Prevalence of *Lawsonia intracellularis*, *Salmonella* spp. and *Eimeria* spp. in Healthy and Diarrheic Pet Rabbits

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**ABSTRACT.** A total of 170 fresh fecal samples (healthy; n=137, diarrheic; n=33) were collected from pet rabbits. By using PCR and formol-ether concentration method, a total 13/137 healthy rabbit feces were positive for *L. intracellularis*, 6/137 for *Salmonella*, and 13/137 for *Eimeria*. On the other hand, a total 17/33 diarrheic rabbit fecal samples were positive for *L. intracellularis*, 10/33 for *Salmonella*, and 21/33 for *Eimeria*. From these results, more than 20% of clinically normal and 97% of diarrheic rabbits were positive for single or concurrent infection of three pathogens. To the best of our knowledge, this is the first report to describe the prevalence of the microorganisms *L. intracellularis*, *Salmonella* and *Eimeria* in pet rabbits.

**KEY WORDS:** diarrhea, *Eimeria*, *Lawsonia intracellularis*, pet rabbit, *Salmonella*.

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Gastrointestinal disease caused by pathogenic microorganisms is a cause of high morbidity and mortality in pet rabbits. The most common pathogens associated with gastrointestinal disease include *L. intracellularis*, *E. coli*, *Salmonella* spp., *Eimeria* spp. and *Listeria* spp. [6]. Proliferative enteropathy is an intestinal infectious disease characterized by thickening of the small and proximal large intestinal mucosa due to enterocyte proliferation associated with *L. intracellularis* [8]. *L. intracellularis* has been associated with colonization of enterocytes in animals such as swine, rabbits, hamsters, rats and guinea pigs [2, 4]. Salmonellosis is still a major cause of zoonosis and food-borne disease in humans, and is usually associated with contaminated food or water; the clinical signs of salmonellosis include septicemia, depression, pyrexia and death and the condition is often accompanied by diarrhea in rabbits [6]. Aside from bacterial pathogens, coccidiosis can result from infections by several species of *Eimeria*, and young rabbits are commonly affected with diarrhea, weight loss and anorexia [6]. In spite of a large pet rabbit population, the prevalence of gastrointestinal pathogenic organisms including *L. intracellularis*, *Salmonella* and *Eimeria* has never been described.

Study material was collected from a local veterinary hospital (Daejeon, Korea). Fresh feces from Lionhead (n=131), Dutch (n=13), Mongrel (n=9), Mini Rex (n=8), Lop Ear (n=5) and cross-breed (n=4) rabbits were collected based on documented clinical cases of diarrheic and healthy pet rabbits, and were kept at 4°C until processed for chromosomal DNA purification.

Bacterial DNA purification from feces and PCR were performed by previously described methods [5, 9]. DNA from *L. intracellularis* (National Veterinary Research and Quarantine Service, Anyang, Korea) and *Salmonella enterica* serovar Typhimurium (ATCC14028) was used as a control and amplified PCR products were 319 and 457 bp (Fig. 1A and 1B). For detection and counting of *Eimeria* cysts, the formol-ether concentration method was used (Fig. 1C), as previously described [1].

Among healthy young rabbit group, 4/67, 1/67 and 6/67 were positive for *L. intracellularis*, *Salmonella* spp. (*Salmonella*) and *Eimeria* spp. (*Eimeria*), respectively. Concurrently, one out of 67 each, was positive for *L. intracellularis* and *Salmonella*, *L. intracellularis* and *Eimeria* or for all the three pathogens. In healthy adult group, 3/39, 2/39 and 3/39 were positive for *L. intracellularis*, *Salmonella* and *Eimeria*, respectively. In addition, 3/31 from healthy old group were positive either for *L. intracellularis* or *Eimeria*.

Among diarrheic young rabbit group, while 6/17, 1/17 and 5/17 were positive for *L. intracellularis*, *Salmonella* and *Eimeria*, and 2/17 each were concurrently positive for either *L. intracellularis* and *Salmonella* or *L. intracellularis* and *Eimeria*. In diarrheic adult group, 1/10 and 3/10 were positive for *Salmonella* and *Eimeria*. Besides, 1/10 each was concurrently positive for *L. intracellularis* and *Salmonella* or for *Salmonella* and *Eimeria*, and 3/10 of the adult group had all the three pathogens. From diarrhoeic old group, 3/6 were from young rabbits (<3 months old), 37 from old rabbits (>12 months old) and the remaining 49 samples from adult rabbits aged 3–12 months. The fecal samples were collected based on documented clinical cases of diarrheic and healthy pet rabbits, and were kept at 4°C until processed for chromosomal DNA purification.

*NOTE. Bacteriology*
were positive for *Eimeria* and 3/6 were concurrently positive for *L. intracellularis* and *Eimeria*. The microorganisms in rabbit feces in this study were summarized in Table 1.

In distribution of microorganisms isolated by age in the healthy rabbit population, 7/67, 3/67 and 8/67 from healthy young rabbit group and 3/39, 3/39 and 2/39 from healthy adult group were positive for *L. intracellularis*, *Salmonella* and *Eimeria*, respectively. In addition, 3/31 and 3/31 from healthy old group were positive for *L. intracellularis* and *Eimeria*. Furthermore, 10/17, 3/17 and 7/17 from diarrheic young rabbit group and 4/10, 6/10 and 7/10 from diarrheic adult rabbit group were positive for *L. intracellularis*, *Salmonella* and *Eimeria*, respectively. In addition, 3/6 and 6/6 from diarrheic old group were positive for *L. intracellularis* and *Eimeria*.

The prevalence of microorganisms in healthy rabbits, 13/137 (9.5%) samples were positive for *L. intracellularis*, 6/137 (4.7%) for *Salmonella*, and 13/137 (9.5%) for *Eimeria*. In diarrheic rabbits, 17/33 (51.5%) samples were positive for *L. intracellularis*, 10/33 (30%) for *Salmonella*, and 21/33 (63.6%) for *Eimeria*.

In view of the established broad host range of *L. intracellularis*, *Salmonella* and *Eimeria*, we should remain alert to the possibility that the range of susceptible species may broaden further and incorporate domestic animals. From this study, the prevalence rate of three pathogens in healthy rabbit was almost similar in terms age. On the other hand, diarrheic adult and old rabbits showed higher susceptible to *Eimeria* than young rabbits (*P*<0.05; Chi-Square Test, SPSS ver 10), suggesting that the age of rabbit seems a factor of gastrointestinal disorder by *Eimeria*. Recently, rabbit infections with *L. intracellularis* were reported [3, 4], but the prevalence of *L. intracellularis* has not been described in rabbits. Considering that *Salmonella* intermittent shedding is observed in animal salmonellosis [7], the proportion of pet rabbits with *Salmonella* may be higher than 9.4% of this study. *Eimeria* are also frequently observed in pet rabbits in this study. This indicates that high prevalence of pet rabbits with *Eimeria* causes intestinal disorders and *Eimeria*-infected rabbits may be a potential reservoir for coccidiosis, which may be transmitted to other animals. The primary mode of transmission for these three agents is a fecal-oral route. To reduce spreading of these three agents in healthy and diarrheic pet rabbits, laboratory test including bacterial isolation, microscopic observation and molecular methods and antimicrobial treatment are necessary. From the high prevalence of these microorganisms in healthy pet rabbits, it is clear that any symptomatic presentations in this population should be evaluated for the presence of these pathogens and treated as necessary.

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**Table 1.** Results of PCR analysis for *L. intracellularis* and *Salmonella* spp., and *Eimeria* cysts observation from the pet rabbit faeces

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Number of PCR and <em>Eimeria</em> cysts positive for (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>S</td>
</tr>
<tr>
<td>Healthy</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>(n=137)</td>
<td>(7.2%)</td>
<td>(2.9%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>(n=33)</td>
<td>(18.1%)</td>
<td>(6.0%)</td>
</tr>
</tbody>
</table>

a) PCR detection for *L. intracellularis* and *Salmonella* spp. b) L; *L. intracellularis*, S; *Salmonella* spp., E; *Eimeria* spp., +; concurrent infection.
REFERENCES