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# Report of Lawsonia intracellularis Infection in Dogs by Polymerase Chain Reaction

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*Short Communication*

# Report of *Lawsonia intracellularis* Infection in Dogs by Polymerase Chain Reaction

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***Abstract***

The purpose of this study was to survey *Lawsonia intracellularis* in dogs in Thailand by using Polymerase chain reaction technique. *Lawsonia intracellularis* is obligatory intracellular bacterium which causes proliferative enteropathy in animals, including dog. Fecal samples from 48 dogs were collected from Bangkok, Pathumthani and Nakhon Ratchasima and tested for *L. intracellularis* by PCR technique. The dogs were classified into two groups according to the presence or absence of diarrhea. In the group of dogs with diarrhea (n= 11), 6 dogs were positive (54.4%) and in the group without signs of diarrhea (n= 37), 15 dogs were positive (40.5%). The overall positivity was 43.8% (21/48). These results indicated that *L. intracellularis* was positive in dogs in Thailand.

**Keywords:** diarrhea, dog, *Lawsonia intracellularis*

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# บทคัดย่อ

## รายงานการติดเชื้อ *Lawsonia intracellularis* ในสุนัขโดยวิธีปฏิกิริยาลูกโซ่พอลิเมอเรส

***ศริยา อัศวกาญจน์* สุพจน์ วัฒนะพันธ์ศักดิ์ ทนง อัศวกาญจน์***

จุดประสงค์ของการศึกษาครั้งนี้ คือเพื่อตรวจหาเชื้อ *Lawsonia intracellularis* ในสุนัขในประเทศไทย โดยใช้วิธีปฏิกิริยาลูกโซ่พอลิเมอเรส *Lawsonia intracellularis* เป็นเชื้อแบคทีเรียที่ต้องอาศัยอยู่ในเซลล์เพื่อเพิ่มจำนวน และเป็นเชื้อที่ก่อให้เกิดโรคลำไส้อักเสบในสัตว์หลายชนิดรวมทั้งในสุนัข การศึกษาครั้งนี้ได้เก็บตัวอย่างอุจจาระจากสุนัขทั้งหมด 48 ตัวในจังหวัดกรุงเทพฯ ปทุมธานี และนครราชสีมา โดยได้สุ่มเก็บและตรวจเชื้อโดยวิธีปฏิกิริยาลูกโซ่พอลิเมอเรส ตัวอย่างอุจจาระที่สุ่มเก็บได้แบ่งออกเป็น 2 กลุ่มคือ กลุ่มที่มีภาวะถ่ายเหลว และกลุ่มที่ไม่มีภาวะถ่ายเหลว ในกลุ่มที่มีภาวะถ่ายเหลว พบว่าให้ผลบวกจำนวน 6 ตัวอย่างจากทั้งหมด 11 ตัวอย่าง (ร้อยละ 54.4) ในกลุ่มสุนัขปกติพบผลบวกจำนวน 15 ตัวอย่างจากทั้งหมด 37 ตัวอย่าง (ร้อยละ 40.5) รวมทั้งหมดทั้ง 2 กลุ่มพบ 21 ตัวอย่างจาก 48 ตัวอย่าง (ร้อยละ 43.8) จากการศึกษาครั้งนี้พบว่าสุนัขในประเทศไทยให้ผลบวกต่อเชื้อ *L .intracellularis*

**คำสำคัญ:** ถ่ายเหลว สุนัข *Lawsonia intracellularis*

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## *Introduction*

*Lawsonia intracellularis* is an obligatory intracellular bacterium which affects the digestive tract particularly at the caudal portion of the small intestine – the ileum of sensitive hosts (McOrist et al., 1995). It has been identified as the etiological agent of hyperplasia of intestinal which is called "proliferative enteropathy". Transmission of pathogen generally occurs through the fecal-oral route (Smith and Lawson, 2001). It is an important enteropathogen of domestic pigs with worldwide distribution, which typically causes diarrhea and growth retardation (Lawson and Gebhart, 2000). This bacterium was detected in a dog with proliferative lesions in gastrointestinal tract and reported in a dog with inflammatory bowel disease. Clinical signs of dogs with positive *L. intracellularis* are vomiting and chronic or intermittent diarrhea causing proliferative enteritis. Proliferative lesions in the gastrointestinal tract, caused by *L. intracellularis* were reported in two Dalmatian pups (Collins et al., 1983) and one German short-haired pointer (Husnik et al., 2003). *L. intracellularis* has also been found in other species of Canidae family, namely the gray wolfs and red foxes (Tomanova et al., 2003). In other animals, however, the infection of these bacterium was reported in horses (Lavoie et al., 2000), ferrets, rats, ostriches (Cooper et al., 1997), rabbits (Hotchkiss et al., 1996), ferrets, hamsters (Fox et al., 1994), rhesus macaques (Klein et al., 1999), white-tailed deer (Drolet et al., 1996) and emus (Lemarchand et al., 1997). In Thailand, the infectious status of *L. intracellularis* in dogs has not been reported. This study conducted a survey of *L. intracellularis* in dogs with and without clinical signs of diarrhea by using PCR technique in Thailand. The PCR technique can be used for diagnosis of the presence or absence of *L. intracellularis* in dogs.

## *Materials and Methods*

Fecal samples were randomly collected from dogs which were brought to veterinary clinics and hospitals in Bangkok and Pathumthani and which were trained of War Dog at Battalion in Nakhon Ratchasima. In total, 48 dog-fecal samples were randomly collected, 11 samples from dogs with clinical signs of diarrhea and 37 dogs without diarrhea. DNA of bacteria was purified from fecal samples using a commercial fecal extraction method QIAmp DNA Stool Mini Kit (Qiagen). Oligonucleotide primers (A: 5'TATGGCTGTCAAACACTCCG-3' and B: 5'TGAAGGTATTGGTATTCTCC-3') were used to amplified 318 bp fragment from the *L. intracellularis* chromosome (Jones et al., 1993). Optimized reaction conditions consisted of 50 µl reaction mixtures using Quantitec probe PCR kit master mix (Qiagen), 600 nM of each Primer and 10 µl template DNA. All reaction were performed in a G-Strom GS1 thermal cycler with the following profiles: initial activation at $95^0$C for 15 min, followed by 35 cycles with denaturation at $94^0$C for 30 sec, annealing at $56^0$C for 60 sec and extension at $72^0$C for 30 sec. Reaction mixtures were chilled at $4^0$C. PCR run included positive and negative

extraction controls which were processed for every batch samples. *L. intracellularis* agents form vaccine was used as positive control. PCR products were separated on 2% agarose gels stained with ethidium bromide.

## *Results and Discussion*

PCR product from positive *L. intracellularis* fecal samples were amplified 318 bp fragments and was separated in 2% agarose gel electrophoresis (Fig 1). This PCR technique was specific for detecting *L. intracellularis* in fecal samples. The inhibitory factors in feces might cause false-negative results**.**

Examination of total 48 fecal samples revealed positive for *L. intracellularis* in 21 dogs (43.8%). In the group with clinical signs of diarrhea, 6 samples were positive from 11 samples (54.5%). One dog was both positive for *L. intracellularis* and canine parvovirus with bloody diarrhea. By using PCR technique, *L. intracellularis* infection in dogs was 43.8%. Some reports suggested that *L. intracellularis* was probably much more widespread in other animal species and thus in the environment than previous thought. Subclinical infected dogs might play a big role in transmission of the bacterium since they can shed it to the environment while they are not showing any clinical signs. The age of positive dogs was from 2 months to 10 years, similar to a previous report which showed that *L. intracellularis* infection was in range of 4 months to 12 years (Klimes et al., 2007). Nevertheless, in pigs *L. intracellularis* affected young growing pigs from 6-20 weeks of age resulting in diarrhea and poor growth rate (Lawson and Gebhart, 2000). *L. intracellularis* infected horses and caused diarrhea and hypoproteinemia in foals 4-7 months of age (Lavoie and Drolet, 2007). Dog population infected by *L. intracellularis* is in wider and older range of age than pigs and horses. Klimes et al. (2007) reported that the prevalence of *L. intracellularis* infection in dog in Czech Republic was 74.7%, which is relatively high compared to this study. Previous studies used indirect fluorescence antibody test (IFAT) for determining the prevalence of the infection. Unlike PCR technique, the serology test determines the presence or absence of the immunoglobulin G (IgG) against *L. intracellularis* infection. The advantage of serology tests is that its sensitivity is better than fecal PCR (Guedes et al., 2002). Although the PCR sensitivity (70%) was affected by sample quality and PCR-inhibitory factors, this technique has been widely used to demonstrate *L.intracellularis* in tissue specimens and fecal samples and considered to have 100% specificity (Gebhart, 2006).

It was reported that PCR technique could detect $10^3$ -$10^4$*L. intracellularis* organisms/g of feces and $10^1$ organisms/mucus (Elder et al., 1997). In summary, this study is the first report on the detection of *L. intracellularis* in dogs in Thailand. Once dogs are suffering from diarrhea and poor growth, *L.intracellularis* infection should be considered and suspected. Fecal PCR can be used for final diagnosis of *L. intracellularis* infection in dogs.

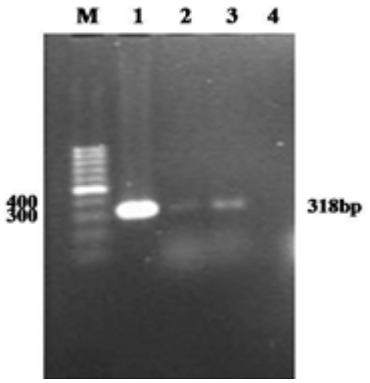


**Figure 1** PCR products from positive *L. intracellularis* fecal samples were amplified 318 bp fragments. (Lane M was 100 bp DNA ladder marker, Lane 1 was positive control, Lane 2 and 3 were positive *L. intracellularis* and Lane 4 was negative control)

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