***Campylobacter* Species Isolated from Swine with Lesions of Proliferative Enteritis**

Tatsuo OHYA, Masanori KUBO, and Hiroshi WATASE

Kyushu Branch Laboratory, National Institute of Animal Health, 2702 Chuzan, Kagoshima 891-01, Japan

(Received 10 September 1984/Accepted 20 January 1984)

ABSTRACT. *Campylobacter* species were isolated from 93 (48.4%) out of 196 pigs in which proliferative enteritis (PE) was detected at an abattoir in Kagoshima City. Three species of *Campylobacter* were identified as *Campylobacter sputorum* ssp. *mucosalis* (CSM), *Campylobacter hyointestinalis* (CHI) and *Campylobacter coli* (CCO) on the basis of biochemical characteristics. From 51 specimens (54.9%) CSM was isolated, CHI was from 23 specimens (24.7%) and CCO was from seven specimens (7.5%). From nine specimens (9.6%) CSM and CHI were isolated simultaneously. Simultaneous isolation of CSM and CCO, and that of CHI and CCO were seen in one (1.1%) and two specimens (2.2%), respectively. Viable bacterial count per gram of affected mucosa ranged 10^4 to 10^8 CFU. More than 80% of the positive specimens were involved in the range of 10^6 to 10^8 CFU. The presence of four different serovars of CSM in addition to serovar A was suggested. Strains other than serovar A had narrow range of growth temperature around 37°C and their growth was inhibited by the presence of triphenyl tetrazolium chloride. Forty-five sera of pigs bred in three farms, where CSM and CHI were thought to be prevailing, were subjected to the agglutination test to determine antibody titers against CSM and CHI. Twenty-one pigs had high agglutinin titer of more than 1:80 against CSM. On the other hand, only 2 pigs had high titer against CHI.—**KEY WORDS:** proliferative enteritis, *Campylobacter sputorum* ssp. *mucosalis*, *Campylobacter hyointestinalis*, *Campylobacter coli*.

Jpn. J. Vet. Sci. 47(2): 285–294, 1985

The characteristic lesions of proliferative enteritis (PE) in swine is the proliferative changes in mucosal epithelium of the ileum and occasionally of the jejunum, cecum and colon [7, 13, 24, 25] in association with intracellular presence of *Campylobacter* sp-like organisms [15].

Campylobacter sputorum ssp. *mucosalis* (CSM) was isolated by Lawson *et al.* and was considered as the causative agent [8]. Although many works have been attempted to clarify the relationship between CSM and PE [12, 14, 15, 16, 20, 21, 23], it has not succeeded in consistent reproduction of the disease. Recently, Gebhart *et al.* isolated *Campylobacter hyointestinalis* (proposed new species) from ileal lesion besides CSM and *Campylobacter jejuni/coli* [2].

The first detection of PE in Japan was reported in 1978 [5, 19], and it is now regarded to have prevailed in many prefec-

tures [3, 6]. *Campylobacter sputorum* ssp. *mucosalis* was isolated from proliferative lesions in ileal mucosa of a pig slaughtered at a prefectural abattoir in Ibaraki Prefecture in 1981 [18]. But the results of bacteriological examination on PE at that time are no longer sufficient to explain the etiology of the disease.

This report deals with the isolation of *Campylobacter* species from pigs which were detected to have lesions of PE at an abattoir in Kagoshima City from April, 1983 to March, 1984. Histopathological study on affected pigs described above has previously been reported in part [7].

MATERIALS AND METHODS

Sampling procedure: Freshly collected portions of caudal part of the ileum from 196 pigs having PE at an abattoir in Kagoshima

City from April, 1983 to March, 1984, were examined for *Campylobacter* species. In addition, 13 ileal specimens without lesion were also subjected to the bacteriological examination. The specimens were opened and gently washed with saline to remove intestinal contents. Then the mucosal layer was obtained by scraping the mucosal surface with sterile spatula. The mucosal sample was weighed, diluted to 1:10 with diluent A [17] and homogenized with Stomacher (Lab-Blender 80, Seward Laboratory UAC House, England) for 30 sec. Serial tenfold dilutions were prepared, and then 0.1 ml each of 10^{-3} , 10^{-4} , 10^{-5} dilutions was inoculated onto selective media.

Media employed: The selective medium employed for the isolation of *Campylobacter* species was essentially the same as that of Lawson *et al.* [8] except that sheep blood was substituted for horse blood and brilliant green was added at the concentration of 1:120,000 instead of 1:60,000. The inoculated plates were placed in an anaerobic jar together with Gas Pak (BBL) without catalyst. Then the jar was evacuated to a negative pressure of 650 mmHg. The jar was placed at 37°C for 3 days.

Isolation and identification: Four colonies suspected to be *Campylobacter* species were picked up and subcultured for each sample. Bacterial counts in samples were made on the primary plates. Tentative identification with *Campylobacter* species was performed by Gram's staining, oxidase test (Cytochrome-oxidase reagent, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), catalase test (employing 3% H_2O_2) and growth under microaerophilic condition. The isolates were cultured in Mueller Hinton Broth (Difco) supplemented by 0.5% of yeast extract (Difco), 0.03% of cysteine hydrochloride, 0.25% of sodium formate and 5.0% of horse serum. The broth culture incubated at 37°C for 3 days under microaerophilic condition was employed as an inoculum for biochemical test. Inocula-

tion onto plates for biochemical test was done by Continuous Inoculator (Ikeda Instrument Company, Tokyo, Japan). Blood agar base No. 2 (Oxoid) added 5.0% of sheep blood was used as a basal medium in the following tests. Growth in the presence of 1.5%, 1.75% and 3.0% sodium chloride, growth in 1.0% glycine and in 0.04% triphenyl tetrazolium chloride (TTC) were tested on the basal medium supplemented by each substance. Hydrogen sulfide production was demonstrated on freshly prepared triple sugar iron agar (Eiken Chemical Co., Ltd., Tokyo, Japan). The effect of temperature on growth of the organism was determined on the basal medium at 25°C and 43°C for 3 days. Susceptibility to nalidixic acid (NA) and cephalothin (CET) were assessed on a plate containing 30.0 µg/ml of these antimicrobial agents. These inoculated media were examined for growth after 3 days' incubation. Sodium hippurate hydrolysis was tested by the method of Hwang and Ederer [4].

Reference strains: *Campylobacter sputorum* ssp. *mucosalis* NKS strain (serovar A) was provided by Dr. M. Nakazawa of our institute. *Campylobacter jejuni* (HP 941) and *Campylobacter coli* (HP 1774) were kindly offered by Dr. T. Itoh of Tokyo Metropolitan Research Laboratory of Public Health.

Antibiotic susceptibility: Basal medium was blood agar base No. 2 supplemented with 5.0% of horse serum. Sixteen antibiotics were added to the basal medium at the concentration of 0.19, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 µg/ml. Thirty strains of CSM and 21 strains of CHI were subjected to the test. After incubation for 3 days the growth on each plate was evaluated and minimum inhibitory concentration (MIC) was determined.

Whole cell (OH) agglutination: Strains identified as CSM were subjected to slide agglutination test with OH antiserum prepared against CSM NKS strain (serovar A) according to the procedure of Lawson *et al.* [8,

Table 1. Monthly incidence of proliferative enteritis (PE) on different farms (A, B, C and Others) for the period of April, 1983 to March, 1984

Year:	Month	A	B	C	Others	Total
1983:	APR	5 ^{a)} (2) ^{b)}	1(1)	0	0	6(3)
	MAY	11 (2)	2(0)	3(2)	1(1)	17(5)
	JUN	23 (11)	7(3)	2(0)	3(1)	35(15)
	JUL	13 (4)	4(3)	2(1)	4(3)	23(11)
	AUG	12 (1)	5(4)	1(0)	5(2)	23(7)
	SEP	5 (2)	2(0)	0	4(2)	11(4)
	OCT	1 (1)	1(0)	2(0)	1(0)	5(1)
	NOV	11 (9)	3(2)	0	2(0)	16(11)
	DEC	3 (2)	6(1)	3(2)	2(1)	14(6)
	JAN	12 (4)	0	2(2)	2(2)	16(8)
	FEB	17 (13)	3(3)	0	1(1)	21(17)
	MAR	2 (1)	2(1)	2(2)	3(1)	9(5)
Total		115 (52)	36(18)	17(9)	28(14)	196(93)

a) No. of pigs detected PE.

b) No. of pigs isolated *Campylobacter* ssp.

9]. Strains agglutinated by the A antiserum were tentatively determined serovar A and abbreviated as CSM+ in this paper. Inagglutinable strains were abbreviated as CSM-. Four CSM+ antisera (against isolates NKS, 27, 34 and 85), five CSM- antisera (against isolates 9, 32, 90, 92 and 304) and two CHI antisera (against isolates 6 and 71) were prepared by the same method. The test was performed on these 11 strains using Microtiter plates (Sanko Junyaku Co., Ltd., Tokyo, Japan). Serum samples (0.05 ml) were dispensed to wells and diluted twofold with phosphate-buffered saline (pH 7.2) using Microdiluter (Sanko Junyaku Co., Ltd., Tokyo, Japan). An equal volume of antigen was added and mixed for 5 min. The plates were sealed with transparent tapes, and then incubated at 37°C for 18 hr before interpretation.

Detection of serum agglutinins: Serum agglutinin was titrated on 45 pigs from three farms, which were slaughtered at an abattoir in Kagoshima City. Incidence of PE was limited to the pigs produced at these three farms [7]. Formalinized antigen [8] of CSM+ strains (NKS and 77), CSM- strain 32 and CHI strain 40 were employed to detect

serum agglutinins. Agglutination test was carried out using microplate. Bacteriological examination was not done for these pigs.

RESULTS

Proliferative enteritis detected at an abattoir in Kagoshima City was limited to the pigs produced in three farms under the same management. The number of PE-positive pigs detected monthly is summarized in Table 1. Most of the farms referred to "others" were composed of relatively small farms and under the same management as the three farms. The number of pigs bred in farms A and B and slaughtered at the abattoir was approximately 1,000 and 500 by month, respectively. That of farm C and "others" was unknown.

Organisms isolated: In 196 pigs examined, 178 had histopathologically apparent PE lesions. The other 18 pigs lacked typical PE lesions, but many of them had the thickness of tunica muscularis. Two of these 18 pigs harbored 10⁵ CFU of CSM and CHI, respectively. *Campylobacter* species were isolated from 93 pigs. Biochemical tests were conducted for 339 isolates derived from 93 speci-

Table 2. Characteristics of *Campylobacter* spp. isolated from intestines of swine

Item	CSM+ ^{a)} (158) ^{e)}	CSM- ^{b)} (47)	CHI ^{c)} (101)	CCO ^{d)} (33)
Catalase	- ^{f)} (0) ^{g)}	-(0)	+ ^{h)} (101)	+(33)
Growth: 25°C	d ⁱ⁾ (96)	-(6)	+(95)	+(1)
43°C	d (78)	-(3)	+(89)	+(33)
1.5% NaCl	+(146)	+(44)	-(8)	-(3)
1.75% NaCl	+(142)	+(44)	-(4)	-(0)
3.0% NaCl	-(0)	-(0)	-(0)	-(0)
1.0% Glycine	d (38)	d(10)	+(90)	d(8)
0.04% TTC ^{j)}	d (119)	d(9)	d (23)	d(28)
H ₂ S on TSI agar	+(158)	+(47)	+(101)	-(0)
Susceptibility to NA ^{k)}	-(15)	-(7)	-(2)	+(30)
Susceptibility to CET ^{l)}	d (103)	d(18)	d (18)	-(4)
Hippurate hydrolysis	-(0)	-(0)	-(0)	-(0)

a) *Campylobacter sputorum* ssp. *mucosalis* of serovar A.b) *Campylobacter sputorum* ssp. *mucosalis* of other than serovar A.c) *Campylobacter hyointestinalis*.d) *Campylobacter coli*.

e) No. of strains subjected.

f) Means less than 15% of strains are positive.

g) No. of strains positive for corresponding item.

h) Means more than 86% of strains are positive.

i) Means 16 to 85% of strains are positive.

j) Triphenyl tetrazolium chloride.

k) Nalidixic acid (30 µg/ml).

l) Cephalothin (30 µg/ml).

Table 3. Isolation rate of *Campylobacter* spp. from 93 out of 196 pigs examined

<i>Campylobacter</i> spp. isolated	No. of sample positive for corresponding <i>Campylobacter</i> spp.
CSM+ ^{a)}	39 (42.1%)
CSM- ^{b)}	9 (9.6%)
CSM+ & ^{e)} CSM-	3 (3.2%)
CHI ^{c)}	23 (24.7%)
CCO ^{d)}	7 (7.5%)
CSM+ & CHI	6 (6.4%)
CSM- & CHI	3 (3.2%)
CSM- & CCO	1 (1.1%)
CHI & CCO	2 (2.2%)

a), b), c), d) Abbreviations are listed in Table 2.

e) Means simultaneous isolation.

mens (Table 2). *Campylobacter* species was not isolated from 13 ileal samples without lesion. The isolated strains were divided into four groups, namely, *Campylobacter sputorum* ssp. *mucosalis* of serovar A (CSM+), *Campylobacter sputorum* ssp. *mucosalis* except

serovar A (CSM-), *Campylobacter hyointestinalis* (CHI) and *Campylobacter coli* (CCO) by biochemical or serological test.

The proportion of isolated organisms is shown in table 3. From 51 specimens (54.9%) CSM was isolated, CHI was from 23

Table 4. Antibiotic susceptibility of *Campylobacter sputorum* ssp. *mucosalis* and *Campylobacter hyointestinalis*

Drug tested	<i>Campylobacter sputorum</i> ssp. <i>mucosalis</i> (30 strains)										
	≤0.19 ^{a)}	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
Penicillin		1 ^{b)}	2	2	5	11	8	1			
Ampicillin	2	4	4	8	11	1					
Cephalothin			12	4	2		1	5	6		
Gentamycin	24	4	2								
Kanamycin	9	2	13	6							
Streptomycin	18		8					3		1	
Neomycin	1	7	12	7	3						
Tetracycline	4	4	4	7	9	2					
Oxytetracycline	14		1	2	6	1	5		1		
Erythromycin	1	4	6	7	12						
Bacitracin											30
Colistin	29	1									
Polymyxin B		1	1	15	11	2					
Novobiocin						2	1	4	14	8	1
Chloramphenicol		19		2	3	2	4				
Nalidixic acid							2	3	9	16	

a) Minimum inhibitory concentration (MIC; µg or unit/ml).

b) Number of strains showing the respective MIC.

Table 4. Continued.

Drug tested	<i>Campylobacter hyointestinalis</i> (21 strains)										
	≤0.19	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
Penicillin					6	5	7	1		2	
Ampicillin			8	3	6	2	2				
Cephalothin						1	1	11	2	3	3
Gentamycin	2	9	10								
Kanamycin				1	3	4			1		12
Streptomycin			1	1			2	4		4	9
Neomycin			1	2	5	1	2	7	2	1	
Tetracycline			4	1			6	5	5		
Oxytetracycline					2	5	1	4	7	2	
Erythromycin				12	2	2			1	1	3
Bacitracin											21
Colistin			6	9	2	2	2				
Polymyxin B							9	9		2	1
Novobiocin								5	11	4	1
Chloramphenicol				1	7	6	5	1		1	
Nalidixic acid										3	18

specimens (24.7%) and CCO was from seven specimens (7.5%). From nine specimens (9.6%) CSM and CHI were isolated simultaneously. At the same time CSM and CCO, CHI and CCO were isolated from one (1.1%)

and two (2.2%) specimens, respectively. Viable count per gram of affected mucosa ranged 10^4 to 10^8 CFU. More than 80% of the positive specimens harbored 10^6 CFU or more of organisms.

Table 5. Homologous and heterologous titers of antisera in agglutination with whole cell antigens of *Campylobacter* spp.

Antisera	CSM+ ^{a)} Antigens					CSM- ^{b)} Antigens				CHI ^{c)} Antigens	
	NKS	27	34	85	9	32	90	92	304	6	71
NKS	1280	640	1280	1280	<10	40	10	<10	40	<10	<10
27	1280	1280	2560	640	10	40	20	<10	40	<10	10
34	1280	1280	2560	320	40	20	10	<10	80	<10	<10
85	2560	1280	640	1280	160	20	20	<10	40	20	10
9	40	640	20	160	2560	1280	160	40	40	20	10
32	10	20	10	<10	2560	1280	640	80	40	20	160
90	<10	<10	10	<10	160	80	1280	160	80	<10	<10
92	40	80	20	80	40	40	160	1280	320	<10	<10
304	10	20	20	20	80	160	160	160	2560	20	20
6	10	10	<10	<10	<10	<10	10	<10	80	1280	320
71	10	10	<10	<10	<10	<10	10	<10	20	1280	1280

a), b), c) Abbreviations are listed in Table 2.

At first 205 of isolates were identified as CSM. The colonies of CSM on the selective medium were about 1.5 to 2.0 mm in diameter, round, umbonate, dark yellow and composed of Gram-negative, short and irregularly curved rods. In biochemical tests, they were catalase-negative, H₂S positive on triple sugar iron agar and sodium hippurate hydrolysis negative. They grew well on media containing 1.75% of sodium chloride, however, not with 3.0% of sodium chloride, and they were resistant to nalidixic acid. Strains of CSM were discriminated in two types by optimum growth temperature and the growth in the presence of 0.04% of TTC, and both of them were related to the serovar. Isolated CSM strains were tentatively divided into serovar A and other serovars. Serovar A strains were relatively tolerant to the incubation temperature at 25°C or 43°C, and grew in the presence of TTC. But 47 strains except serovar A did not grow at 25°C and 43°C, and under the presence of TTC.

The next 101 isolates were identified as CHI. The colonies of CHI were about 1.0 to 1.5 mm in diameter, round, umbonate and light brick-red. The organisms were catalase-positive, H₂S positive on triple sugar iron agar and grew well on media with 1.0%

glycine, but not on media with 1.5% sodium chloride. They grew well at 25°C and 43°C, and were resistant to nalidixic acid. All the strains did not hydrolyze sodium hippurate.

The remaining 33 isolates were identified as CCO. These organisms were catalase-positive, failed to produce H₂S on triple sugar iron agar, intolerant to 1.5% sodium chloride, but grew on media containing TTC. They grew abundantly at 43°C, but not at 25°. They were susceptible to nalidixic acid, but not to cephalothin. Hydrolysis of sodium hippurate was negative for all strains examined.

Antibiotic susceptibility: Susceptibility of CSM and CHI to 16 antibiotics are shown in Table 4. Strains of CSM were susceptible to ampicillin, gentamycin, kanamycin, neomycin, tetracycline, erythromycin, colistin and polymyxin B. On the other hand, CHI strains were less susceptible to these antibiotics except ampicillin and gentamycin. Bacitracin, novobiocin and nalidixic acid were inactive to both CSM and CHI. Susceptibility to kanamycin, neomycin, tetracycline, erythromycin and polymyxin B was prominently different between CSM and CHI. The resistant strains to these antibiotics were more common in CHI than CSM. As a whole, CSM and CHI showed different susceptibility patterns.

Table 6. Serum agglutinin titers of pigs produced in three farms (A, B, and C) against four antigens

No. of serum	A				B				C			
	CSM+ ^{a)}		CSM- ^{b)}	CHI ^{c)}	CSM+		CSM-	CHI	CSM+		CSM-	CHI
	NKS	77			NKS	77			NKS	77		
1	0 ^{d)}	1	0	0	3	4	1	3	4	4	4	1
2	2	4	1	1	3	4	1	1	3	2	0	1
3	3	4	0	3	3	2	0	3	3	1	0	1
4	2	1	2	0	3	5	1	3	5	5	0	3
5	3	3	2	2	1	4	2	1	2	4	2	3
6	2	2	1	1	3	4	0	1	3	3	0	0
7	3	5	5	2	3	5	1	2	3	3	3	4
8	3	2	0	2	2	2	1	1	2	2	0	1
9	3	3	3	2	2	4	0	0	3	4	0	3
10	3	3	0	1	2	4	0	0	2	3	1	3
11	2	2	2	2	2	4	0	2	2	1	3	1
12	2	4	0	1	2	2	0	0	3	0	0	1
13	1	3	0	2	1	2	3	0	1	2	0	2
14	2	2	0	2	2	4	0	0	6	6	5	5
15	2	4	1	1	3	3	0	2	2	1	5	2

a, b, c) Abbreviations are listed in Table 2.

d) Agglutinin titers are shown by scores as follows:

0=<1:10, 1=1:10, 2=1:20, 3=3:40, 4=1:80, 5=1:160, 6=1:320.

OH agglutination: Isolated CSM strains were distinguished into serovar A (CSM+) and other serovars (CSM-) by slide agglutination test. The presence of four serologically different groups (9 and 32; 90; 92; and 304) was suggested in CSM- by agglutination test as shown in Table 5. Strains 6 and 71 of CHI were serologically distinct from CSM.

Detection of serum agglutinins: Presence of serum agglutinins against CSM and CHI was examined on 45 pigs bred in three farms (A, B and C). Serum agglutinin titers <1:10 to 1:320 are converted to scores 0 to 6 in Table 6. Pigs having high agglutinin titer more than 4 in score (>1:80) were found in every farm. High score was obtained when the antigen prepared from strain 77 was used in the test to detect agglutinin against CSM+. This strain was isolated from the pig produced in farm A. Serum agglutinin was more frequent in high titer against CSM+ than CSM- or CHI.

DISCUSSION

No notable characteristics were observed as for the monthly incidence of PE. The occurrence of PE in farms A and B was approximately 0.96% (115/12,000) and 0.6% (36/6,000), respectively.

Campylobacter species were isolated from 93 (7.4%) in 196 pigs examined. They were identified into three species, *Campylobacter sputorum* ssp. *mucosalis*, *Campylobacter hyointestinalis* and *Campylobacter coli* by biochemical tests.

From 61 pigs CSM ws isolated, in which serovar A was from 46 pigs and the other serovars were from 12 pigs. Simultaneous isolation of them was made in 3 pigs. From 9 pigs CSM and CHI were isolated at the same time. Three serovars (A, B and C) have been proposed by Roberts *et al.* [22] and Lawson *et al.* [11], and it has been reported that serovars B and C do not react with A antisera [12]. Strains of CSM isolated in this study and were inagglutinable with A antiserum may be

involved in serovar B or C, or in other serovars. Such strains referred to CSM- had a narrow range of growth temperature. Since many of the CSM+ strains grew well at 25°C and 43°C, it is presumed that the difference in temperature sensitivity depends on the serovars.

Strains of CHI isolated from 38 pigs had essentially the same characteristics as described by Gebhart *et al.* in 1983 [2]. The colonies on the selective agar were relatively smaller than that of CSM, and distinguishable from CSM and CCO by the difference in color tone.

Simultaneous isolation of CHI and CSM was made in 9 pigs, and that of with CCO was in 2 pigs.

Antibiotic susceptibility is apparently different between CSM and CHI. Gentamycin was most active against CSM and CHI. Their difference in antibiotic susceptibility may offer new tool for the identification of CSM and CHI.

Nine representative strains of CSM were subjected to cross agglutination test and it was demonstrated that there were four serologically different groups in addition to serovar A. An agglutinin absorption test of these antisera with cross reacting antigen is under study. Serologically, CSM and CHI were differentiated clearly. It is reported that CHI does not share specific antigens with CSM [1].

Results of testing of swine sera from three farms indicate that 21 in 45 pigs had high agglutinin titers against CSM. High agglutinin titer was obtained when CSM+ strain 77 which was isolated in farm A was employed as antigen. It is suggested that six heat labile surface antigens of serovar A which have been reported [10, 12] might be relevant to above mentioned results. Distribution of these heat labile antigens may be different between strain NKS and strain 77.

Widespread presence of CSM agglutinin

titers in slaughterhouse pigs is also reported by Lawson *et al.* [12]. Chang *et al.* reported that the majority of adult pigs from production herd had high agglutinin titers to CHI and CSM in the U.S. [1]. In our results, only 2 of 45 pigs had high agglutinin titer against CHI. It is suggested that the prevalence of CSM was more frequent than CHI in these farms. Although a survey to point out some reasons why the occurrence of PE was limited in these farms was attempted from the standpoint of animal husbandary sanitation, no special cause was disclosed.

Campylobacter species were not isolated from 13 normal gut examined. This result strongly supports the view that *Campylobacter* species recovered in this study are deeply related with etiopathogenesis of PE, however, the definitive etiologic agent of PE in swine is not proven yet. Medication of benzetimide (an antiperistaltic) allowed CSM infection to be established but there were no lesions [23], while oral dosage with *E. coli* and *Streptococcus bovis* merely caused an increase in the total number of CSM in the gut [16].

Very little is known about the factors that enhance the intracellular multiplication of *Campylobacter* in intestines. Viral infections or co-resident microorganisms have possibly deep concern with the elicitation of PE. In addition, since CSM, CHI and CCO were isolated from the same sample in several combinations, it is suggested that association of more than one species of *Campylobacter* might have some effects on the etiology of PE.

Further studies to reveal the significance of CSM or CHI on the etiology of PE are being carried out, considering factors responsible for the manifestation of PE.

ACKNOWLEDGEMENTS. We are grateful to the meat inspectors of the abattoir in Kagoshima City for supplying the samples.

REFERENCES

1. Chang, K., Kurtz, H. J., Ward, G. E., Gebhart, C. J. 1984. *Campylobacter* microagglutination tests of swine with proliferative enteritis. *Am. J. Vet. Res.* 45: 1373-1378.
2. Gebhart, C. J., Ward, G. E., Chang, K., and Kurtz, H. J. 1983. *Campylobacter hyointestinalis* (new species) isolated from swine with lesions of proliferative ileitis. *Am. J. Vet. Res.* 44: 361-367.
3. Hayashi, K., Naitou, Y., Ikehashi, A., Nishima, T., Ikumi, A., and Tanaka, K. 1982. Porcine intestinal adenoma complex detected in meat inspection. *J. Jpn. Vet. Med. Assoc.* 35: 449-453. (in Japanese).
4. Hwang, M., and Ederer, G. M. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. *J. Clin. Microbiol.* 1: 114-115.
5. Kubo, M., Okuda, H., Sugiyama, T., and Konno, S. 1979. A pathological study on adenomatous intestinal hyperplasia in the pig. *Natl. Inst. Anim. Health Q. (Jpn.)* 19: 134-135.
6. Kubo, M., Osada, M., Nakazawa, M., and Inui, S. 1982. Pathological study on proliferative hemorrhagic enteropathy. *Bull. Natl. Inst. Anim. Health* 84: 19-27 (in Japanese).
7. Kubo, M., Ohya, T., and Watase, H. 1984. Proliferative hemorrhagic enteropathy detected at an abattoir in Kagoshima. *Jpn. J. Vet. Sci.* 46: 413-417.
8. Lawson, G. H. K., and Rowland, A. C. 1974. Intestinal adenomatosis in the pig: A bacteriological study. *Res. Vet. Sci.* 17: 331-336.
9. Lawson, G. H. K., Rowland, A. C., and Roberts, L. 1976. Studies on *Campylobacter sputorum* subspecies *mucosalis*. *J. Med. Microbiol.* 9: 163-171.
10. Lawson, G. H. K., Rowland, A. C., and Roberts, L. 1977. The surface antigens of *Campylobacter sputorum* subspecies *mucosalis*. *Res. Vet. Sci.* 23: 378-832.
11. Lawson, G. H. K., Rowland, A. C., Roberts, L., Fraser, G., and McCartney, E. 1979. Proliferative hemorrhagic enteropathy. *Res. Vet. Sci.* 27: 46-51.
12. Lawson, G. H. K., Roberts, L., McCartney, E., Rowland, A. C., and Luckins, A. G. 1982. Presence of serum agglutinins to *Campylobacter sputorum* subspecies *mucosalis* in pigs. *Res. Vet. Sci.* 32: 89-94.
13. Lomax, L. G., and Glock, R. D. 1982. Naturally occurring porcine proliferative enteritis: Pathological and bacteriological findings. *Am. J. Vet. Res.* 43: 1608-1614.
14. Lomax, L. G., Glock, R. D., and Hogan, J. E. 1982. Experimentally induced porcine proliferative enteritis in specific-pathogen-free pigs. *Am. J. Vet. Res.* 43: 1615-1621.
15. Lomax, L. G., Glock, R. D., Harris, D. L., and Hogan, J. E. 1982. Porcine proliferative enteritis: Experimentally induced disease in cesarean-derived colostrum-deprived pigs. *Am. J. Vet. Res.* 43: 1622-1630.
16. McCartney, E., Lawson, G. H. K., and Rowland, A. C. 1984. Behaviour of *Campylobacter sputorum* subspecies *mucosalis* in gnotobiotic pigs. *Res. Vet. Sci.* 36: 290-297.
17. Mitsuka, T. 1980. A color atlas of anaerobic bacteria. 317-327, Sobunsha, Tokyo (in Japanese).
18. Nakazawa, M., Kubo, M., Sugimoto, C., Kikuchi, Y., and Isayama, Y. 1982. Isolation of *Campylobacter sputorum* subspecies *mucosalis* from proliferative hemorrhagic enteropathy in pig. *Jpn. J. Vet. Sci.* 44: 787-689.
19. Okuda, H., Sugiyama, T., Yabe, H., Yamada, Y., Nanba, F., Harada, H., Noya, H., Kubo, M., and Konno, S. 1981. An outbreak of proliferative hemorrhagic enteritis in swine. *J. Jpn. Vet. Med. Assoc.* 34: 584-589 (in Japanese).
20. Rajasekhar, M., Lawson, G. H. K., and Fraser, G. 1982. Infection of cell cultures by *Campylobacter sputorum* subspecies *mucosalis*. *Proc. Int. Pig Soc. (Mexico City, Mexico)*, 56.
21. Roberts, L., Rowland, A. C., and Lawson, G. H. K. 1977. Experimental reproduction of porcine intestinal adenomatosis and necrotic enteritis. *Vet. Rec.* 100: 12-13.
22. Roberts, L., Lawson, G. H. K., and Rowland, A. C. 1977. Porcine intestinal adenomatosis associated with serologically distinct *Campylobacter sputorum* subspecies *mucosalis*. *Res. Vet. Sci.* 23: 257-258.
23. Roberts, L., Lawson, G. H. K., and Rowland, A. C. 1980. The experimental infection of pigs with *Campylobacter sputorum* subspecies *mucosalis*. Weaned pigs, with special reference to pharmacologically mediated hypomotility. *Res. Vet. Sci.* 28: 148-150.
24. Rowland, A. C., and Lawson, G. H. K. 1975. Intestinal adenomatosis in the pigs: A possible relationship with hemorrhagic enteropathy. *Res. Vet. Sci.* 18: 263-268.
25. Rowland, A. C., and Lawson, G. H. K. 1975. Porcine intestinal adenomatosis: A possible relationship with necrotic enteritis, regional ileitis and proliferative enteropathy. *Vet. Rec.* 97: 178-180.

要 約

豚の増殖性腸炎から分離された *Campylobacter* 属菌について：大宅辰夫，久保正法，渡瀬 弘（家畜衛生試験場 九州支場）——鹿児島市食肉センターにおいて検出された豚の増殖性腸炎（PE）例から *Campylobacter* 属菌の分離を試みた。196例中93例（47.4%）から菌が分離された。菌量は腸管粘膜 1 g あたり $10^4 \sim 10^8$ CFU であり，80%以上の例では 10^6 CFU 以上であった。分離株339株は生化学的性状により *Campylobacter sputorum* ssp. *mucosalis* (CSM) *Campylobacter hyointestinalis* (CHI) および *Campylobacter coli* (CCO) の3菌種に分けられた。3菌種の分離状況は，CSMのみ分離51例，CHIのみ分離23例，CCOのみ分離7例，CSMおよびCCOの分離9例，CSMおよびCCOの分離1例，CHIおよびCCOの分離2例であった。CSMについては血清学的検討によりA型以外の4種の血清型の存在が示唆され，これらの菌株はA型に比べて発育温度域が狭く，テトラゾリウム塩の存在下で発育が抑制された。また，CSMとCHIは異なる抗生物質感受性パターンを示した。PEの検出は特定の養豚場からの出荷豚に集中し，屠殺時採取血清45例中21例がCSMに，2例がCHIに対して高い凝集素価を示した。