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NECROTIZING INFECTIOUS ENTERITIS IN PIGLETS, CAUSED BY CLOSTRIDIUM PERFRINGENS TYPE C

IV. BACTERIOLOGICAL DIAGNOSIS*)

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The diagnosis of necrotizing enteritis in infant piglets is based on clinical observations, patho-anatomical changes, and bacteriological and bacterio-toxicological examinations.

Previous works (*Høgh* 1967 a, b, 1969) have dealt with the aetiology, symptomatology and pathology of the disease.

The purpose of this paper is to present the results of bacteriological examinations of pigs from spontaneous outbreaks.

PREVIOUS INVESTIGATIONS

The objects of such bacteriological examinations are the demonstration of specific toxin in the intestinal contents and the isolation and typing of Cl. perfringens from that material.

In their bacteriological examinations, *Barnes & Moon* (1964), *Bergeland* (1965) and *Høgh* (1965) used direct spreading of scrapings from intestinal contents on to the surface of agar plates, followed by anaerobic incubation. *Field & Gibson* (1955) and *Szent-Iványi & Szabó* (1956) employed pre-incubation of intestinal contents in fluid medium, from which agar plates were subsequently inoculated and incubated anaerobically.

With a few exceptions, Cl. perfringens type C was isolated by both methods from the intestinal canal of pigs with characteristic postmortem findings.

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Szent-Iványi & Szabó found the infection limited to the intestinal canal and the mesenteric lymph nodes. *Barnes & Moon* and *Bergeland* isolated Cl. perfringens type C from the heart blood both of pigs that had died of the disease and of such that had been killed in a moribund state. *Bakhtin* (1956) found septicaemia in some cases of Cl. perfringens type B infection, and in all instances the clostridium could be isolated from the peritoneal cavity.

The presence of β-toxin in intestinal contents was demonstrated by *Field & Gibson, Szent-Iványi & Szabó* and *Høgh* (1965). *Barnes & Moon* reported that β-toxin could be found in the peritoneal fluid both of pigs that had died of the disease and of pigs killed in the moribund state. *Field & Gibson* stated that, in neutralization experiments with specific Cl. perfringens antitoxins, toxic intestinal filtrates could give unspecific results. This was ascribed to insufficient clarification of the intestinal contents or to the presence of decomposition products.

Szent-Iványi & Szabó isolated non-pathogenic aerobic bacteria from pigs with necrotizing enteritis. *Høgh* (1965) found haemolytic Escherichia coli in 3 out of 5 pigs, but concluded that this was a fortuitous or secondary finding. *Moon & Bergeland* (1965) also reported the finding of haemolytic E. coli in a number of pigs with necrotizing enteritis. Those writers point to the risk of erroneous diagnosis to which such bacteriological findings can give rise.

MATERIAL AND METHODS

The material consisted of 339 piglets with necrotizing enteritis sent in for examination from 125 herds of swine. In order to elucidate the diagnostic significance of Cl. perfringens type C in necrotizing enteritis, 111 infant piglets with catarrhal enteritis or negative postmortem findings were included in the examination. Sixty-one of these pigs originated from 22 herds where necrotizing enteritis had been diagnosed, and 50 from 34 herds where the disease had not been found.

Media. The solid media employed were Zeissler's dextrose blood agar (2 % dextrose and 2 % agar), 5 % blood agar, and egg-yolk agar, produced as described by *McClung & Toabe* (1947).

Robertson's cooked-meat medium (*Robertson* 1915—16), made from minced beef heart and meat-infusion-peptone broth, was used for enrichment of pure cultures for typing.

Inoculation. Scrapings from intestinal contents, mesenteric lymph

nodes, liver and spleen were inoculated on dextrose blood agar and 5 % blood agar, and incubated under anaerobic (Fielde & McIntosh's jar) and aerobic conditions at 37°C for 18—20 hrs. For material from lymph nodes, liver and spleen, generally 1 agar plate of each kind was used, while 3 or 4 dilution plates were made from intestinal contents. In addition to this direct inoculation, intestinal contents from 48 pigs were inoculated into cooked-meat medium and incubated for 18—20 hrs. at 37°C with a view to demonstrating β-toxin in filtrates of mixed cultures.

Isolation of pure cultures. Colonies showing double haemolysis zones on dextrose blood agar were isolated in cooked-meat medium and controlled for purity. Pure cultures of haemolytic E. coli and haemolytic streptococci were obtained by subcultivation of suspect colonies on 5 % blood agar.

Examination for formation of toxin. The presence of toxin in intestinal contents and mixed cultures was demonstrated by intravenous injection on mice (weight 18—22 g).

Intestinal contents. The small intestines of 173 pigs were emptied of their contents, to which equal parts of sterile saline were added. After careful mixing and centrifugation at 5000 r.p.m. for 15—20 min., the supernatant was clarified by filtration (Seitz K5 filter). The clarified supernatant was divided into 2 fractions, 1 of which was trypsinized. Each fraction was tested for toxicity by injection of 0.5 ml intravenously into each of 2 mice. Filtrates found to be toxic in 1 or both fractions after observation for 24 hrs. were typed.

Mixed cultures. After incubation for 18—20 hrs., cultures of intestinal contents in cooked-meat medium were clarified by filtration. The filtrates were tested for toxicity as in the case of intestinal contents, and toxic filtrates were typed.

Fluid from peritoneal and pleural cavities. After centrifugation, serial 2-fold dilutions were made of the supernatant, and 0.5 ml of each dilution injected i. v. into each of 2 mice. The MLD was determined as the highest dilution that would kill both mice within 24 hrs.

Trypsinization. Pancreatic Trypsin Novo, salt-free, containing 6 Anson trypsin units per gram, was dissolved in sterile saline in an amount of 10 mg per ml. For trypsinization 0.1 ml solution was added to 1 ml filtrate, which was thereafter incubated at 37°C for 1 hr.

Typing

Toxic filtrates. The presence of Cl. perfringens major, i.e., lethal antigens in toxic filtrates of intestinal contents and mixed cultures was demonstrated by intravenous inoculation into mice after treatment with diagnostic Cl. perfringens sera Types A, C and D (Burroughs Wellcome & Co.). Typing was performed according to the principles described by *Oakley & Warrack* (1953). The filtrates were examined priliminary for the presence of β-toxin by neutralization

Table 1. Neutralization experiments with types A and C sera on toxic filtrates.

	Filtrate ml	Saline ml	Undiluted serum type A ml	Undiluted serum type C ml	Results 1	Results 2	Results 3
Non-trypsinized	1.0	0.2	0	0	2+	2+	2+
	1.0	0.1	0.1	0	2+	2+	2+
	1.0	0	0.1	0.1	2—	2—	2—
Trypsinized	1.0	0.2	0	0	2—	+/—	2+
	1.0	0.1	0.1	0	—	2—	2—
Toxins present					β	(α),β	α,β

+: Died.
—: Survived.

tests with Types A and C sera on toxic, non-trypsinized filtrates, and with Type A serum on toxic, trypsinized filtrates (see Table 1).

After standing at room temperature for 30 min., 0.5 ml of each mixture was injected intravenously into each of 2 mice, which were observed for 24 hrs. If the neutralization test gave one of the results shown in Table 1, the toxins demonstrated were considered to have been produced by Cl. perfringens type C. If Type A serum neutralized a non-trypsinized filtrate, the test was repeated with normal horse serum instead of Type A serum. In the cases in which non-trypsinized filtrates were not neutralized by Type C serum, and trypsinized filtrates not by Type A serum, the test was repeated with the combinations of sera used by *Oakley & Warrack*.

The filtrates that were neutralized by normal horse serum and those that did not give unequivocal results in the neutralization tests were designated as unspecific.

Clostridium perfringens. Single colonies were subcultured in cooked-meat medium at 37°C for 18—20 hrs., and the cultures sterilized by filtration (Seitz EK filter). The filtrates were examined for the presence of major, i.e., lethal antigens as described by *Høgh* (1967a). The experimental animals used were depilated white guinea pigs which were injected intracutaneously with 0.2 ml of each filtrate-serum mixture. Strains of Cl. perfringens that did not provoke skin necrosis were grown on egg-yolk agar for demonstration of a possible lecithinase reaction (*Nagler* 1939; *Macfarlane et al.* 1941).

Haemolytic Escherichia coli. The strains of haemolytic E. coli isolated were initially examined by slide agglutination tests with OL serum of Types O8, O138, O139, O141ab and O141ac. Strains that were not agglutinated by any of these sera were examined by tube agglutination tests against 24 pooled sera, each representing 6 O-types. In the event of agglutination with a pooled serum, the coli culture in question was tested against each of the 6 type sera included in the pool. Strains that were not agglutinated by any of the sera employed were designated type —.

Haemolytic streptococci. The haemolytic streptococci isolated were examined by precipitation against group-specific sera. The antigen was produced by hydrochloric-acid extraction as described by *Lancefield* (1933). Group C streptococci were tested for fermentation of sorbit and trehalose in order to distinguish between Groups Ca and Ch.

RESULTS

The diagnostic significance of the demonstration of Clostridium perfringens type C in necrotizing enteritis will appear from Table 2.

Table 2. The presence of Cl. perfringens type C in piglets with necrotizing enteritis and in piglets without necrotic or haemorrhagic lesions of the small intestine.

	No. of piglets examined	Piglets with no growth of Cl. perfringens		Piglets with growth of only type A and non-necrotizing strains		Piglets with growth of type C	
		no.	%	no.	%	no.	%
a	339	14	4.1	24	7.1	301	88.8
b	61	19	31.1	33	54.1	9	14.8
c	50	15	30.0	35	70.0	0	0

a. Piglets with necrotizing enteritis.
b. Piglets without necrotizing enteritis but originating from infected herds.
c. Piglets from herds where the disease has not been diagnosed.

Table 3. Results of typing pure cultures of Cl. perfringens.

	No. of strains examined	Non-necrotizing strains	Cl. perfringens type A	Cl. perfringens type C
a	847	198	8	641
b	154	141	3	10
c	123	121	2	0

a. Piglets with necrotizing enteritis.
b. Piglets without necrotizing enteritis but originating from infected herds.
c. Piglets from herds where the disease has not been diagnosed.

It will be seen that Cl. perfringens type C was isolated from 301 piglets (88.8 %) with necrotizing enteritis (a). Cl. perfringens type C was also isolated from 9 piglets (14.8 %) which originated from infected herds (b) but showed no necrotic or

haemorrhagic lesions of the small intestine. Cl. perfringens type C was not demonstrated in the intestinal contents of 50 piglets from herds where the disease had not been diagnosed (c). It will also be seen from the table that Cl. perfringens was not isolated from 14 pigs (4.1 %) with necrotizing enteritis (a), nor from respectively 19 and 15 pigs (about 30 %) without specific lesions of the small intestine (b and c).

By typing of 1124 pure cultures of Cl. perfringens (Table 3) only types A and C were found besides non-necrotizing lecithinase-positive strains.

Table 4. The presence of Cl. perfringens type C in relation to patho-anatomical changes in the jejunum.

Group	No. of piglets examined	Piglets with no growth of Cl. perfringens		Piglets with growth of only type A and non-necrotizing strains		Piglets with growth of type C	
		no.	%	no.	%	no.	%
I	146	0		10	6.8	136	93.2
II	95	5	5.3	4	4.2	86	90.5
III-1	22	0		1	4.5	21	95.5
III-2	76	9	11.9	9	11.9	58	76.3
Total	339	14	4.1	24	7.1	301	88.8

In Table 4 the presence of Cl. perfringens type C in the intestinal contents of pigs with necrotizing enteritis is related to the character of the patho-anatomical changes in the small intestine (cf. *Høgh* 1969). It will be seen that Cl. perfringens type C was demonstrated in 90.5—95.5 % of the pigs in the patho-anatomical Groups I, II and III-1, and in 76.3 % of the pigs in Group III-2. This latter group also contained the largest number of pigs showing no growth of Cl. perfringens from the intestinal contents.

Thirty-two pigs were known to have been treated with antibiotics or chemotherapeutics during the disease; Cl. perfringens type C was isolated from the intestinal contents of only 19 of these pigs (59.4 %). In 5 piglets Cl. perfringens was not demonstrated, and in 8 piglets only Cl. perfringens type A and non-necrotizing strains were found.

Cl. perfringens type C was isolated from the liver in 7.3 %, from the spleen in 8 %, and from the mesenteric lymph nodes

Table 5. The presence of Cl. perfringens type C in liver, spleen and mesenteric lymph nodes.

Cl. perfringens type C isolated from	Group				Total	%
	I	II	III-1	III-2		
Jejunum only	26	33	3	28	84	56.0
Jejunum and lfnd.*)	16	8	3	5	32	21.3
Jejunum and liver or spleen	10	2	0	2	14	9.3
Jejunum, lfnd. and liver or spleen	5	1	0	0	6	4.0
Jejunum, lfnd., liver and spleen	1	0	0	0	1	0.7
Lfnd. only	1	1	0	0	2	1.3
No growth	3	2	0	6	11	7.3
No. of piglets examined	62	47	6	35	150	—

*) lfnd.: Mesenteric lymph nodes.

in 27.3 % of 150 piglets examined. Generally only very few colonies appeared on dextrose blood agar, and only rarely was the growth abundant. As will be seen from Table 5, in 56 % of the pigs the infection was limited to the small intestine. In a further 21.3 % Cl. perfringens type C was isolated also from the mesenteric lymph nodes, but not from the other organs examined. In 2 out of 13 cases in which Cl. perfringens type C was not isolated from the intestinal contents, the infection was present in the mesenteric lymph nodes. In the remaining 11 cases the clostridium was not demonstrated in any of the organs examined. Only in a single instance was Cl. perfringens type C isolated from both intestine, liver, spleen and mesenteric lymph nodes. In 20 cases the clostridium was found in only 1 or 2 organs besides the intestinal canal. It further appears from the table that the finding of Cl. perfringens type C in liver, spleen and

Table 6. Demonstration and typing of toxin in filtered intestinal contents.

Group	No. of piglets examined	Intestinal contents				%
		non-toxic	unspecific	α-toxin	β-toxin	β-toxin
I	94	16	8	2	68	72.3
II	48	22	6	2	18	37.5
III-1	8	7	0	0	1	12.5
III-2	23	18	4	1	0	0
Total	173	63	18	5	87	50.3
%	100.0	36.4	10.4	2.9	50.3	—

mesenteric lymph nodes was more frequent in Group I than in the other groups.

Filtrates of mixed cultures from the intestinal contents of 48 pigs were examined for toxins; β-toxin was demonstrated in 34 (70.8 %) and α-toxin in 3 cases. Five filtrates gave unspecific results and 6 were atoxic.

Table 6 shows the occurrence of toxin in filtrates of intestinal contents of 173 pigs, and the types of toxin demonstrated. β-toxin was found in 87 specimens (50.3 %) and α-toxin in 5. Eighteen toxic filtrates gave unspecific results on typing, and 63 filtrates were found to be atoxic. It will also be seen that β-toxin was present in the intestinal contents of 72.3 % of the pigs in Group I and in 37.5 % of the animals in Group II. Only 1 out of 8 pigs in Group III-1 and none of the 23 pigs in Group

Table 7. Comparison between the amounts of β-toxin in intestinal contents and in pleural and peritoneal fluids.

Case no.	β-toxin MLD per ml		
	Intestinal contents	Peritoneal fluid	Pleural fluid
145-1 67/68	32	2	1
145-2 —	16	1	0
145-3 —	32	8	0
170-1 —	<2	8	0
176-1 —	<4	8	2
177-1 —	32	2	0
177-2 —	16	2	0
186-1 —	2	1	0
187-1 —	2	<2	<2
187-2 —	2	2	0
187-3 —	8	4	<2
201-1 —	<2	<4	<4
201-2 —	2	2	0
4-1 68/69	16	4	0
7-1 —	<2	<2	<2
13-1 —	2	1	0
14-2 —	4	1	0
P 86	16	16	0
P 119	16	16	0
P 120	32	8	0
P 121	32	8	1
P 122	8	4	0
P 124	8	8	1
P 125	16	8	0
P 160	32	32	4

III-2 had demonstrable amounts of β-toxin in their intestinal contents.

The results of a parallel examination for the presence of β-toxin in intestinal contents and in pleural and peritoneal fluids of 25 pigs with haemorrhagic necrotizing enteritis (Group I) are shown in Table 7. It will be seen that the amount of β-toxin in intestinal contents and peritoneal fluid varied from < 2 to 32 MLD per ml. Apart from 2 cases in which the amount of β-toxin was highest in the peritoneal fluid, the amount of toxin found there was about 40 % of that found in the intestinal contents. β-toxin was found in only 5 cases in pleural fluid in amounts of from 1 to 4 MLD per ml.

Table 8. Comparison between isolation of Cl. perfringens type C after inoculation on to solid medium and demonstration of β-toxin in mixed cultures and intestinal contents.

Group	No. of piglets examined	Cl. perfringens type C isolated	β-toxin present in	
			mixed cultures	intestinal contents
I	15	15	11	10
	1	0	0	0
II	16	16	12	9
III-2	13	13	8	0
	3	0	3	0
Total	48	44	34	19

Table 8 shows a comparison based on 48 cases, between the results obtained with the various diagnostic methods. Cl. perfringens type C was isolated from the intestinal contents of 44 pigs and β-toxin was present in 34 filtrates of mixed cultures and in 19 filtrates of intestinal contents. It will also be seen that the examination for β-toxin in mixed cultures revealed the presence of Cl. perfringens type C in 3 cases (Group III-2), even though that type was not isolated from the intestinal contents by inoculation on dextrose blood agar.

Secondary infection

In 105 (37.7 %) of 286 pigs with necrotizing enteritis, haemolytic Escherichia coli or haemolytic streptococci could be demonstrated in the intestinal contents. In the majority of cases the growth appeared as single or few haemolytic colonies in a mas-

sive growth of non-haemolytic colonies. In 30 pigs the growth could be characterized as massive, and in 6 cases pure cultures of haemolytic E. coli were obtained under aerobic conditions.

With the exception of a few cases in which haemolytic colonies were also isolated from liver, spleen and mesenteric lymph nodes, the sole localization of these organisms was the small intestine.

Among pigs referred to the patho-anatomical Groups I and III-1, haemolytic streptococci and haemolytic E. coli occurred with equal frequency. In Groups II and III-2 haemolytic E. coli were present 3—4 times more frequently than haemolytic streptococci.

Among the 38 non-verified cases of necrotizing enteritis there were 14 in which haemolytic E. coli or haemolytic streptococci were found. In only 5 of these cases could the growth be characterized as abundant.

Thirty-seven of 70 strains of E. coli were distributed among 23 known serotypes, while 33 were designated as type — . Types O8, O139, O141 ab and O141 ac were each isolated from 1 pig. The rest of the typable strains belonged to O-groups not known to be pathogenic for pigs.

Ten of 30 strains of haemolytic streptococci belonged to Group Ch and 20 to Group L.

DISCUSSION

By bacteriological examination of intestinal contents Cl. perfringens type C was found in a few pigs without necrotic or haemorrhagic lesions of the small intestine. As the pigs in question all originated from herds where necrotizing enteritis had been diagnosed in other pigs, these findings are probably an expression of the widespread occurrence of this clostridium in an infected invironment. The isolation of Cl. perfringens type C from the intestinal contents of pigs with necrotizing enteritis must therefore be of diagnostic significance.

Cl. perfringens type C was not isolated from the intestinal contents of about 11 % of pigs with necrotizing enteritis. The majority of these unverified cases occurred in pigs with postmortem findings corresponding to Groups II and III-2.

Provided the anamnestic information has been adequate, only 32 of the pigs examined had been treated with antibiotics or chemotherapeutics. Even though such treatment was found to

reduce the number of verified cases to about 60 %, it did not have any noteworthy effect on the differences between the 3 patho-anatomical groups as regards the rate of recovery of Cl. perfringens type C from intestinal contents of the pigs.

Fuller & Moore (1967) reported that lipids in the small intestine would have a bactericidal effect on Cl. perfringens if the pigs had fasted for at least 6 hrs.

In acute to sub-acute cases (Groups II and III-2) the disease often lasts for several days, and the lack of, or a strongly reduced, desire to suck is a prominent symptom. Thus, in certain cases the course of the disease might provide the possibility of a lipid-reduced bactericidal effect in the small intestine. It is not possible to determine to what extent this could explain the observed difference in the rate of recovery of Cl. perfringens type C, since the sensitivity of type C to lipids is not known with certainty.

In evaluating the frequency of generalized infection, it must be taken into account that, due to the time required for shipment, the pigs were not examined bacteriologically until 1 or 2 days after death. Yet, in almost 80 % of cases it was found that the infection was limited to the intestinal canal, or to the intestinal canal and the regional mesenteric lymph nodes. Cl. perfringens type C was demonstrated in the liver in 7.3 % and in the spleen in 8.0 % of the pigs, but in only 1 case was the clostridium found in both of these organs. This shows that the presence of Cl. perfringens type C in the liver and spleen must be regarded as fortuitous, and presumably it is an expression of a postmortem invasion from the intestinal canal. On the basis of this assumption, which is substantiated by the afebrile course of the disease and the leukopenia observed (*Høgh* 1967b), the disease must be regarded as a local intestinal infection. This has also been shown by *Szent-Iványi & Szabó* (1956).

Mainly β-toxin and, in a few cases, α-toxin were present in filtered intestinal contents, while ε-toxin was not found. This is in agreement with the fact that Cl. perfringens type B was not isolated by the bacteriological examinations. The 18 cases of unspecific reaction are (cf. *Field & Gibson* 1955) presumably due to decomposition products of insufficient clarification; in fact, the intestinal contents often remained turbid even after repeated filtrations.

With the exception of 1 case, β-toxin was found only in the intestinal contents of pigs with haemorrhagic lesions of the

small intestine (Groups I and II). This might indicate either a greater stability or a more abundant production of β-toxin in the pigs of these 2 groups than in those of Group III. *Niilo* (1965) found in in vitro experiments that the half-life of β-toxin was 2 days at 22°C. Since, however, all the pigs in the present material were examined at approximately the same time after death (1—2 days) such "normal" degradation should influence the presence of β-toxin to the same degree in all pigs, irrespective of the patho-anatomical changes. More likely, in view of the fact that, on the one hand, β-toxin is inactivated by trypsin (*Dalling & Ross* 1938) while, on the other hand, a trypsin inhibitor is present in the colostrum during the first 5 days after farrowing (*Laskowski et al.* 1957) the difference observed is associated with variations in the age of the pigs at the time of death (*Høgh* 1969).

Titrations of β-toxin in intestinal contents and peritoneal and pleural fluids showed that, with a few exceptions, there was good agreement between the amounts of β-toxin in the 2 materials first mentioned, while only few specimens of pleural fluid contained demonstrable amounts of β-toxin. In cases in which there is sufficient exudate in the peritoneal cavity, this can be used with advantage for diagnostic purposes, as stated by *Barnes & Moon* (1964).

The difference between the β-toxin levels in peritoneal and pleural fluids would indicate that for the greatest part, the presence of toxin in fluid from the peritoneal cavity is due to filtration from the small intestine. The β-toxin demonstrated in fluid from the pleural cavity must be presumed to originate from the blood. Even though the majority of the pleural specimens did not contain demonstrable amounts of β-toxin, this does not necessarily imply an atoxaemic course of the disease. The microscopic lesions of the brain (*Høgh* 1969), the leukopenia and the presence of juvenile heterophils (*Høgh* 1967b) would indicate that the disease is, in fact, accompanied by toxaemia.

Isolation and typing of pure cultures were found to be more reliable for diagnosis purposes than examination for the presence of β-toxin in intestinal contents and in mixed cultures from such. Thus examination for β-toxin has not as often as direct inoculation revealed the presence of Cl. perfringens type C. nevertheless this examination is of value whenever a rapid verification of the disease is desirable. Furthermore, mixed cultures have

been found to contain β-toxin in a few cases in which Cl. perfringens type C was not demonstrated by direct inoculation (Group III-2). Examination for the presence of β-toxin is therefore a valuable supplement to direct inoculation on dextrose agar.

In only 36 cases (12.6 %) did cultures from intestinal contents show such a massive growth of haemolytic E. coli or haemolytic streptococci that it might lead to an erroneous diagnosis (cf. *Moon & Bergeland* 1965).

The presence of haemolytic E. coli and haemolytic streptococci must be considered to be fortuitous or secondary. This is suggested by the observation, that massive growth of these organisms was seen in but very few of the cases of necrotizing enteritis that were not verified as cases of Cl. perfringens type C infection, and, as far as E. coli is concerned, by the fact that on serological examination the strains isolated were found to be distributed over a wide range of known and unknown serotypes.

A further good reason to believe that haemolytic E. coli and streptococci are without causal relation to necrotizing enteritis is provided by the fact that oral infection experiments with Cl. perfringens type C on new-born pigs will provoke a disease with clinical and postmortem features indistinguishable from those found in spontaneous cases (*Szent-Iványi & Szabó; Field & Goodwin* 1959; *Bergeland* 1965; *Høgh* 1967b).

The demonstration of β-toxin in 3 cases and the isolation of Cl. perfringens type C from mesenteric lymph nodes in 2 cases in which this organism was not isolated by culture from intestinal contents on dextrose blood agar, lends support to the assumption that Cl. perfringens type C is the causal agent even in those cases of necrotizing enteritis in which this cannot be verified bacteriologically.

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SUMMARY

The diagnostic significance of Cl. perfringens type C was elucidated by an examination for the presence of this clostridium in the intestinal contents of 339 piglets with necrotizing enteritis and 121 piglets without necrotic or haemorrhagic lesions of the small intestine.

Typing of 1124 pure cultures revealed the presence of Cl. perfringens type C in the intestinal contents of 301 piglets with necrotizing enteritis and in 9 piglets without demonstrable lesions of the intestinal canal. The latter animals all originated from herds where the disease had been found to occur (Table 2). Besides Cl. perfringens type C, only type A and non-necrotizing strains occurred (Table 3).

In the majority of cases, the infection was localized to the intestinal canal and the mesenteric lymph nodes. The clostridium was also found in a few instances in the liver (7.3 %) or spleen (8.0 %), but in only 1 case it was found in both organs (Table 5).

Cl. perfringens type C was demonstrated more often in the intestinal contents of pigs with haemorrhagic lesions of the small intestine than in pigs with severe necrotic lesions but no haemorrhages (Table 4). The same was the case as regards the presence of β-toxin, which was found in filtered intestinal contents in 87 of the 173 specimens examined (Table 6). Twice as much β-toxin was found in intestinal content as in peritoneal fluid, while pleural fluid only rarely contained demonstrable amounts of toxin (Table 7).

Isolation of pure cultures gave better diagnostic results than demonstration of β-toxin in intestinal contents and mixed cultures (Table 8). However, examination for β-toxin provides an important supplement to direct inoculation on dextrose blood agar plates, particularly in cases in which rapid verification of the diagnosis is desirable.

Haemolytic Escherichia coli and haemolytic streptococci were found in the intestinal contents of 105 of 286 pigs with necrotizing enteritis. The presence of these agents, which in the majority of cases was only sparse, must be considered to be fortuitous or secondary.

ZUSAMMENFASSUNG

Infektiöse nekrotisierende Enteritis bei Saugferkeln verursacht von Clostridium perfringens Typ C. IV. Bakteriologische Diagnose.

Die diagnostische Signifikanz des Clostridium perfringens Typ C wurde durch eine Untersuchung über das Auftreten dieser Clostridie

im Darmgehalt von 339 Saugferkeln mit nekrotisierender Enteritis und von 121 Saugferkeln ohne nekrotische oder hämorrhagische Veränderungen im Dünndarm beleuchtet.

Bei einer Typenbestimmung von 1124 Reinkulturen wurde Cl. perfringens Typ C im Darmgehalt von 301 Saugferkeln mit nekrotisierender Enteritis und bei 9 Saugferkeln ohne nachweisbare Veränderungen im Darmkanal festgestellt. Diese letzeren 9 Saugferkel entstammten alle Beständen, in denen die Krankheit konstatiert worden war (Tabelle 2). Ausser Cl. perfringens Typ C kamen nur Typ A sowie nicht nekrotisierende Stämme vor (Tabelle 3).

Im grössten Teil der Fälle war die Infektion im Darmkanal und in den mesenterialen Lymphdrüsen lokalisiert. In vereinzelten Fällen kam die Clostridie ausserdem in der Leber (7,3 %) oder in der Milz (8,0 %) vor, jedoch nur in einem einzelnen Fall gleichzeitig in beiden Organen (Tabelle 5).

Der Cl. perfringens Typ C wurde mit grösserer Häufigkeit im Darmkanal von Ferkeln mit hämorrhagischen Veränderungen im Dünndarm festgestellt als bei Ferkeln mit ernstlicher nekrotisierender Enteritis ohne Hämorrhagie (Tabelle 4). Das Vorkommen von β-Toxin, das in filtriertem Darmgehalt in 87 von 173 untersuchten Proben festgestellt wurde (Tabelle 6), zeigte ein entsprechendes Verhältnis. Das β-Toxin kam im Darmgehalt in etwa doppelt so grosser Menge wie im Bauchhöhlenexsudat vor, während das Brusthöhlenexsudat nur ausnahmsweise nachweisbare Toxinmengen enthielt (Tabelle 7).

Die Isolation von Reinkulturen gab bessere diagnostische Ergebnisse als die Feststellung von β-Toxin im Darmgehalt und in Mischkulturen von diesem (Tabelle 8). Die Untersuchung auf das Vorkommen von β-Toxin ist jedoch ein wichtiges Supplement zu der direkten Aussaat auf Dextroseblutagar, besonders in den Fällen, in denen eine schnelle Verifikation der Diagnose von Bedeutung ist.

Hämolytische Escherichia coli und hämolytische Streptococcen kamen im Darmgehalt von 105 von 286 Ferkeln mit nekrotisierender Enteritis vor. Dieses Vorkommen, das im grössten Teil der Fälle sehr sparsam war, ist als zufällig oder sekundär anzusehen.

SAMMENDRAG

Infektiøs nekrotiserende enteritis hos pattegrise forårsaget af Clostridium perfringens type C. IV. Bakteriologisk diagnose.

Den diagnostiske signifikans af Clostridium perfringens type C blev belyst ved en undersøgelse for forekomst af denne clostridie i tarmindhold af 339 pattegrise med nekrotiserende enteritis og 121 pattegrise uden nekrotiske eller hæmorrhagiske forandringer i tyndtarmen.

Ved typebestemmelse af 1124 renkulturer blev Cl. perfringens type C påvist i tarmindhold af 301 pattegrise med nekrotiserende enteritis og i 9 pattegrise uden påviselige forandringer i tarmkanalen. Disse 9 pattegrise hidrørte alle fra besætninger, hvor sygdommen er konstateret (Tabel 2). Foruden Cl. perfringens type C forekom kun type A og ikke-nekrotiserende stammer (Tabel 3).

I hovedparten af tilfældene var infektionen lokaliseret til tarmkanalen og de mesenteriale lymfekirtler. I få tilfælde forekom clostridien tillige i leveren (7,3 %) eller i milten (8,0 %), men kun i et enkelt tilfælde i begge organer samtidig (Tabel 5).

Cl. perfringens type C blev påvist med større hyppighed i tarmindhold af grise med hæmorrhagiske forandringer i tyndtarmen end hos grise med en voldsom nekrotiserende enteritis uden hæmorrhagi (Tabel 4). Det samme forhold gjorde sig gældende med hensyn til forekomst af β-toksin, som blev påvist i filtreret tarmindhold i 87 af 173 undersøgte prøver (Tabel 6). β-toksinet forekom i tarmindhold i ca. dobbelt så stor en mængde som i bughuleexsudat, mens brysthuleexsudat kun undtagelsesvis indeholdt påviselige mængder toksin (Tabel 7).

Isolation af renkulturer gav bedre diagnostiske resultater end påvisning af β-toksin i tarmindhold og i blandingskulturer fra dette (Tabel 8). Undersøgelse for forekomst af β-toksin er imidlertid et vigtigt supplement til den direkte udsæd på dextroseblodagar, ikke mindst i tilfælde, hvor en hurtig verifikation af diagnosen er ønskelig.

Hæmolytiske Escherichia coli og hæmolytiske streptococcer forekom i tarmindhold af 105 af 286 grise med nekrotiserende enteritis. Denne forekomst, der i hovedparten af tilfældene har været meget sparsom, må betragtes som tilfældig eller sekundær.

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