

## Detection of *Cryptosporidium* oocysts in canine fecal samples by immunofluorescence assay

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**Abstract:** Fecal samples were collected from 257 dogs in four areas in Korea during the period of January 1996 to November 1997 and examined by immunofluorescence assay for *Cryptosporidium* oocysts using a commercial diagnostic kit (Meridian Diagnostics, Cincinnati, Ohio). Of the 257 samples, 25 (9.7%) were positive for *Cryptosporidium*. Differences were noted in the prevalence of canine cryptosporidiosis in both areas and dog types. The results provide a further evidence of environmental contamination and widespread distribution of the parasite in Korea.

**Key words:** *Cryptosporidium*, dog, Korea

*Cryptosporidium* species is a coccidian parasite of worldwide distribution, with infection reported in a wide range of hosts including man, many other mammalian species, birds, reptiles and fish (Current, 1985; Moon and Woodmansee 1986; Angus, 1987; Dubey *et al.*, 1990). The parasite mainly causes diarrhea in several mammalian species, especially in calves. Canine cryptosporidiosis, however, was rarely reported.

It has been relatively common to find *Cryptosporidium* oocysts in the fecal samples from men and animals in Korea. Using Sheather's floatation technique, Kinyoun's modified acid-fast staining and osmic acid prefixed Giemsa staining, Rhee *et al.* (1991) reported the prevalence of cryptosporidiosis ranging from 13.3% to 29.6% in laboratory mice, rats, chickens, pigs, and dairy cows. Later, Wee *et al.* (1996) found that 11.4% of

diarrheal calves were positive for *Cryptosporidium* oocysts by a commercial diagnostic kit (Meridian Diagnostics, Cincinnati, Ohio). The parasite was first detected in Korean people by Cho *et al.* (1993), who reported a prevalence rate of 22%. An epidemiological survey conducted later by Chai *et al.* (1996) in the people in Seoul and Chollanam-do revealed that the overall oocyst positive rate was 7.9%.

The diagnosis of cryptosporidiosis has been relied on the identification of oocysts in fecal materials. Several types of serological diagnostic methods using monoclonal antibodies are used more often lately (Arrowood and Sterling, 1989; Rusnak *et al.*, 1989). In the present study the prevalence of canine cryptosporidiosis was determined by immunofluorescence assay using the commercial diagnostic kit.

Fecal samples were collected from 257 dogs in four areas during the period of January 1996 to November 1997 (Table 1). They were preserved in 10% neutral formalin solution

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**Table 1.** Prevalence of *Cryptosporidium* infection based on fecal oocyst detection in 257 dogs by areas

Area	No. of dogs examined	No. of positive dogs (%)
Chunchon	144	19 (13.2)
Sangju	11	0 (0.0)
Kwachon	29	5 (17.2)
Songnam	73	1 (1.4)
Total	257	25 (9.7)

until used. From each non-concentrated fecal sample, 20 µl was taken and applied to a slide provided in the commercial *Cryptosporidium* diagnostic kit (Meridian Diagnostics). The slides were then processed and examined as mentioned in the manufacturer's instructions.

Of the 257 fecal samples, 25 (9.7%) were positive for *Cryptosporidium* oocysts (Table 1). The prevalence was higher in Kwachon area, followed by Chunchon and Songnam areas in decreasing order. None of the dogs in Sangju area was infected by the parasite.

The size of the oocyst was measured on the fecal samples stained with DMSO-modified acid-fast staining (Bronsdon, 1984). The average size of the oocyst was  $4.7 \pm 0.5 \times 4.4 \pm 0.6 \mu\text{m}$  with the shape index (length/width ratio) of  $1.07 \pm 0.06$ . Morphologically they were compatible with *C. parvum*.

The results in the present study clearly indicate that the canine cryptosporidiosis is present in Korea and that the disease is endemic in those three areas surveyed. el-Ahraf *et al.* (1991) examined 200 stray dogs impounded at the San Bernardino City and County animal shelters for *Cryptosporidium* oocysts and reported the infection rate of 2%. The authors concluded that human and canine populations in the area are at low risk for the development of cryptosporidiosis. Canine infection with *Cryptosporidium* was studied by Bauer (1994); after examining canine fecal samples collected from 10 different halting sites in Dublin, he reported that the occurrence of canine zoonosis was not established.

Cho *et al.* (1993) examined fecal samples of 230 human patients, most of them with no

**Table 2.** Infection rates of dogs with *Cryptosporidium* according to the type of rearing

Type	No. examined	No. positive (%)
Companion <sup>a)</sup>	80	11 (13.8)
Watch dog <sup>b)</sup>	79	8 (10.1)
Farm <sup>c)</sup>	98	6 (6.1)
Total	257	25 (9.7)

<sup>a)</sup>Yorkshire-Terrier, Poodle, Maltese, Dachshund, Pekingese, Pug, Shih-Tzu, Chihuahua, Schnauzer, and Pomeranian. <sup>b)</sup>Beagle, Tosa, Korean Jindo, Dalmatian, Pointer, Great-Dane, Spitz, Shepherd, Rottweiler, and PitBull-Terrier. <sup>c)</sup>Mixed breed.

diarrheal illnesses; more than 20% of the samples were positive for *Cryptosporidium* oocysts by both acid-fast staining and auramine-rhodamine staining. The authors suggested a high level of environmental contamination and widespread distribution of the parasite in Korea. An epidemiological survey conducted later in humans also indicated a widespread distribution of the organism (Chai *et al.*, 1996).

According to the type of rearing, the prevalence of cryptosporidiosis was higher in companion dogs (13.8%), followed by watchdog and farm dogs in decreasing order (Table 2). The organisms can be transmitted directly from man to man or from animal to man (Moon and Woodmansee, 1986). Thus any animal, once infected, can act as a source of infection for others (Augus, 1987). The high infection rate in companion dogs of the present study suggests a further evidence of environmental contamination with the parasite. Meaningful results would be obtained in the future studies if dogs and their owners are included together.

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=초록=

## 형광항체법을 이용한 개의 와포자충 감염 실태 조사

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국내에서 사육되고 있는 개의 와포자충 감염 실태를 알아보기 위하여 1996년 1월부터 1997년 11월까지 4개 지역 (강원도 춘천, 경상남도 상주, 경기도 과천 및 성남)의 개 257두의 분변을 수집하였다. 와포자충의 확인은 현재 상품화되어 판매되고 있는 *Cryptosporidium* 진단 키트 (Meridian Diagnostics, Cincinnati, Ohio)를 사용하였다. 총 257두 중 25두에서 와포자충 오시스트가 확인되어 9.7%의 검출률을 나타내었는데, 과천지역이 17.2%로 가장 높았고, 춘천지역 13.2%, 성남지역이 1.4%인 반면, 상주지역은 오시스트가 검출되지 않았다. 개의 사육형태별 검출률은 사람과 함께 생활하는 실내견이 13.9%로 가장 높았고, 집지키는 개가 10.1%, 그리고 잠견 (또는 사육견)이 6.1%로 밝혀졌다. 이상의 결과로 보아 국내에 있는 여러 종류의 사육견들이 와포자충에 감염되어 있음을 확인할 수 있었으며, 특히 사람과 함께 생활하는 애완견에서 감염률이 높다는 사실은 환경의 오염에 의하여 애완견에서 사람으로 와포자충의 전파가 가능할 수도 있다는 점을 시사하는 것으로 이에 대한 대책이 필요할 것으로 생각된다.

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