A Survey of the Alimentary Tract of Cattle for Clostridium Perfringens

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SUMMARY

Contents of the rumen, abomasum, ileum, and colon of 100 fattened cattle were examined for the presence of *Cl. Perfringens.* Liquid medium iniculated with each sample of gut content was tested for the presence of toxins of *Cl. Perfringens.*

Identification of *Cl. Perfringens* was based on atmospheric requirements for growth, colonial morphology, and stormy fermentation in litmus milk. Identification of toxins was based on neutralization tests in guinea pigs and mice.

***Cl. Perfringens* was isolated from 202 of 399 samples. In 105 additional cultures, colonies characteristic of *Cl. Perfringens* were present but could not be isolated in pure culture.**

***Cl. Perfringens* type D toxin was identified in only one culture, which was inoculated with ileum contents. Type A toxin was identified in eight of the 24 samples from the one lot of samples in which no type A antitoxin was used. There were no identifications of toxigenic types B, C, or E.**

The results indicate that an isolation from necropsy specimens of untyped *Cl. Perfringens* or type A *Cl. Perfringens* is in itself of little significance. The infrequency of occurrence of the other toxigenic types in this survey of healthy cattle indicates that recovery of these types from necropsy specimens may be of more significance in determining the cause of death.

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Introduction

In past years various workers have associated *Cl. perfringens* with a variety of diseases of cattle. During the last ten years, "sudden death" has been reported frequently in the literature, particularly in feedlots, and is considered by many clinicians to be of common occurrence. In most cases it has been suspected that *Cl. perfringens,* type D is the cause, however, the validity of evidence incriminating *Cl. perfringens* is not always convincing (9).

The significance of isolations of *Cl. perfringens* or its toxins from bovine tissues would be more clear if data were available on the occurrence of the bacterium in normal cattle. While the organism is considered to be widely distributed in nature, no extensive studies have been made concerning its presence in normal cattle. The objective of this study was to find the occurrence of *Cl. perfringens* in the alimentary tract of domestic bovines coming from feedlots where an "all-grain" ration was being fed.

The results of previous surveys in normal and diseased cattle are summarized in Table I.

Materials and Methods

Collection of Samples — Three hundred ninety-nine samples of alimentary tract content were examined. They were drawn from the rumen, abomasum, ileum, and colon of 100 cattle.

Following evisceration of the cattle, the stomachs and intestines were placed in a cart. The samples were then taken after

TABLE I. Results of Surveys for Occurrence of Clostridium Perfringens in Animals

Author	Country	Species	No. Examined	Types Isolated					Not Typed
				A	B	C	D	E	
Borthwick (2)	U.K.	Dog	?	2			2		
		Rabbit	?	2					
		Guinea Pig	?				3		
Taylor and Gordon (8)	U.K.	Cattle	25	23			1		
		Sheep	23	23					
		Rabbit	15	13	1		1		
		Swine	28	28					
		Dog	21	17					
		Cat	7	7					
		Guinea Pig	17	16					
		Poultry	13	12					
Bullen (4)	U.K.	Sheep	100			46			
Estola and Stenberg	Sweden	Cattle	79						15
		Horse	2						2
		Swine	27	4					8
		Sheep	9						3
		Poultry	7						5
Niilo and Avery (7)	Canada	Cattle	278	142		3	2		131
		Sheep	56	34		1			21
		Swine	13	6					7
Maki and Picard (6)	U.S.A.	Cattle	15						2

the surface of each collection site had been seared with a propane torch. Inoculation of media was generally begun within 30 minutes of collection.

The samples were recorded as lot numbers one to 17, with 20 samples in lots one and two, and 24 samples in lots three to 17.

Examination of Samples

The methods used were adapted from techniques employed by Taylor and Gordon (8) and Bullen (4) in similar surveys.

Isolation and identification of *Cl. perfringens.* — Each sample was inoculated into the horsemeat-papain digest broth medium suggested by Brooks, Sterne, and Warrack as suitable for toxin production (3). After 24 hours incubation at 37 degrees centigrade, a loopful of broth culture was streaked onto a blood agar plate (Difco tryptic soy agar with five percent bovine blood). The plates were then placed in an air-tight incubator and vacuum applied until the chamber pressure was brought to minus 20 pounds per square inch. Nitrogen was then introduced to return the chamber to atmospheric pressure. This procedure was repeated three times except that on the final introduction of nitrogen the chamber pressure was returned to only minus six pounds pressure. The plates were then incubated at 37 degrees centigrade for 24 hours.

Following incubation, the plates were examined for colonies with morphology typical of *Cl. perfringens.* If such colonies were well-isolated, they were inoculated into tubes of litmus milk containing ferrous sulfate as a reducing agent. If colonies suspected of being *Cl. perfringens* were not well-isolated on primary plates, they were picked and replated. If primary plates were covered with spreading or swarming growth, the primary broth cultures were brought to a boil in a water bath and replated. In some cases, colonies showing characteristics of *Cl. perfringens* could not be isolated in pure culture, in which case they were recorded as suspected *Cl. perfringens.*

Demonstration of toxins of *Cl. perfringens.* — Supernatant fluid collected from centrifuged primary broth cultures was used for toxin demonstration. Broth cultures were centrifuged for 30 minutes at 3,000 r.p.m.. One ml. of supernatant fluid was pipetted into each of two glass vials. To both vials, ten units of *Cl. perfringens* type A antitoxin was added, with the ex-

TABLE II. Distribution of Clostridium Perfringens Isolants from Alimentary Tracts of One Hundred Cattle

Animal No.	Site				Animal No.	Site				Animal No.	Site				Animal No.	Site			
	R	A	I	C		R	A	I	C		R	A	I	C		R	A	I	C
1	P	P	P	N	26	N	N	P	P	51	S	N	S	P	76	P	N	P	P
2	N	S	N	P	27	P	S	P	P	52	S	N	P	P	77	P	N	N	N
3	P	N	N	N	28	P	P	P	P	53	S	N	P	N	78	P	S	P	P
4	P	P	P	P	29	S	N	P	P	54	S	S	P	P	79	P	N	P	P
5	N	P	P	N	30	N	N	N	P	55	P	S	S	P	80	P	P	P	P
6	P	P	P	P	31	S	N	P	P	56	S	P	P	P	81	P	N	P	P
7	P	N	P	P	32	—	S	P	P	57	N	S	P	P	82	P	P	P	P
8	P	P	P	P	33	S	N	P	S	58	N	N	S	S	83	S	P	P	P
9	N	P	N	P	34	N	N	P	P	59	P	N	S	P	84	S	P	P	S
10	S	P	N	N	35	P	S	N	P	60	P	P	P	P	85	N	P	P	P
11	P	P	N	P	36	S	S	N	S	61	P	S	N	P	86	S	P	P	N
12	P	P	N	P	37	P	N	P	S	62	S	S	P	P	87	S	S	P	N
13	P	P	P	P	38	N	N	N	S	63	S	N	P	P	88	S	S	S	P
14	P	P	P	P	39	S	S	S	N	64	S	P	P	S	89	N	N	P	P
15	P	P	P	P	40	N	N	N	N	65	S	N	P	P	90	P	N	P	P
16	P	P	P	P	41	S	P	P	N	66	S	S	S	S	91	N	P	P	P
17	P	N	N	S	42	S	S	N	S	67	S	S	N	N	92	N	S	P	P
18	S	P	N	P	43	N	P	S	N	68	P	S	N	S	93	N	N	P	S
19	P	P	P	S	44	S	P	N	S	69	P	N	P	S	94	P	N	P	P
20	S	P	S	S	45	S	N	S	S	70	S	P	S	N	95	S	N	S	S
21	S	S	P	S	46	S	S	N	P	71	P	P	N	N	96	P	S	P	P
22	S	S	S	S	47	N	P	P	N	72	P	P	P	N	97	S	N	S	S
23	N	S	P	P	48	S	N	P	N	73	N	P	P	P	98	S	P	S	S
24	S	P	P	P	49	P	P	P	S	74	P	N	P	P	99	S	P	P	P
25	P	P	P	P	50	S	P	S	P	75	P	N	P	S	100	P	S	P	S

Sites — R = rumen, A = abomasum, I = ileum, C = colon Cultures — P = positive, S = suspicious, N = negative

ception of lot four (24 samples) to which no antitoxin was added. To one vial of each pair, sufficient trysin (1:250 Difco) was added to make a 0.25 percent solution. The trypsintreated supernatant fluid was incubated at 37 degrees centigrade for 45 minutes, while the non-trypsinized vial of each pair was held at room temperature for the same length of time.

A portion from each vial was then injected intravenously into a mouse. The amount injected varied from 0.2 to 0.3 ml. according to the estimated weight of the mice, which varied from 17 to 28 grams.

Portions of the supernatant fluids caussing death of mice in the screening procedure were injected into additional pairs of mice to re-check the original result. If these supernatant fluids were lethal on the repeat test, filtrates were prepared and

TABLE III. Isolations of Clostridium Perfringens by Site

Culture	Rumen	Abomasum	Ileum	Colon	Total
Positive	41	41	63	57	52
Suspicious	39	25	16	25	105
Negative	19	34	21	18	92

subjected to the typing procedure suggested by the Burroughs-Wellcome Company, whose antitoxins were used for the procedure. In the typing procedure, intravenous inoculation of mice was the principal technique, although intradermal inoculation of guinea pigs was used occasionally.

Results

One sample was not processed due to damaged equipment. The results therefore cover a total of 399 samples, comprised of 100 each from the abomasum, ileum, and colon, and 99 from the rumen.

Isolations of *Clostridium perfringens.* — Table II illustrates the isolations of *Cl. perfringens* from each animal while Table III shows the total isolations from each site. There was a statistically significant larger number (p .05) of isolates from the ileum than other sites when positive cultures only were considered. There was no statistically significant difference in frequency of isolation from any one of the four sites (p .05) when the totals of confirmed and suspicious cultures for each site were compared. *Cl. perfringens* was isolated in pure culture from 202 of 399 specimens of gastrointestinal tract contents. There were only six animals from which positive cultures were not obtained. In five of these, suspicious colonies were recovered but could not be confirmed as *Cl. perfringens.*

Recovery of Toxins of Clostridium perfringens — The screening procedure involved inoculation of 796 mice. Thirty-four of these died within 48 hours of injection. In 16 instances, only the trypsin-treated portion of a sample caused death; in 12, only the non-trypsinized portion; in three, both portions of the sample caused death.

On repeat injections into pairs of mice, toxicity was confirmed in 20 of the samples, and neutralization procedures with antitoxins were carried out on these 20. Eight were typed as A and one as D, while 11 were not neutralized by any of the antitoxins used. All of the type A strains were demonstrated from lot number four in which no antitoxin was used in the screening procedure.

Type D toxin was demonstrated in the trypsin-treated portion of supernatant fluid. Type A toxin was demonstrated with only the non-trypsinized samples. All deaths of mice significant to the factors in this survey occurred within 24 hours of injection. These results are summarized in Table IV.

TABLE IV. Distribution of Clostridium Perfringens Toxins Demonstrated

Animal number	Sites	Trypsin treated	Time of death	"A" antitoxin treated	Type
17	R.C.	No	7 hrs	No	A
18	C	No	7 hrs.	No	A
19	R.I.C.	No	22 hrs.	No	A
20	I	No	22 hrs.	No	A
22	R	No	22 hrs.	No	A
88	I	Yes	24 hrs.	Yes	D

Sites — R = rumen, A = abomasum, I = ileum, c = colon.

Discussion

Isolation of *Cl. perfringens.* — The results in Table III indicate that *Cl. perfringens* is usually present in the alimentary tract of cattle in this area while on an "all-grain" ration. While the rapidity with which *Cl. perfringens* invades other viscera was not studied, the organism's consistent presence in the gut indicates that mere isolation is of no diagnostic significance. Several factors, including variation in permeability of tissues, hours since death, sites of isolation, frequency of terminal bacteremia, and the possibility of the presence of spores in normal tissues, bear on the conclusions which may be made when the organism is isolated.

Demonstration of toxins of *Cl. perfringens.* — In lot four, type A toxin was demonstrated in eight of 19 cultures which had colonies suspicous of *Cl. perfringens.* Projection of these results to the isolations in other lots would indicate that approximately half of the *Cl. perfringens* isolates would have been identified as type A, had antitoxin (type A) not been used routinely. The other half would therefore be nontoxigenic, i.e. not produce sufficient toxin to be demonstrated under the conditions of this survey. This proportion of toxigenic and nontoxigenic strains of *Cl. perfringens* is in accord with a previous investigation using similar methods on isolates from necropsy specimens (7). In the eight samples from which type A toxin was demonstrated, only untrypsinized fluids were lethal for mice. It has been reported previously that trypsin destroys type A toxin (Batty, personal communication). These results clearly indicate that even isolates identified as *Cl. perfringens* type A are of little significance without supporting evidence of their pathogenicity.

The single type D isolate, and absence of any of types B, C, or E, indicates that these types infrequently inhabit the alimentary tract of normal cattle in the area sampled. Demonstration of their toxins in the gut, or isolations of *Cl. perfringens* of these types from gut content or tissues, may therefore be considered as more significant than isolations of type A.

The finding of only a single type D culture in this study is quite different from the results reported by Bullen in a very similar survey of sheep in Britain in which 46 of 100 animals sampled harbored the type D organisms (4). In a survey of soil samples, type D organisms were found only on farms where the disease was known to have occurred in sheep (1). It is possible then that the occurrence of type D strains is more closely associated with animal disease then is type A. If so, the results of this survey tend to indicate that enterotoxemia due to *Cl. perfringens* type D is not common in cattle in the area surveyed.

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