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# Studies on isosporosis in dogs I: Isolation and sporulation of *Isospora ohioensis*

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**Abstract:** Based on the morphologic characteristics of oocysts of the genus *Isospora*, we have demonstrated that *I. ohioensis* is a relatively common *Isospora* species in dogs which are resident in Chonbuk province, Korea. The prepatent period of *I. ohioensis* was four days. The size of the unsporulated oocysts in fresh stool specimens was 22.9 × 19.8 μm (R = 1.16). The size of the contracted sporonts was 17.4 × 16.3 μm (R = 1.06). By 96 hours, sporulation is complete and the ratio of length/width was constant relatively. And the sizes of oocysts and sporocysts were 22.8 × 20.5 μm (R = 1.11) and 15.0 × 10.8 μm (R = 1.39), respectively. At this time it is most reliable for the measurements of oocyst sizes of *I. ohioensis* to provide with the identifiable clues against the others. It is therefore recommended that the clinical fecal specimens suspected of isosporosis should first be incubated and aerated for 96 hours before a definitive parasitological diagnosis can be reached. These and other observations contribute to the understanding of the biological characteristics and laboratory and clinical diagnosis of isosporosis.

**Key words:** *Isospora ohioensis*, isosporosis, oocyst, sporocyst, sporulation, dog.

## INTRODUCTION

Coccidiosis in the dog is an enteric disease caused by protozoa predominantly in the genus *Isospora* and can result in a serious, or even fatal colitis (Dunbar & Foreyt, 1985 ) and enteritis (Corea *et al.*, 1983). In severe infection, the clinical signs include catarrhal hemorrhagic enteritis with diarrhea, severe anemia, rapid emaciation, central nervous system involvement and lethargy. Coccidiosis remains one of the most formidable parasitic diseases in kennel pups on commercial pup-producing farms. This is particularly important in farms where pups are kept at ground level without any wire mesh. Under these production circumstances, diagnosis and prevention become a major aspect of management on the dog farms. The oocysts of *Isospora* sp. can be easily confused with one another, since members of this genus are morphologically very similar each other. Jang (1972 & 1975) discussed the problems of coccidiosis in domestic animals but never mentioned precise data on the species of the genus *Isospora* among dogs or any other domestic animal in Korea. Accordingly, when we diagnosed a case of isosporosis in Chonbuk, Korea, we decided to fully characterize it with respect to morphology and pathogenicity in order to fulfill Koch's postulates.

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This report describes the identification and diagnosis of *Isospora* infection in a dog from which a single oocyst was isolated from the stool and inoculated into a susceptible puppy. From the experimentally infected dog the heavy preparation of fecal oocysts was made and inoculated into susceptible puppies in order to increase the total harvest of oocysts. The latter preparation was then inoculated into five four-week old puppies in order to investigate the kinetics of appearance and morphogenesis of the oocysts during the course of sporulation under highly controlled and defined conditions.

## MATERIALS AND METHODS

**Fecal examination:** The stool specimens of 412 apparently clinically normal dogs ranging in age from one month to 4 years were used for fecal examination. The dogs living in Chonbuk province and Chonju suburbs were investigated in this study.

**Separation of oocyst from pooled stools:** Stool samples were collected from all the dogs in their respective locations and brought to College of Veterinary Medicine Laboratory, Chonbuk National University. The samples were individually screened for different parasites by Sheather's floatation method (sucrose; 5 lbs, water; 1470 ml and phenol; 30 ml) and then pooled together.

The pooled sample was allowed to develop sporogony over a period of 5 days with optimal aeration at room temperature. The pool was further analyzed by the same floatation method to concentrate the oocysts.

**Isolation of *I. ohioensis*:** The oocysts of *Isospora* sp. and the eggs of other intestinal parasites were differentiated morphologically according to Levine (1977) and Soulsby (1982).

The single oocyst of *I. ohioensis* was isolated according to its characteristic size and shape. In order to get a single oocyst, the oocysts were spread out on 1% agarose gel and the single oocyst of *I. ohioensis* picked up from the agarose plate using a pasteur pipette.

**Experimental inoculation of a dog:** The single oocyst was then inoculated into the individual of group No. 1. Starting from Day 1 through Day 10 post-inoculation, feces were individually collected 4 to 5 times daily as fresh as possible.

**Sporogony:** The feces were suspended in 2.5% potassium dichromate solution and passed through a series of sieves. The oocyst suspension was poured into a 1 L graduated cylinder and aerated at room temperature for 5 days (Baek *et al.*, 1988). The diameter (Length/Width = R) of the sporoblasts, sporocysts and oocysts were measured on at least 100 oocysts daily.

**Sub-inoculation:** The accumulated oocysts from group No. 1 were washed three times in distilled water to remove the potassium dichromate, concentrated by centrifugation at 1,500 × g for 10 minutes. The concentrate adjusted to a concentration of $0.3 \times 10^3$ oocysts. Three ml of the concentrate was then administered per os to each of the three dogs in group No. 2. Feces from this group were again collected and processed as above.

**Animals:** The dogs were of mixed breed but each sub-inoculation group was derived from the same liter. They were housed in individual wire-bottom cages. All the animals were supplied with water and commercial puppy starter *ad libitum* throughout the experiment.

## RESULTS

This report is confined to investigations on *I. ohioensis* isolated from pooled stool specimens from 412 dogs.

**Prepatent period:** Especially, a dog which received single oocyst began showing the oocysts from five days after the inoculation. But generally, the dogs of group No. 2 had an prepatent period of four days.

**Oocyst measurements during sporulation:** The sizes of the oocysts, sporonts and sporocysts of *I. ohioensis* kinetically changed depending on aeration time (Figs. 1 and 2), and also the length/width ratio of them were evident depending on the stage of their development (Fig. 3).

**Sporogony:** Fresh specimens generally contained unsporulated oocysts of *I. ohioensis*. Fig. 4 shows a typical resting nucleus prior to sporulation. The size of the unsporulated oocysts ranged between 20.3 - 26.0 × 15.6 - 22.9 μm (Mean: 22.9 × 19.8 μm, R = 1.16). The size of the contracted sporonts ranged

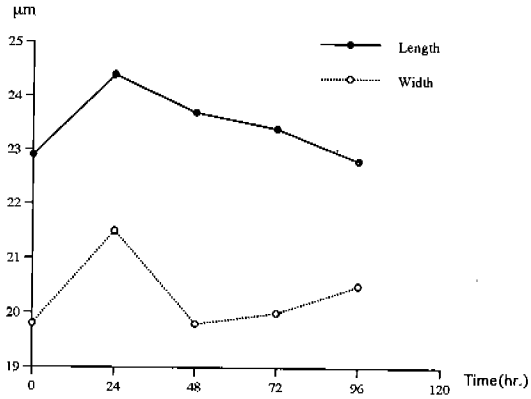


**Fig. 1.** The changes of the oocyst sizes of *I. ohioensis* according to sporulation.

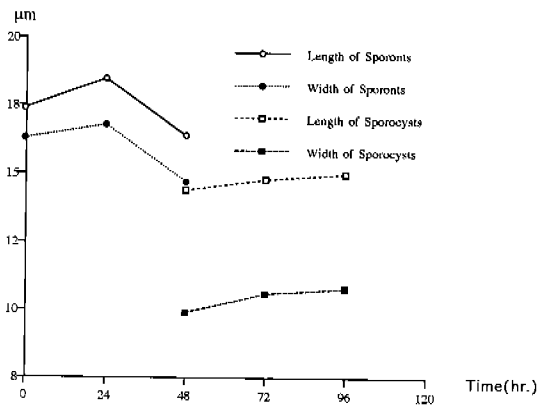


**Fig. 2.** The changes of the sporont and sporocyst sizes of *I. ohioensis* according to sporulation.

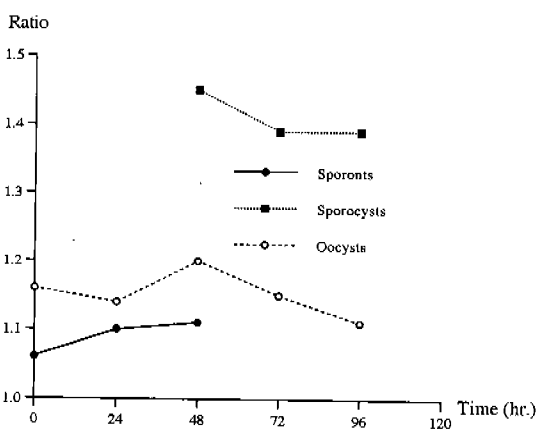


**Fig. 3.** The length/width ratio of the oocysts, sporonts and sporocysts of *I. ohioensis* according to sporulation.

between 15.6 - 21.1 × 14.6 - 18.2 μm (Mean: 17.4 × 16.3 μm, R = 1.06). Situated between the sporulant and oocyst wall, hazy (translucent) bodies were observed.

After 24 hours, the oocysts measured 22.8 - 26.0 × 19.0 - 23.4 μm (Mean: 24.4 × 21.5 μm, R = 1.14) indicating slight enlargement. The sporonts were slightly elongated and there was evidence of nuclear division with the size of the nuclei ranging between 16.1 - 21.3 × 15.6 - 20.3 μm (Mean: 18.5 × 16.8 μm, R = 1.1) as shown in Figs. 5 and 6. Daughter nuclei migrated to the opposite poles of the sporont (Fig. 6) with evidence of constriction in the sporont. The sporont remained in cleavage status (Fig. 7) until two sporoblasts were formed (Fig. 8).

After 48 hours the sporocysts and oocysts measured 15.0 - 20.5 × 13.0 - 16.9 μm (Mean: 16.3 × 14.6 μm, R = 1.11) and 20.5 - 26.0 × 15.6 - 22.9 μm (Mean : 23.7 × 19.8 μm, R = 1.20), respectively. The sporonts measured 15.1 - 20.5 × 13.0 - 16.9 μm (Mean: 16.4 × 14.7 μm, R = 1.11). The nuclear division of the sporoblast occurred with two nuclei appearing at the opposite poles of the sporoblast and with a well-demarcated sporocyst (Fig. 10). During the same period, the sporocyst membrane became closely associated with the protoplasm as the sporocyst developed (Fig. 11).

After 72 hours, as shown in Fig. 12, the sporocysts in the oocysts were slightly elongated. The sporocysts and oocysts measured 12.7 - 18.4 × 8.3 - 12.7 μm (Mean: 15.5 × 10.7 μm, R = 1.39) and 21.1 - 23.9 × 18.5 - 21.5 μm (Mean: 23.4 × 20.4 μm, R = 1.15), respectively.

After 96 hours, upon completion of sporozoite formation, a granular sporocyst residuum was visible (Figs. 12 and 13), with the sizes of the oocysts and sporocysts being 21.1 - 23.9 × 19.0 - 21.6 μm (Mean: 22.8 × 20.5 μm, R = 1.11) and 13.0 - 16.6 × 10.1 - 11.7 μm(Mean: 15.0 × 10.8 μm, R = 1.39), respectively. The sporocyst residuum consisted of a spherical hyaline body surrounded by several smaller granules. No micropyle was seen in the oocysts and no stieda bodies were seen in the sporocysts. The residuum became more compact and somewhat elongated and eventually contracted into an oval granular

body (Fig. 13).

## DISCUSSION

Canine coccidiosis has been studied extensively (Jang, 1972; Levine, 1977; Corea *et al.*, 1983; Dunbar and Foreyt, 1985). Levine (1977) described the various intestinal protozoa in the family Eimeriidae which includes *Isospora* sp. namely: *I. bigemina*, *I. ohioensis* and *I. canis*. Our study in Korea, has for the first time, demonstrated that dogs in this country are infected with multiple intestinal parasites. Among these parasites four kinds of *Isospora* sp. were observed in the course of mass screening of dog stools using Sheather's floatation technique. They were identified on the basis of size and morphology of oocysts. *Isospora ohioensis* showed the highest prevalence rate (16.0%). Differentiation between *I. ohioensis* and *I. burrowsi* on a morphological basis is complicated by the similarity in the size and shape of their oocysts. However, the oocysts of *I. ohioensis*

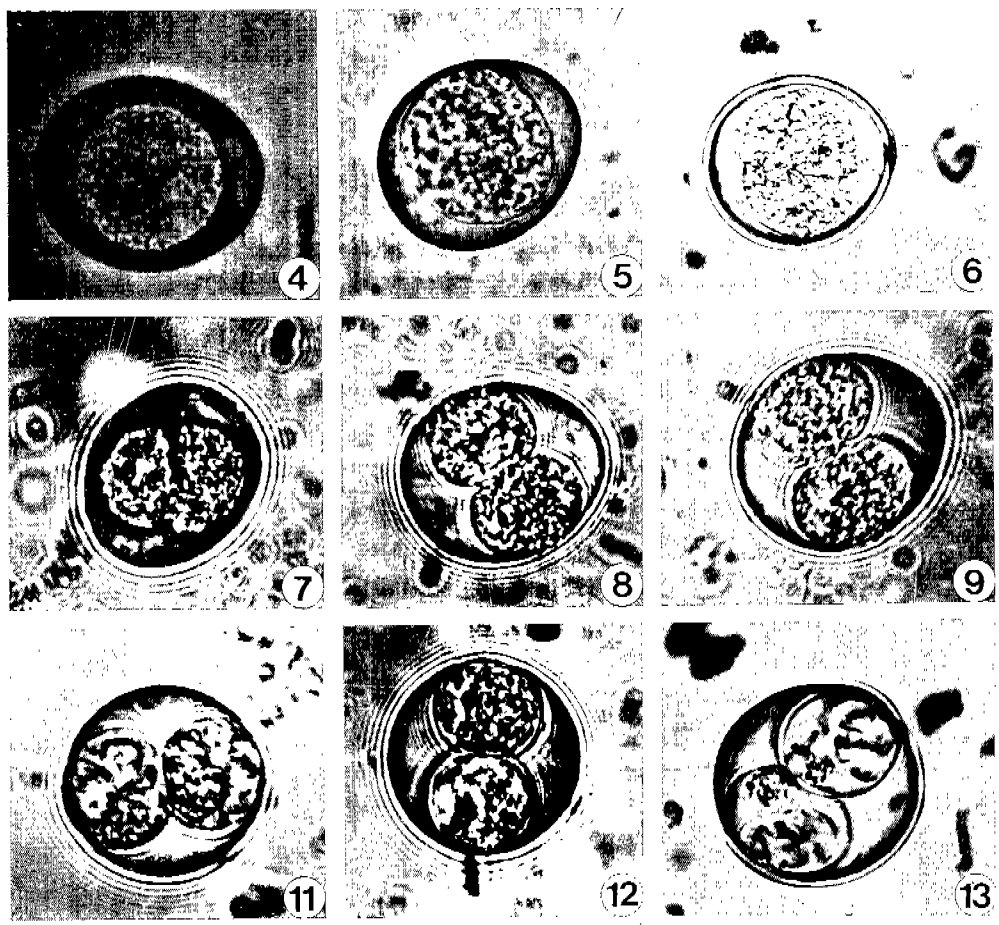


**Fig. 4:** Unsporulated oocyst in the sporont stage with hazy bodies of *I. ohioensis* (× 1,000). **Figs. 5 and 6:** Oocyst of *I. ohioensis* developing a binucleated sporont and the sporoblast (× 1,000). **Fig. 7:** Oocyst of *I. ohioensis* containing a sporont in the developing sporoblast (× 1,000). **Fig. 8:** Oocyst exhibiting a line of constriction in sporont (× 1,000). **Fig. 9:** Oocyst containing two binucleated sporoblasts with polar nuclei (× 1,000). **Fig. 10:** Oocyst contain two elongated sporocysts (× 1,000). **Fig. 11:** Oocyst with the developing sporozoite in late sporocyst stage (× 1,000). **Fig. 12:** Oocyst in early sporozoite stage. Each sporocyst contains fully formed sporozoites and dispersed granular residuum (× 1,000). **Fig. 13:** Oocyst in sporozoite stage each containing a hyaline body and a few dispersed granules (× 1,000).

are 24 × 21 μm whereas those of *I. burrowsi* are relatively smaller, measuring 20 × 17 μm (Dubey, 1975; Trayser and Todd, 1978 ). The oocysts of *I. neorivolta* are much smaller than either of the above species and readily distinguishable by an experienced parasitologist.

The precise measurement of the dimensions of oocysts is critical in definitive identification of the oocysts of various *Isospora* sp. Levine and Ivens (1965) have established guide lines based on accurate measurements of the oocysts. Thus the sporulated oocysts of *I. bigemina* should measure 10 - 14 × 10 - 12 μm, those of *I. neorivolta* should be 20 - 27 × 15 - 24 μm, whereas those of *I. canis* range between 35 - 42 × 27 - 33 μm. The prevalence rate and host specificity of the family Eimeriidae also provide additional parameters for determining the genus and species of a given oocyst. However, they also pointed out that the size could be misleading in some situations. For example, the sporulated oocysts of *I. rivolta* could change in their shape ellipsoidal to ovoid. This observation is consistent with our data on *I. ohioensis* in Korea. In our case, oocysts measured 20 - 27 × 15 - 24 μm with a mean of 23 - 19 μm; the length/width ratio ranged from 1.0 to 1.3 with a mean of 1.17.

Our data on *I. ohioensis* is further verified by the consistency of the prepatent period (4 - 5 days), sporulation time (96 hours) and size of the oocyst (22.8 - 20.5 μm) with previously published data (Levine, 1977; Dubey and Mahrt, 1978), including the characteristic smooth, colorless or pale yellow oocyst wall, the presence of a polar granule and an oocyst residuum, and the presence of a thin membrane without a micropyle.

Our observations in this study generally agree with reported data (Mahrt, 1968; Dubey and Mahrt, 1978; Dubey, 1979), including the sporulation time being completed in 96 hours after optimal aeration at room temperature as shown in Figs. 4 - 13. At this stage the morphology and measurements are relatively stable and this is the most reliable time to take the differential measurements since significant alterations in the size do occur between defecation and sporulation under optimal aeration conditions. Therefore, we recommend that routinely samples should be aerated and incubated for 96 hours prior to definitive identification purposes.

This study has successfully demonstrated the endemicity and prevalence of isosporosis due to *I. ohioensis* in dogs in Korea. The population of animals studied was predominately from urban and periurban locations of Chonju. Most of the dogs investigated were not confined to kennels, but the fact that the prevalence was high indicates that there may not be any difference between dogs kept in kennels and those residing in human households. Our observations have further strengthened the reliability of accurate measurements as criteria for identification of species of the genus *Isospora*.

Further, the demonstration of *I. ohioensis* in Korea strongly indicates that routine parasitological examinations of canine fecal specimens should routinely include differential diagnosis for members of the genus *Isospora*. Similarly, the potential clinical significance of these parasites in the dog and other species is high lighted by our findings.

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=국문초록=

## 개의 Isosporosis에 관한 硏究 I : *Isospora ohioensis*의 분리 및 胞子形成

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한국산 개로부터 *Isospora ohioensis*를 分離하기 위하여 形態學的 特性을 觀察하였던 바 다음과 같은 結果를 얻었다. 즉, 全北地方의 개의 糞便에서 검출된 *Isospora* spp. 중 *I. ohioensis*의 oocyst 하나를 分離하여 강아지에 經口感染시켜 얻은 oocyst를 다시 5두의 개에 感染시킨 후 形態學的 特性을 顯微鏡下에서 觀察하였다. 이의 潛伏基는 4일이었으며 糞便內에 排出된 oocyst의 크기는 22.9 × 19.8 μm (R = 1.16)이었고 sporont의 크기는 17.4 × 16.3 μm (R = 1.06)이었다. 96시간에 있어서 胞子가 완전히 形成되었으며 oocyst 및 sporocyst의 크기는 각각 22.8 × 20.5 μm (R = 1.11)과 15.0 x 10.8 μm (R = 1.39)이었다. 胞子形成 後에 oocyst 및 sporocyst의 크기는 거의 變化가 없었던 점으로 보아 개의 糞便檢査를 통한 *Isospora*속의 鑑別診斷을 위하여 96시간 정도의 胞子形成期間이 필요할 것으로 예상되는 바이다.

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