FULL PAPER *Pathology*

Relationship between Bacterial Translocation and Isolation of Enteric Bacteria from Extraintestinal Organs in Slaughtered Pigs

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(Received 6 April 2011/Accepted 11 July 2011/Published online in J-STAGE 25 July 2011)

ABSTRACT. Enteric bacteria, especially *Escherichia coli*, have been isolated from porcine livers affected with ascariasis. We hypothesized that reason for this bacterial isolation was due to bacterial translocation (BT). In order to clarify the association between ascariasis and isolation of *Enterobacteriaceae* in the livers,12 pigs with ascariasis (ascariasis group), 12 pigs without ascariasis (non-ascariasis A group) were used. Jejunum, ileum, cecum, colon, livers, mesenteric lymph nodes (MLN), and portal blood were obtained from these pigs. Furthermore, in order to confirm the presence of BT in pigs, the samples mentioned above as well as spleens were obtained from 11 pigs without ascariasis (non-ascariasis B group) and 6 specific pathogen free (SPF) pigs without ascariasis (non-ascariasis SPF group). In the first experiment, the Gram-negative bacteria were isolated from livers (66.7%), MLN (91.7%), and portal blood (55.6%) regardless the presence of ascariasis. In the second experiment, isolation rates of Gram-negative and -positive bacteria were 52.9 and 66.7% for livers, 52.9 and 80% for MLN, 11.8 and 26.7% for spleens, and 40 and 20% for portal blood of the pigs examined, respectively. *E. coli* and *Staphylococcus* were the predominant isolates from these pigs. A large number of antigens immunodetected by *E. coli* polyclonal antibody were found in the damaged cecal mucosa. These findings present evidence that BT is generally observed in slaughtered pigs regardless the presence of ascariasis. This has challenged our notion that extraintestinal organs of pigs are usually maintained as sterile.
KEY WORDS: ascariasis, bacterial translocation, enteric bacteria, gastrointestinal tract, swine.

J. Vet. Med. Sci. 73(12): 1553–1560, 2011

Microbes, suspected to be of intestinal origin, such as *Escherichia coli* [12, 13], *Lactobacillus* sp. [12], *Lactobacillus casei* [13], *Lactobacillus salivalius* [13], *Propionibacterium* sp. [12], *Propionibacterium acnes*, *Propionibacterium granulosum*, *Propionibacterium avidum* [15], *Enterococcus* sp. or *Streptococcus* sp. and *Staphylococcus* sp. [12] have been isolated from livers of pigs with ascariasis. John [10] suspected that the infective *Ascaris* larvae carried the intestinal bacteria from the intestine to the liver in the ascariasis pigs with the severe hepatic inflammatory lesions. However, these intestinal bacteria were also isolated from the site of normal hepatic parenchyma without milk-spotted lesions, which is characteristic to an ascariasis [12, 13, 15]. Furthermore immunohistochemical detection of antigens using polyclonal antibodies against non-transformed *E. coli* strain K12 C600 (DakoCytomation, Inc., Tokyo, Japan) did not show a relationship between the distributions of positive antigens and hepatic white spot lesions [12]. These findings have raised the possibility of other factors associated with the bacteria isolated from porcine livers affected with ascariasis.

Translocation from the gastrointestinal (GI) tract to the mesenteric lymph nodes (MLN) and other extraintestinal organs has been known for intestinal microbes, such as *Enterobacteriaceae*, *Pseudomonas*, *Enterococcus* or *Streptococcus* and *Staphylococcus*, and this phenomenon has been defined as bacterial translocation (BT) [2–4]. Although BT is caused by intestinal bacterial overgrowth, physical damage to intestinal mucosa, and defects in host immunity, these three factors is often interrelated to each other [2, 3]. So far two routes have been considered as main routes for the translocating bacteria. They are via lymphatics to MLN and directly via blood to the liver [2].

We hypothesized that the enteric bacteria isolated from livers from animals with ascariasis were due to BT, because there was no relationship between the hepatic lesions and the presence of enteric bacteria. In the present study we firstly investigated in order to clarify the difference between conventional fattening pigs with ascariasis and without ascariasis in regard to the isolation of *Enterobacteriaceae*. Secondly we performed pathological, immunohistochemical, and bacteriological investigations in order to confirm BT of Gram-negative and -positive enteric bacteria in slaughtered fattening pigs.

MATERIALS AND METHODS

Animals and experimental design: Experiment 1 in 2007 and 2008. In order to clarify whether there is the difference of *Enterobacteriaceae* isolation rates between conventionally raised fattening pigs with ascariasis and without ascariasis, 24 conventionally raised fattening pigs with ascariasis and without ascariasis were obtained from a slaughterhouse in Kanagawa Prefecture. Among them 12 were diagnosed to have ascariasis (ascariasis group) and 12 were without ascariasis (non-ascariasis A group). Ascariasis group and

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non-ascariasis A group were originated from 6 farms in 3 prefectures and 7 farms in 4 prefectures, respectively. Livers were sampled from all 24 pigs, but small and large intestines, MLN, and portal blood were obtained from 6 pigs each of two groups.

Experiment 2 in 2008 and 2009. In order to conduct detailed investigation of BT in slaughtered pigs in regard to isolation of facultative, anaerobic, and aerobic Gram-negative and -positive enteric bacteria, 11 conventional fattening pigs without ascariasis (non-ascariasis B group) and 6 fattening pigs raised as specific pathogen free (SPF) without ascariasis (non-ascariasis SPF group) were obtained from the same slaughterhouse in Kanagawa and a slaughterhouse in Toyama Prefecture. Non-ascariasis B and non-ascariasis SPF pigs were originated from 5 farms in 4 prefectures and one SPF farm in Toyama Prefecture, respectively.

Livers, small and large intestines, MLN, portal blood, and spleens were obtained from all 17 pigs.

After pigs were slaughtered, samples were obtained immediately and they were kept in cooler boxes. These samples of ascariasis, non-ascariasis A and B groups were transferred from the slaughterhouse in Kanagawa Prefecture to our laboratory. Non-ascariasis SPF group pigs were transferred from the slaughterhouse to the meat inspection center in Toyama Prefecture.

Histopathology: Liver, MLN, a middle portion of jejunum, distal ileum, cecum and colon were immediately fixed with 20% formalin, in parallel with macroscopic observation as well as sampling for bacteriological examination. Fixed tissues were then embedded in paraffin, sectioned by 3 to 4 μm thick, and stained with hematoxylin and eosin (HE). Selected sections were stained by the Gram's method.

Immunohistochemistry: In order to detect the translocation of enterobacteria to the intestines, and livers selected sections mentioned above were used in this analysis. With regard to the livers, the sections from 4 cases of livers which the number of Gram-negative enteric bacteria were isolated at more than $\log_{10}3.8$ in ascariasis group and non-ascariasis A group were selected for this analysis. After consecutive deparaffinization, sections were immunostained by mean of the streptavidin-biotin conjugate (SAB) method using a Histofine SAB-PO (R) Kit (Nichirei, Inc., Tokyo, Japan) following manufacturer's instructions. Rabbit anti-*E. coli* strain K12 C600 polyclonal antibody (DakoCytomation, Inc., Tokyo, Japan) and rabbit polyclonal antibody specific for single stranded-DNA (anti-ssDNA) antibody (DakoCytomation) were used as the primary antibodies. Sections using anti-ssDNA antibody were initially treated with 0.25% trypsin in order to activate the antigen. The treated sections and other non-treated sections were overlaid at 1:1,600 dilution of anti-ssDNA antibody, and 1:3,200 dilution of anti-*E. coli* antibody in 0.01 mol/*l* phosphate-buffered saline with 0.05% Tween 20. The sections were incubated overnight at 4°C in a humidified chamber. The sections were counterstained with Krazzi's hematoxylin. Specimens obtained from the same block were stained without these primary antibodies and used as negative controls. The sections of murine lung, which received direct injection of *E. coli* (IFO 3301, K12), were used as positive controls.

Electron microscopy: Small tissue blocks from the cecum, which contained mucous membrane accompanied by necrotic epithelial cells and immunodetected enteric bacteria, were used for the electron microscopic examination. Small pieces of paraffin blocks were immersed in xylen overnight. These deparaffinized samples were rinsed with ethanol, fixed using 1% osmium tetroxide, and embedded in epoxy resin (Epok 812, Okenshoji Co., Ltd., Tokyo, Japan). Ultrathin sections were stained with uranyl acetate and lead citrate.

Bacteriology: One block from each lobe of liver, except for caudal lobe and quadrate lobe, in total three blocks were aseptically obtained by burning the surface with heated spatula. MLN blocks were collected from pancreatico-duodenal lymph node (pd-ly), jejunal lymph node (j-ly), and ileocolic lymph node (ic-ly), one for each pig. These blocks were rinsed with 90% ethanol and the surface was lightly seared. After weighing these samples, 10% homogenate was made with 2% buffered peptone water. These homogenates were immediately used for bacterial analysis. The bacterial analyses were performed using two methods: In Experiment 1, 100 μl each of homogenates was evenly applied onto desoxycholate-hydrogen sulphide-lactose (DHL) agar plates (Eiken Inc., Tokyo, Japan). After incubation at 37°C overnight, the number of colonies was counted and the number of bacteria was calculated for 1 g of original tissue sample. If no enteric bacteria were detected on the DHL plates, each of 0.5 m*l* homogenate was added into 5 m*l* heart infusion broth, and then enrichment culture was carried out at 37°C overnight. A loopful of the heart infusion broth was streaked onto a DHL agar plate, and then incubated at 37°C overnight. In Experiment 2, the number of *Enterobacteriaceae* was estimated using the most probable number (MPN) technique. The homogenates were divided into 3 aliquots (1 m*l*, 0.1 m*l*, and 0.01 m*l* each in volume), which were diluted with brilliant green lactose bile broth (BGLB) (EikenInc, Tokyo, Japan), and then these aliquots were incubated at 37°C for 48 hr. The detections of *Enterobacteriaceae* were confirmed by gas accumulations in Durham tube. The number of bacteria was calculated for 1 g of original tissue sample. After incubation, a loopful of the broth culture was streaked onto a DHL agar plate. In addition, 100 μl each of homogenate, except samples of two pigs in the non-ascariasis B group, was evenly applied onto 5% horse blood agar (BA) plates for the detection of facultative, anaerobic, and aerobic Gram-positive enteric bacteria. Both plates were incubated at 37°C overnight.

Aseptically obtained portal blood samples (0.5 m*l*) were inoculated into a culture bottle of Versa TREK REDOX 1 EZ DRAW™ with Stir Bar for aerobic broth (Trek Diagnostic Systems Inc., Cleveland City, OH, U.S.A.). The culture bottles were incubated at 37°C overnight. After incubation, a loopful of broth culture was streaked onto a DHL agar in Experiment 1, whereas onto a DHL agar as well as BA

plates in Experiment 2, and then all plates were incubated at 37°C overnight. In addition, spleen samples were also cultured at 37°C overnight using MPN technique and BA plates mentioned above.

Isolates from DHL agar plates further underwent biochemical examinations in order to identify *Enterobacteriaceae*. Isolates suspected to be *Salmonella* were serotyped by the slide agglutination test using anti-*Salmonella* antisera (Denka Seiken, Tokyo, Japan) to determine the presence of *Salmonella* specific somatic O antigens. The bacteriological species except for *Salmonella* were identified using kits api 20E or api 20NE following manufacturer's instructions (Nippon BioMerieux, Tokyo, Japan).Gram-positive bacteria were identified based on their morphology, catalase and oxidase productions. The genus *Staphylococcus* was examined for biochemical properties: coagulase and deoxyribonuclease products, the resolution of mannitol, and the isolation from egg-yolk salt agar in order to differentiate *S. aureus* from coagulase negative *Staphylococcus*.

Statistical analysis: Histopathological findings were graded from 1 to 4 according to the degree of intestinal mucosal lesions (Table 1). Statistical analysis was conducted using unpaired Mann-Whitney U-test with Bonferroni correction using the software ystat 2006 [18]. The numbers of isolates from liver and MLN of ascariasis and non-ascariasis A groups were statistically evaluated using the same multiple comparison analysis. Probabilities less than 0.05 were considered significant.

RESULTS

Pathological findings: Macroscopical lesions in twelve ascariasis livers were classified into two types: multiple compact white spots often accompanied with petechial hemorrhages at the center (41.7%); multifocal mesh-worked white spots of irregular shape often with lymphonodular white spots (58.3%). The histopathological hepatic lesions in ascariasis group were characterized as eosinophilic interstitial hepatitis accompanied by connective tissue proliferation with varying degree of milk-spotted lesions, but larva of *Ascaris suum* was not found in these hepatic tissues. Livers from non-ascariasis groups did not show any microscopic changes.

Although there were no macroscopic changes in small and large bowels in all groups, histopathological examination of the mucosal membranes revealed variable degrees of epithelial cell desquamation, pyknosis, karyorrhexis and strong eosinophilic staining of epithelial cells accompanied by edematous laminapropria (Fig. 1A). In the cecum edematous lamina propria was exposed due to denuded necrotizing epithelial cells (Fig. 1C). Gram-negative rod type bacteria were clearly detected on the surface of the necrotic epithelial cell layers, while the presence of Gram-positive bacteria was not so clear on the cell layers. These lesions were more prominent in the cecum than the other intestinal regions. Electromicroscopic examination revealed Gram-negative bacteria, which were passing through cecal epithelial cell tight junction (Fig. 2). The degrees of intestinal mucosal lesions were graded one to four. The distributions of the grades in ascariasis and non-ascariasis groups are shown in Table 1. Other lesions included suppurative ileitis infected with *Balantidium* in 4 pigs of ascariasis group and 3 pigs of non-ascariasis groups. In MLN, mild swelling was macroscopically seen in 51.7% of 29 pigs sexamined. The histological changes were the enlargements of the lymphoid follicles accompanied with starry sky appearances.

Immunohistochemical findings: The antigens detected by immunohistochemistry were distributing from the surface of mucosal epithelial cell layer (Fig. 3) to the mucosal lamina propria (Fig. 1B and 1D) in the intestinal mucosal tissues examined. The antigens were not detected in the suppurative mucosal lesions infected by *Balantidium*. In the livers, the antigens were detected in the hepatic sinusoids of 2 ascariasis pigs (Fig. 4A), and one non-ascariasis A pig (Fig. 4B). The nuclei of the mucosal epithelial cells, revealed pyknosis and karyorrhexis, were immunolabeled with anti-ssDNA antibody. The detection rates using anti-*E.coli* polyclonal antibody in the intestines of ascariasis and non-ascariasis groups are presented in Table 1.

Bacteriological findings: The data of *Enterobacteriaceae* and the other Gram-negative enteric bacteria isolated from MLN, extraintestinal organs, and portal blood in Experiments 1 and 2 are summarized in Tables 2 and 3. In Experiment 1, these bacteria were isolated from the livers (66.7%), MLN (91.7%), and the portal blood (55.6%) regardless the presence of ascariasis, and the isolation rate

Table 1. The degree of intestinal mucosal lesions and the distribution of positive antigens detected by anti-*E. coli* polyclonal antibody in the intestinal mucosa (n=29 pigs)

Mucosal lesion	Grade	Ascariasis group (n=6)				Non-ascariasis group (n=23)			
		Jejunum	Ileum	Cecum	Colon	Jejunum	Ileum	Cecum	Colon*
Normal	1	6**	2	0	1	23	18	3	7
Localized necrosis in epithelial cell layer	2	0	3	2	4	0	4	14	6
Widespread necrosis in epithelial cell layer and subepithelial edema	3	0	0	3	1	0	1	5	6
Necrosis both in epithelial cells and subepithelial lamina propria	4	0	1	1	0	0	0	1	0
Detection rates of the antigens (%)		0 (0)	1 (16.7)	5 (83.3)	3 (50.0)	0 (0)	10 (43.5)	18 (78.3)	2 (10.5)

*: Four pigs were not examined. **: Total number of pigs.

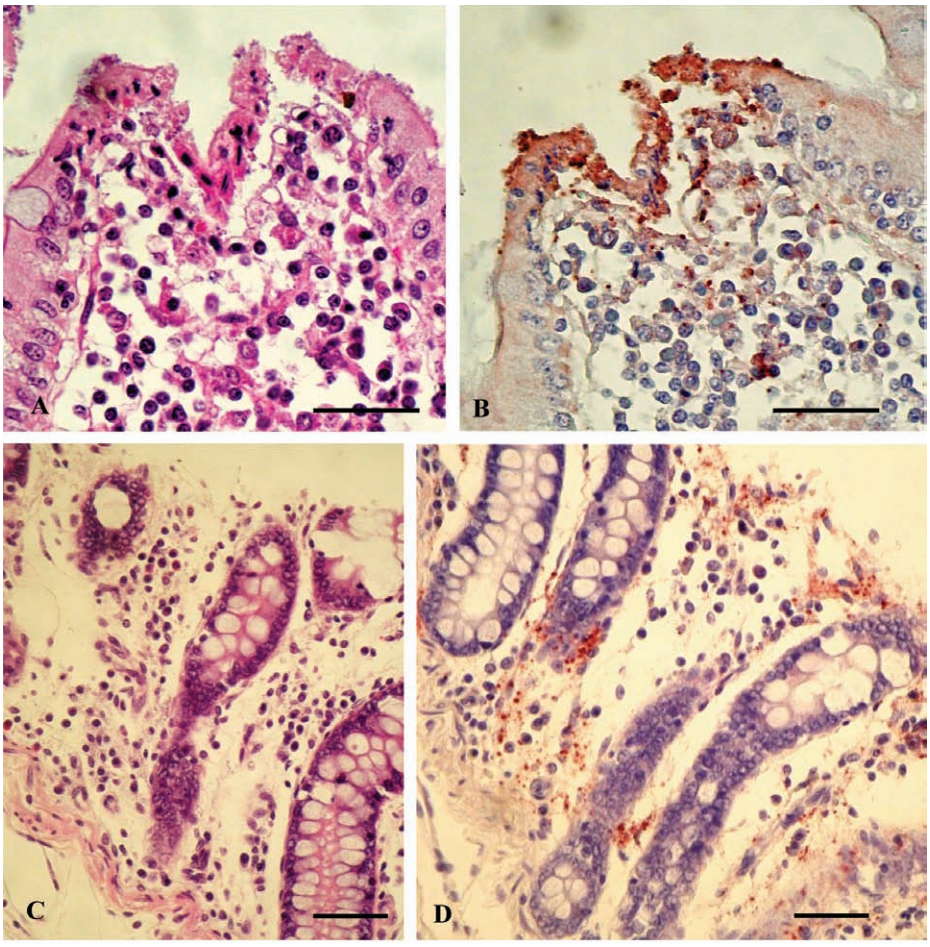

Fig. 1. A: Epithelial cell necrosis and mild subepithelial edema in the cecum. HE. Bar=25 μm. B: Some-immuno-positive antigens for anti-*E. coli* polyclonal antibody detected in the necrotic and edematous area in the serial section of A. Bar=25 μm. C: Edematous lamina propria exposed due to denuded epithelial cell layer in the cecum. HE. Bar=25 μm. D: A large number of immuno-positive antigens for anti-*E. coli* polyclonal antibody detected in the edematous area in the serial section of C: Bar=25 μm.

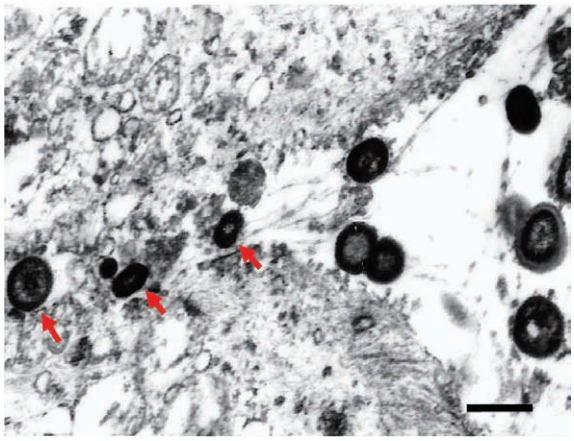

Fig. 2. Transmission electron micrograph of Gram-negative bacteria (arrows) passing through the cecal epithelial cell tight junction. Bar=1 μm.

for each group was 83.3 and 100% for ascariasis, and non-ascariasis A groups, respectively. In Experiment 2, Gram-negative bacteria were isolated from 16 pigs (94.1%) except for one pig of non-ascariasis B group. The isolation rates of Gram-negative bacteria were 52.9% for MLN, 52.9% for the livers, 11.8% for the spleens, and 40% for the portal blood of 17 pigs examined. For MLN the bacterial isolation rate from ic-ly in each group was the highest among 3 locations of MLN in the both experiments. The species of isolated *Enterobacteriaceae* were *Burkholderia cepacia, Citrobacter koseri/amalonaticus, Escherichia coli, Escherichia fergusonii*, *Escherichia vulneris, Enterobacter aerogenes*, *Enterobacter cloacae, Klebsiella oxytoca*, *Salmonella* sp. serotye O4, *Pantonea* sp., and *Yersinia enterocolitica.* The other aerobic isolates were *Acinetobacter baumannii, Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia, Pasteurella pneumotropica* and non-identified Gram-negative bacteria. Among isolates, *E. coli*

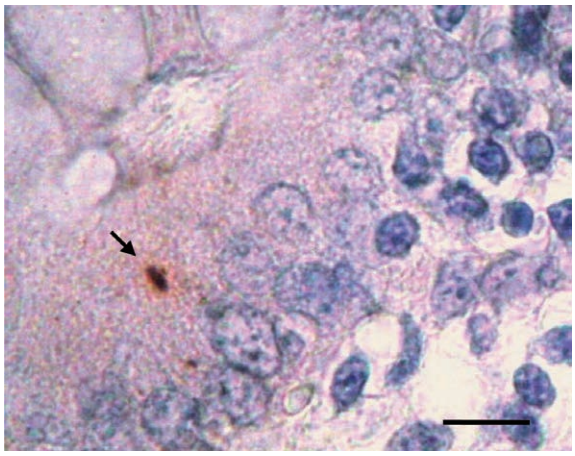

Fig. 3. Bacterial antigens in the epithelial cell layer (arrow) in the cecum immunodetected by streptavidin-biotin conjugate method using anti-*E. coli* polyclonal antibody. Bar=10 μm.

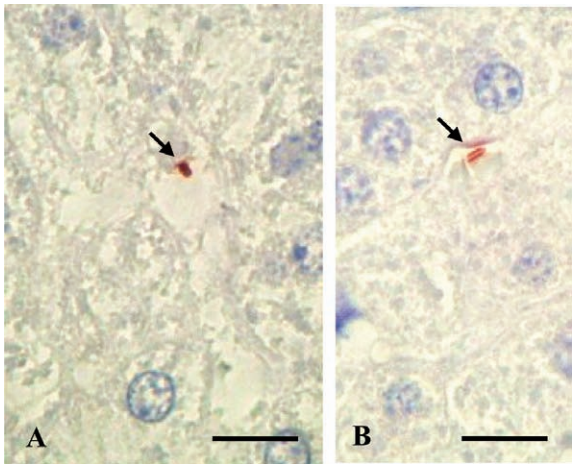

Fig. 4. The bacterial antigens (arrows) in hepatic sinusoid immunodetected by streptavidin-biotin conjugate method using anti-*E. coli* polyclonal antibody. A: Liver of ascariasis pig. Bar=10 μm. B: Liver of non-ascariasis A pig. Bar=10 μm.

was the most frequently isolated from 30 out of 41 pigs (73.2%) examined: 11 pigs (91.7%) of ascariasis group, 14 pigs (60.9%) of non-ascariasis A and B groups, and 5 pigs (58.3%) of non-ascariasis SPF group, respectively.

The isolation rates of facultative anaerobic, and aerobic Gram-positive enteric bacteria in Experiment 2 are summarized in Table 4. These bacteria were isolated from MLN (80%), the livers (66.7%), the spleens (26.7%), and the portal blood (20%) of the 15 pigs examined. Among them, isolation rate of the genus *Staphylococcus* was higher than other genus with isolation rate 86.7% (13/15 pigs).

In the pigs with the cecal mucosal lesions more than grade 2, isolation rates of Gram-negative and -positive enteric bacteria from livers and portal blood were higher than those from MLN: Gram-negative bacteria isolation rates of livers and portal blood, and MLN was 55.2% (16/29 pigs) and 24.1% (7/29 pigs), respectively; Gram-positive bacteria isolation rates from those same samples mentioned above was 66.7% (10/15 pigs) and 33.3% (5/15), respectively.

Statistical findings: Although there was no statistical difference in the grades of mucosal lesions between intestinal sites of ascariasis group, the significant difference was found in non-ascariasis groups: jejunum vs. cecum (P>0.01), ileum vs. cecum (P>0.01), and jejunum vs. colon (P>0.01). There were no statistical differences in the number of isolates between the two groups.

DISCUSSION

The liver lesions of ascariasis group were characterized macroscopically as well as histopathologically and determined to be caused by *Ascaris suum*. Gram-negative bacteria were not only isolated from livers, MLN, and portal blood regardless the presence of ascariasis, but also Gram-positive bacteria, *Enterococcus*, *Streptococcus* and *Staphylococcus*, were isolated from livers in Experiment 2. Furthermore, the bacterial antigens in these livers were confirmed by immunohistochemistry using anti-*E. coli* polyclonal antibody. Thus, it is reasonable to conclude that the presence of enteric bacteria in the livers was due to BT rather than that the bacteria were carried by larval migration of *Ascaris suum*.

Immunohistochemically, the antibody used in this study could detect not only *E. coli* but also several *Enterobacteriaceae* to the similar degree. *E. coli* (IFO 3301 K12), *Klebsiella pneumoniae* (ATCC 12658), *Enterobacter cloacae* (ATCC 13047) and *Citrobacter freundii* (ATCC 8090) were immunolabeled similarly using this anti-*E. coli* antibody (unpublished data). Thus, detection of *E. coli* in this study may not be accurate. However, there is a high possibility that immunodetected antigens might be *E. coli*, because among *Enterobacteriaceae*, *E. coli* was isolated at a greater ate from the samples examined. These and the present electron microscopic findings agree with the definition of BT [2, 3, 6].

It is well known that the postmortem changes in intestine occur very rapidly. However, necrosis distributed from epithelial cell layer to subepithelial lamina propria could be attributed to lesions existed prior to the slaughter, because increase of necrosis in intestine was not found in pigs [7] and cattle [11] when time course changes in necrosis were examined up to 4 hr after being slaughtered. It is known that nuclei of necrotic and apoptotic cells are immunolabeled with anti-ssDNA antibody [9]. In the cecum, the epithelial cell necrosis and the detection rates of antigens were more abundant and higher compared to other intestinal regions regardless ascariasis. Furthermore, the Gram-negative bacteria isolation rate from ic-ly was higher than pd-ly and j-ly within the same group. Thus, cecum seems to be the preferred site of tissue penetration for *Enterobacteriaceae* in the slaughtered pigs. This finding is similar to that of Wells and Erlandsen [17] who used germ-free mice to establish

Table 2. The isolation rates and mean bacterial counts of facultative, anaerobic, and aerobic Gram-negative enteric bacteria from the livers, MLN and portal blood, and species of the isolated bacteria from these samples in the ascariasis and the non-ascariasis A groups

Group		Positive pig numbers/ the number of pigs examined (%)	Mean bacterial counts [$\log_{10}$ colony forming units (CFU) per one g sample]	*Enterobacteriaceae*			Other Gram-negative bacteria*	Non-identified bacteria
				Citrobacter	*Escherichia*	*Enterobacter*		
Ascariasis								
MLN	pd-ly	2/6 (33.3)	1.6**		2***	1		1
	j-ly	1/6 (16.7)	1.6		1			
	ic-ly	4/6 (66.7)	1.7		3			2
Liver		8/12 (66.7)	2.3		8		5	7
Portal blood		3/4 (75)	NT		3			
Non-ascariasis A								
MLN	pd-ly	3/6 (50)	1.7		3		1	3
	j-ly	3/6 (50)	1.6		1			3
	ic-ly	6/6 (100)	1.9		6			4
Liver		8/12 (66.7)	2	3	7	4		4
Portal blood		2/5 (40)	NT		2			

*: The isolated bacterial genus were *Pseudomonas, Stenotrophmonas* and *Acinetobacter* from ascariasis group, and were *Pseudomonas* from non-ascariasis A group. **: The minimal number of isolated bacteria was calculated as 1.6, even if the bacteria were not isolated from the sample. ***: Total number of pigs. NT: Not tested.

Table 3. The isolation rates and mean bacterial counts of facultative, anaerobic, and aerobic Gram-negative enteric bacteria from livers, MLN and portal blood, and species of the isolated bacteria from these samples in the non-ascariasis B and the non-ascariasis SPF groups

Group		Positive pig numbers / the number of pigs examined (%)	Mean bacterial counts [most probable number (MPN) for one g original tissue sample]	*Enterobacteriaceae*			Non-identified bacteria
				Enterobacter	*Escherichia*	*Salmonella*	
Non-ascariasis B							
MLN	pd-ly	0/7 (0)	<3				
	j-ly	0/11 (0)	<3				
	ic-ly	4/11 (36.4)	18.4		2*		2
Liver		7/11 (63.6)	47.5		2		5
Portal blood		4/9 (44.4)	NT				4
Spleen		0/11 (0)	<3				
Non-ascariasis SPF							
MLN	pd-ly	2/6 (33.3)	9.8		1	1	
	j-ly	2/6 (33.3)	10.2			2	
	ic-ly	5/6 (83.3)	210.3		5	2	
Liver		2/6 (33.3)	3.3	1		1	4
Portal blood		2/6 (33.3)	NT	1	1		1
Spleen		2/6 (33.3)	9.9		1		1

*: Total number of pigs. NT: Not tested.

monoassociated mice colonized either by *E. coli*, *Proteus mirabilis*, and *Enterococcus faecalis*.

The antigens were not detected in inflammatory lesions infected with *Balantidium*. This suggests that *Enterobacteriaceae* do not translocate from the foci simply through localized intestinal mucosal damages.

If the intestinal epithelial cell layers are not physically damaged, as seen in the case of intestinal bacterial overgrowth, the enteric bacteria translocate through the epithelial cells (intracellularly), subsequently the bacteria travel via the lymphocytic vessels from lamina propria to MLN. Furthermore, when the translocating bacteria were not able to eradicate by the immunodefenses in MLN, the bacteraspread from MLN to other extraintestinal organs [2, 3]. On the other hand, when the epithelial cell layers are denuded by intestinal permeability and actual mucosal damage, as seen in the case of endotoxic or hemorrhagic shock, the bacteria can spread directly via portal vain to the liver [2, 3]. Thus, the present results suggested that a number of enteric bacteria isolated from the livers have translocated via the blood directly from the mucosal lesions rather than via the MLN.

Spontaneous BT occurs continuously at a low level in healthy animals and humans [14]. But translocating bacte-

Table 4. The isolation rates of facultative, anaerobic, and aerobic Gram-positive enteric bacteria from livers, MLN, portal blood and spleens, and species of the isolated bacteria from these samples in the non-ascariasis B and the non-ascariasis SPF groups

Group		Positive pig numbers / the number of pigs examined (%)	*Bacillus*	*Enterococcus* or *Streptococcus*	*Staphylococcus aureus*	coagulase negative *Staphylococcus*	Non-identified bacteria
Non-ascariasis B							
MLN	pd-ly	1/5 (20)				1*	
	j-ly	5/9 (55.6)	1		1	4	
	ic-ly	3/9 (33.3)	1	1	1	1	
Liver		8/9 (88.9)	3	1		5	3
Portal blood		3/9 (55.6)	3				
Spleen		3/9 (33.3)				3	
Non-ascariasis SPF							
MLN	pd-ly	4/6 (66.7)	1			3	
	j-ly	2/6 (33.3)	1			1	
	ic-ly	3/6 (50)	2			1	
Liver		2/6 (33.3)	1	1		2	
Portal blood		0/6 (0)					
Spleen		1/6 (16.7)				1	

*: Total number of pigs.

ria are essentially cleared by the reticuloendothelial system in healthy immunocompetent host; consequently MLN and extraintestinal organs are usually sterile [3]. However, our findings in the current study have challenged our notion that extraintestinal organs of conventional pigs are usually maintained as sterile. According to the review by Berg [2, 3], the incidence of BT from the MLN to extraintestinal organs was observed when the host immune system is compromised.

Several factors have been known to cause BT, such as; overgrowth of *Enterobacteriaceae* due to decreased cecal populations of indigenous obligate anaerobes after administration of antibiotics [1], intestinal mucosal damage in the ileum and the cecum associated with small amount of endotoxin [8], host immunodeficiency [5], and intestinal damage due to generation of hydrogen peroxide during early phase of hemorrhagic shock [16]. The reason for prevalence of BT observed in slaughtered pigs may be associated with similar factors mentioned above, such as poor feeding management, immunological disorders due to infectious agents, transport stress, and hemorrhagic shock at slaughter. Therefore, BT may reflect a weak immune function in pigs. It is very important to keep pigs healthy in order to secure food safety. Further research is needed to identify the exact causes of BT in order to decrease this incidence.

ACKNOWLEDGMENTS. We are grateful for Drs. T. Tani and T. Shimizu, Surgery Department, School of Medicine, Shiga University of Medical Science, and also for Dr. K. Kimura, Veterinary Microbiology and Preventive Medicine, Iowa State University, U.S.A., for helpful advices. We would like to thank Mr. K. Nishimura, Kanagawa Meet Center, for his collaboration of this study. We are also thankful for students of our school, for their technical supports.

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