Jpn. J. Infect. Dis., 54, 23-26, 2001

Original Article

Prevalence of *Cryptosporidium parvum* Infections in Weaned Piglets and Fattening Porkers in Kanagawa Prefecture, Japan

Shinji Izumiyama, Ichiro Furukawa[1], Toshiro Kuroki[1], Shiro Yamai[1], Hiromu Sugiyama[2], Kenji Yagita[2] and Takuro Endo[2]*

Tsukuba Technical Center, Ikeda Scientific Co. Ltd., Oaza-Akatsuka Aza-Ushigafuchi 586-9, Tsukuba, Ibaraki 305-0062,
[1]Department of Bacteriology and Pathology, Kanagawa Prefectural Public Health Laboratory, Nakao-machi 52-2, Asahi-ku, Yokohama, Kanagawa 241-0815 and
[2]Department of Parasitology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan

(Received February 15, 2001. Accepted March 8, 2001)

SUMMARY: Fecal samples from 232 weaned piglets (1 and 3 months old) and 252 fattening porkers (6 months old) in 8 stock-raising farms located in Kanagawa Prefecture, Japan, from June 1998 to June 2000 were examined to determine the prevalence of *Cryptosporidium* infection. Detection of oocysts was performed using the ethyl acetate fecal concentration method and immunofluorescent staining. *C. parvum* oocysts were identified in 77 (33.2%) 1-3 months old weaned piglets from four farms. The odds of excreting among 1-3 months old piglets were more than 100 times greater than among 6 months old porkers (95% confidence interval: 17-902). This strongly suggests that weaned piglets are important reservoirs of pathogenic microbes whose potential contamination of drinking water has epidemiological implications for human health.

INTRODUCTION

The increasing world population has led to increasing fresh water requirements for drinking, hygiene and household purposes. Population growth also brings in its train high loads of municipal sewage, livestock excreta and industrial wastewater which threaten the sources of drinking water. While water treatment technology can successfully process water from poor quality sources, thereby producing potable water that meets the accepted drinking water standards, there is still serious concern that the barrier to microbial breakthrough in the finished water may fail to measure up to our expectations. Any momentary or intermittent break(s) in the water treatment chain could allow substantial levels of pathogenic microbes to enter potable water–the final product. The recent large-scale outbreaks of *Cryptosporidium* in many industrialized countries have demonstrated that conventional drinking water treatment may not always be adequate to prevent waterborne disease transmission (1). To date, there have been two waterborne outbreaks due to *Cryptosporidium* infection reported in Japan. The first took place in Hiratsuka city in August and September of 1994 and consisted of 461 diarrhea cases (2). The other occurred at a small town in Saitama Prefecture approximately 50 km northwest from Tokyo, in early June and mid-July of 1996 and consisted of 8,812 patients who met the case definition (3). The unusual resistance of oocysts such as *Cryptosporidium* to chlorine-based disinfectants (4) allows for penetration of viable oocysts into the treated water supply.

Since the first report of cryptosporidial infections in pigs in 1977 (5,6), porcine cryptosporidiosis has been reported in various locations worldwide. However, due to the generally accepted opinion that *C. parvum* is not a serious cause of enteritis in either neonatal or weaned piglets, only a limited number of epidemiological studies have yet been reported, making it difficult to know the prevalence of infection in many countries including Japan. As distinct from the case of cryptosporidiosis in ruminants, wherein infection is generally established during the first 2 weeks after birth (7,8), cryptosporidiosis in pigs has been reported to be delayed until after weaning. *Cryptosporidium* infection, at the same time, has been detected in pigs ranging from 1 to 30 weeks old by histological examinations in Canada (9).

In the present study, the prevalence of *Cryptosporidium* infection in asymptomatic, 1-3 months old weaned piglets and 6 months old fattening porkers, the manure of which has been recognized to be one of the major sources of contaminating surface water in Japan, was investigated in the central part of Japan.

MATERIALS AND METHODS

Fecal specimens: Fecal samples were obtained at the slaughterhouse from 232 weaned piglets (1 and 3 months old) and 252 fattening porkers (6 months old) in 8 stock-raising farms located in Kanagawa Prefecture, Japan, from June 1998 to June 2000 (Table 1). One of the stock-raising farms (Farm A) is located near a tributary of the Sagami river system. Pigs were assigned to one of three age categories: 1, 3 and 6 months according to information provided by the farmers. Intestinal (fecal) samples were collected either from the area between the cecum to the upper part of the colon of the piglets or from the rectum of the porkers at sacrifice. Cryptosporidial oocysts were detected in fecal samples from both the cecum/colon and the rectum of infected animals. Fecal samples were stored

*Corresponding author: Tel: +81-3-5285-1111, Fax: +81-3-5285-1173, E-mail: tendo@nih.go.jp

Table 1. A list of samples examined in the present study

Farm	Group (age)	No. sampled
A	1-3 months old	148
B		24
C		48
D		12
Subtotal		232
A	6 months old	187
E		40
F		6
G		12
H		7
Subtotal		252
Total		484

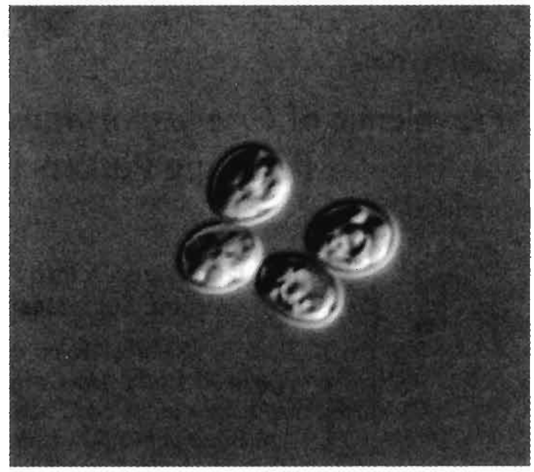

Fig. 1. Differential interference contrast image of oocysts of *Cryptosporidium parvum*.
Cryptosporidial oocysts obtained from fresh feces were fully sporulated with four sporozoites, either spherical or ovoid, with diameters of 4.5-5.4 × 4.2-5.0 μm.

in a refrigerator until examination.

Analysis of *C. parvum* oocysts: Detection of oocysts was performed using the formalin ethyl acetate (FEA) centrifugal concentration method, followed by immunofluorescent staining. Briefly, the intestinal content (fecal) sample was diluted twice with water, mixed thoroughly, and filtered with double-sheeted gauze to remove large debris. The filtrate ($\leqq$ 2 ml) was diluted to 10 ml with water, supplemented with 3 ml of ethyl acetate and mixed thoroughly by shaking vigorously in a screw-capped centrifuge tube. It was immediately centrifuged at 3,000 rpm for 10 min. The sediment was collected, resuspended with a small amount of water ($\leqq$100 μl), smeared onto a slide glass and dried. The smear was then fixed with methanol and stained using a direct immunofluorescent staining kit, FITC-conjugated mouse monoclonal antibody specific for *C. parvum* oocyst (MELIFLUOR®, Meridian, Ohio, USA). The stained specimen was examined using fluorescent microscopy with the excitation wavelength at 450-490 nM.

Statistical analysis: In the present study we examined demographic and environmental factors potentially associated with the excretion of *C. parvum* oocysts in the feces of 1-6 months old pigs. These included age of the animal, geographic location (farms) and month of fecal collection. The distribution of oocyst infection in pigs was calculated for each variable; the associated odds ratios for levels within each variable were calculated using two-sided 95% confidence intervals (CI). Differences in proportions observed were compared by Fisher's exact test or χ2-test, and probability values less than 0.05 were considered significant.

RESULTS

The study was undertaken to determine the prevalence of *C. parvum* oocysts infection in pigs with respect to the age of the animal, locations (farms) and month of fecal collection. Fresh fecal samples were collected from 232 weaned piglets (1 and 3 months old), most of which were specially produced for Chinese restaurants, and from 252 porkers at 6 months of age, the body weights of which were approximately 100 kg.

The oocysts detected by immunofluorescent staining were confirmed to be *C. parvum* by their morphological features, including the size, shape, and internal structure of oocysts, and the thickness of the oocyst wall, as observed under differential interference optics (Fig. 1).

Infection with *C. parvum* oocysts was associated with age

Table 2. Prevalence and crude odds ratio for excretion of *Cryptosporidium parvum* oocysts in porkers

Factor		Prevalence of *C. parvum* oocysts (%)	Crude odds ratio (95% CI)	*P* value
Weaned piglets	Farm A	52/148 (35.1%)	2.7 (0.6-12.8)	ns**
	B	10/24 (41.7%)	3.6 (0.6-20.0)	ns
	C	13/48 (27.1%)	1.9 (0.4-9.6)	ns
	D	2/12 (16.7%)	1.0 *	
	Age (month)			
Weaned piglets	1	69/213 (32.3%)	89.6 (12.3-652.9)	<0.001
	3	8/19 (42.1%)	136 (15.61-186.4)	<0.001
Fattening porkers	6 (Farm A)	1/187 (0.5%)	1.0 *	
Weaned piglets	1-3	77/232 (33.2%)	125.2 (17.2-909.2)	<0.001
Fattening porkers	6	1/252 (0.4%)	1.0 *	

*Referent category for the odds of excreting *C. parvum* oocysts.
**Not significant.

Table 3. Point prevalence and crude odds ratio for excretion of *Cryptosporidium parvum* oocysts according to month of fecal collection in weaned piglets on Farm A

Month of sample acquisition		Prevalence of *C. parvum* oocysts	Crude odds ratio	*P* value
1999	Jul	6/20 (30.0%)	1.0 *	
	Aug	8/12 (66.7%)	4.7 (1.0, 21.7)	<0.05
	Sept	1/8 (12.5%)	0.3 (0.03, 3.3)	ns**
	Oct	5/8 (62.5%)	3.9 (0.7, 21.7)	ns
	Nov	4/8 (50.0%)	2.3 (0.4, 12.6)	ns
	Dec	9/12 (75.0%)	7.0 (1.4, 35.3)	<0.02
2000	Jan	2/8 (25.0%)	0.8 (0.1, 5.0)	ns
	Feb	1/12 (8.3%)	0.2 (0.02, 1.9)	ns
	Mar	0/20 (0%)	0.0	ns
	Apr	9/20 (45.0%)	1.9 (0.5, 7.0)	ns
	May	5/12 (41.7%)	1.7 (0.4, 7.4)	ns
	Jun	2/8 (25.0%)	0.8 (0.1, 5.0)	ns

* Referent category for the odds of excreting *C. parvum* oocysts.
** Not significant.

of pigs but not geographic location or month of fecal collection. The overall prevalence of infection with *C. parvum* oocysts among weaned piglets from four different farms was 33.2% (77/232), ranging from 16.7% (2/12) to 41.7% (10/24). Point prevalences of infection among 1 month and 3 months old weaned piglets were 32.3% (69/213) and 42.1% (8/19), whereas that among 6 months old fattening porkers was 0.3% (1/252), ranging from 0% to 0.5% (1/187) (Table 2). The age of animals was significantly associated with the odds of infection by *C. parvum* oocysts. The odds of infection among 1 and 3 months old piglets were approximately 90 to 136 times greater than that among 6 months old porkers from Farm A, where 95% CI were 12.3-652.9 and 15.6-186.4, respectively (Table 2). The odds ratio calculated between 1 and 3 months was 0.6 (95% CI: 0.3 to 1.7), suggesting that the categories of 1 and 3 months of age could be combined into one category. Although there were slight differences in point of prevalence of fecal excretion of *C. parvum* among weaned piglets on different farms (Farms A-D), the odds ratio of excretion of oocysts among piglets on Farm B was 3.6 (95% CI: 0.6 to 20.0) times greater than that among piglets on Farm D.

To evaluate the oocyst excretion in feces with respect to month of fecal collection, the prevalence of infection among weaned piglets on one of the raising farms (Farm A) was measured monthly from July 1999 to June 2000 (Table 3). The point prevalence of oocyst infection fluctuated according to the month of fecal collection, ranging from 0% in March of 2000 to 75.0% in December of 1999. The odds of *C. parvum* infection among piglets tested in August and December of 1999 were 4.7 and 7.0 times greater, respectively, than among piglets tested in June 1999.

DISCUSSION

Detecting the primary sources of *C. parvum* in the environment will be the first step toward watershed management as a strategy to protect source water and, ultimately, drinking water quality from this parasitic contamination. As in many industrialized countries, in Japan there are an abundance of pig- and cattle-raising farms in mountainside areas in close proximity to rivers, and the manure from such livestock can contaminate, directly or indirectly, the watershed with *C. parvum* oocysts.

Porcine cryptosporidiosis, first reported in 1977 (5,6), has since been found in many countries around the world. Pigs ranging from 1 to 30 weeks have been shown to excrete *Cryptosporidium* oocysts in Canada (9). Based on examination of rectal swabs, 10 of 200 (5%) market pigs in California were found to excrete oocysts in their feces (10). In other studies, *C. parvum* infection has been reported to occur in pigs of all age groups, but in ruminants only before weaning (7,8). In the present study, 1-3 months old piglets were shown to excrete *C. parvum* oocysts in their feces. On Farm A, excretion of oocysts was detected in an overall average of 35.1% of animals, ranging from 0% in March of 2000 to 75% in December of 1999. The odds of *C. parvum* infection were significantly greater in 1-3 months old piglets than in porkers of 6 months old, whereas there was no significant difference in odds of infection between 1 month and 3 months old piglets. Other epidemiological surveys have also reported that infection is prevalent in recently weaned piglets and at the first stages of fattening; the rates were 32.6% in Nigeria (11), 19.9% in Korea (12), 41.5% in Trinidad and Tobago (13) and 59.2% in Spain (14). On the other hand, management of farm operations has been shown to influence the course of cryptosporidial infection in both nursing and weaned piglets (15).

In summary, the results obtained in the present study demonstrate that *Cryptosporidium* infection is prevalent in weaned piglets of 1-3 months old in Japan, supporting the idea that cryptosporidiosis is prevalent among young farm animals. It is thus not unreasonable to assume that weaned piglets, together with other young farm-animals, are the major reservoirs and transmission sources of this microbe, a conclusion with major epidemiological implications for humans due to potential contamination of drinking water. The finding that *Cryptosporidium* oocysts have contaminated drinking water sources suggests that there is hydrologic connection between livestock manure and such water bodies. And in fact, our recent epidemiological finding that a larger number of *Cryptosporidium* oocysts occurred in tributaries running through catchment areas where many hoggeries are located might lend circumstantial evidence to support this idea (16).

ACKNOWLEDGMENTS

We thank Dr. Yukiya Fukuma, Dr. Yuichiro Nozawa, and the staffs of the Meat Inspection Stations in Hiratsuka and Atsugi, Kanagawa, for providing fecal specimens during the study.

This study was partly supported by a Health Science Research Grant on Emerging and Re-emerging Infectious Diseases (12103101) from the Ministry of Health, Labour and Welfare, Japan.

REFERENCES

1. Rose, J. B., Lisle, J. T. and LeChevallier, M. (1997): Waterborne cryptosporidiosis: incidence, outbreaks, and treatment strategies. p. 93-109. *In* Fayer, R. (ed.), *Cryptosporidium* and Cryptosporidiosis. CRC Press, Boca Raton, Fla.
2. Kuroki, T., Watanabe, Y., Asai, Y., Yamai, S., Endo, T., Uni, S., Kimata, I. and Iseki, M. (1996): An outbreak of waterborne cryptosporidiosis in Kanagawa, Japan. J. Jpn. Assoc. Infect. Dis., 70, 132-140 (in Japanese with English summary).

3. Yamamoto, N., Urabe, K., Takaoka, M., Nakazawa, K., Gotoh, A., Haga, M., Fuchigami, H., Kimata, I. and Iseki, M. (2000): Outbreak of cryptosporidiosis after contamination of the public water supply in Saitama Prefecture, Japan, in 1996. J. Jpn. Assoc. Infect. Dis., 74, 518-526.
4. Fayer, R., Speer, C. A. and Dubey, J. P. (1997): The general biology of *Cryptosporidium*. p.1-42. *In* Fayer, R. (ed.), *Cryptosporidium* and Cryptosporidiosis. CRC Press, Boca Raton, Fla.
5. Bergeland, M. E. (1977): Necrotic enteritis in nursing piglets. Proc. Am. Assoc. Vet. Lab. Diagn., 20, 151-158.
6. Kennedy, G. A., Kreitner, G. L. and Strafuss, A. C. (1977): Cryptosporidiosis in three pigs. J. Am. Vet. Med. Assoc., 170, 348-350.
7. Angus, K. W. (1990): Cryptosporidiosis in ruminants. p. 83-104. *In* Dubey, J.P., Speer, C.A. and Fayer, R. (eds), Cryptosporidiosis of Man and Animals. CRC Press, Boca Raton, Fla.
8. Xiao, L. and Herd, P. R. (1994): Review of equine *Cryptosporidium* infection. Equine Vet. J., 26, 9-13.
9. Sanford, S. E. (1987): Enteric cryptosporidial infection in pigs: 18 cases (1981-1985). J. Am. Vet. Med. Assoc., 190, 695-698.
10. Tacal, J. V., Sobieh, M. and El-Ahraf, A. (1987): *Cryptosporidium* in market pigs in southern California, U.S.A., Vet. Rec., 120, 615-616.
11. Ayeni, et al., 1985 The occurrence and effect of *Cryptosporidium* species on livestock in Ile-Ife, Nigeria Trop. Vet., 3: 96-100.
12. Rhee, J. K., Seu, Y. S. and Park, B. K. (1991): Isolation and identification of *Cryptosporidium* from various animals in Korea 1. Prevalence of *Cryptosporidium* in various animals. Korean J. Parasitol., 29, 139-148 (in Korean with English summary).
13. Kaminjolo, J. S., Adesiyun, A. A., Loregnard, R. and Kitson-Piggott, W. (1993): Prevalence of *Cryptosporidium* oocysts in livestock in Trinidad and Tobago. Vet. Parasitol., 45, 209-213.
14. Quilez, J., Sanchez-Acedo, C., Clavel, A., del Cacho, E. and Lopez-Bernad, F. (1996): Prevalence of *Cryptosporidium* and *Giardia* infections in cattle in Aragon (northeastern Spain) Vet. Parasitol., 67, 83-88.
15. Xiao, L., Herd, P. R. and Bowman, G. L. (1994): Prevalence of *Cryptosporidium* and *Giardia* infections on two Ohio pig farms with different management systems. Vet. Parasitol., 52, 331-336.
16. Izumiyama, S., Furukawa, I., Kuroki, T., Yagita, K. and Endo, T. (2001): Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in two river systems, the Sagami and Sakawa, in Kanagawa Prefecture. Environ. Conserv. Engineer., 30 (in press. in Japanese with English summary).