. (1957)
J. Path. Bact., **74**, 185

12- STERNE, M. & WARRACK, G.H. (1964)
J. Hyg. Vol. **88**, No 1, 279