NOTE *Bacteriology*

Isolation of *Lawsonia intracellularis* in Korea and Reproduction of Proliferative Enteropathy in Pigs and Hamsters

Jung-Yong YEH[1)], Tae-Jong KIM[2)], Seung-Yong PARK[2)], Chang-Seon SONG[2)], Yong-Dhuk YOON[3)], Soo-Ki KIM[4)], Joong-Bok LEE[2,4)]* and In-Soo CHOI[2,4)]*

[1)]*National Veterinary Research and Quarantine Service, 480 Anyang 6-dong, Manan-gu, Anyang, Gyeonggi-do 430–824,*
[2)]*Department of Infectious Diseases, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143–701,*
[3)]*Green Cross Veterinary Institute, Green Cross Veterinary Product. Co., Ltd., 227–5 Kugal-ri, Giheung-eup, Yongin, Gyeonggi-do and*
[4)]*Institute of Biomedical Science and Technology, Konkuk University, #1 Hwayang-dong, Gwangjin-gu, Seoul 143–701, Korea*

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ABSTRACT. *Lawsonia intracellularis* (*L. intracellularis*) was isolated from a Korean pig suffering acute proliferative enteropathy. *In vitro* culture conditions of *L. intracellularis* were established in McCoy cells. Pigs and hamsters experimentally infected with the pure culture of *L. intracellularis* reproduced clinical signs and intestinal lesions of proliferative enteropathy. The presence of *L. intracellularis* in the intestinal lesions was confirmed by immunohistochemistry with *L. intracellularis*-specific monoclonal antibodies.
KEY WORDS: Korean pig, *Lawsonia intracellularis*, proliferative enteropathy.

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Proliferative enteropathy (PE) is an enteric disease of pigs caused by the obligate intracellular bacterium, *Lawsonia intracellularis* (*L. intracellularis*) [11, 13]. It is estimated that about 30% of pig herds suffer from clinical or sub-clinical PE [9]. A study in Korea indicated that 53% of finisher pigs in the disease-harboring farms were infected with *L. intracellularis* [8]. Since *L. intracellularis* is a fastidious bacterium for pure culturing *in vitro*, its isolation and culturing has been achieved in only a few laboratories [6, 7]. In this study, we report the isolation of *L. intracellularis* for the first time in Korea and the reproduction of PE in pigs and hamsters that were infected with the pure culture of *L. intracellularis*.

A hemorrhagic region of the small intestine was obtained from a 20-week-old finisher pig in Korea that showed typical signs of acute hemorrhagic PE [3, 13]. Isolation of *L. intracellularis* was performed by the previously reported methods with some modifications [6, 13]. Briefly, the mucosa taken from the PE lesion of the small intestine was partially digested with 1% trypsin (Gibco BRL, U.S.A.) and homogenized with a blender. The homogenized tissue was diluted 1:20 vol/vol with sucrose-potassium-glutamate solution (pH 7.0) containing 0.218 M sucrose, 0.0038 M KH_2PO_4, 0.0049 M potassium glutamate and 10% fetal bovine serum (FBS), and then filtered through 0.8-μm-pore sized filter. One milliliter of the sample suspension was added to McCoy cells (ATCC CRL 1696) grown in DMEM medium supplemented with 1% L-glutamine, 2.5 μg/m*l* amphotericin B and 5% FBS without antibiotics. The cell-containing flasks were immediately centrifuged at 2,090 × g for 10 min and the atmosphere of the flasks was replaced with hydrogen gas for 10 min. Each flask was then refilled with a gas mixture containing 8.0% oxygen, 8.8% carbon dioxide, and 83.2% nitrogen as described elsewhere [7]. After 3-hr incubation at 37°C, the culture medium of the flask was changed with DMEM containing 100 μ/m*l* of gentamicin and 100 μg/m*l* vancomycin. The cell culture medium was changed at days 2 and 4 post-inoculation. On day 7, *L. intracellularis* was harvested from the McCoy cells by lysis with hypotonic solution as described elsewhere [7]. After five passages in the McCoy cells, *L. intracellularis* present in the cytoplasm of the cells was identified by immunostaining with monoclonal antibodies IG4 and 2001 MAb [2, 10]. Six 3-week-old pigs were used in this study. They all tested negative for *Brachyspira* species and *L. intracellularis* as determined by serologic tests and PCR [1, 5]. Three of them were orally infected with 4.5 × 108 *L. intracellularis* that had been purified from the bacteria-infected McCoy cells after nine passages. The other three pigs were used as non-infected controls. In addition, eight 4-week-old Syrian hamsters (*Mesocricetus auratus*) were used in this study, all of which were negative for *L. intracellularis* when tested by PCR [4, 5]. Four hamsters were orally inoculated with 1.5×10^7 *L. intracellularis* and the other four were used as non-infected controls.

A Korean strain of *L. intracellularis*, designated as PHE/KK421, was isolated by infecting the McCoy cells with the intestinal homogenate of a PE-suffering pig. The presence of *L. intracellularis* was identified both in the cell culture supernatants and in the cytoplasm of the McCoy cells from five passages (Fig. 1A). Replication of *L. intracellularis* in the cytoplasm of the infected cells continued to day 7 post infection (PI) without cytopathic effects such as cell rounding, fusion, detachment or vacuoles. The identity of *L. intracellularis* was also confirmed by PCR reaction with the bacterium-specific primers (data not shown) [5]. *L. intracellularis* was not identified in the uninfected McCoy cells

* CORRESPONDENCE TO: CHOI, I. S. Department of Infectious Diseases, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143–701, Korea.
* Footnote: Choi, I. S. and Lee, J. B. equally contributed to corresponding authors.

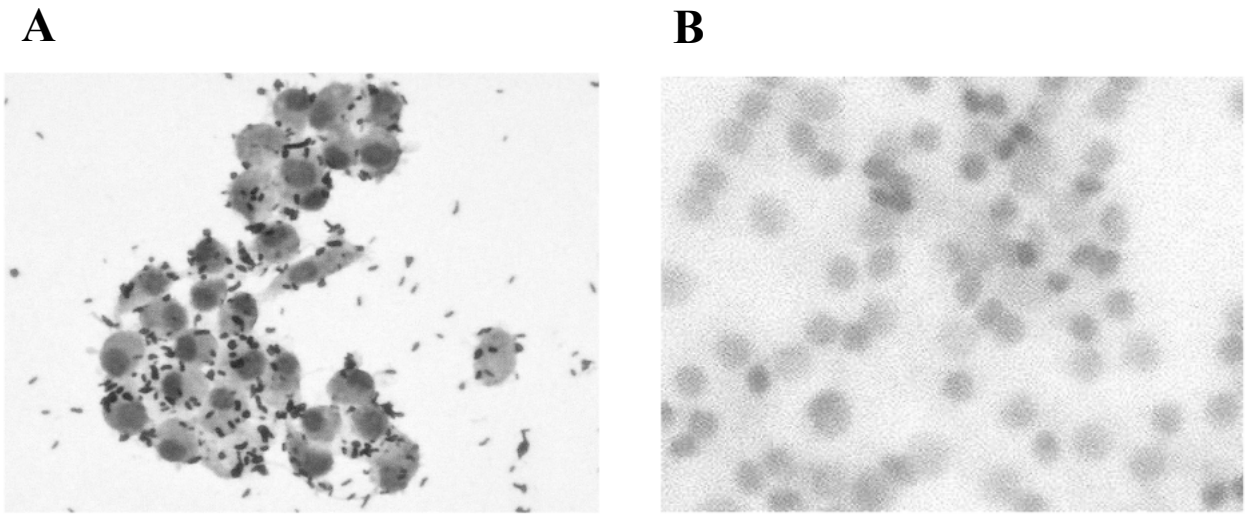

Fig. 1. Identification of *L. intracellularis* in McCoy cells. (A) McCoy cells infected with *L. intracellularis* were immunostained with *L. intracellularis*-specific monoclonal antibodies and counterstaining was performed with hematoxylin. (B) Uninfected control McCoy cells. Magnification, × 200.

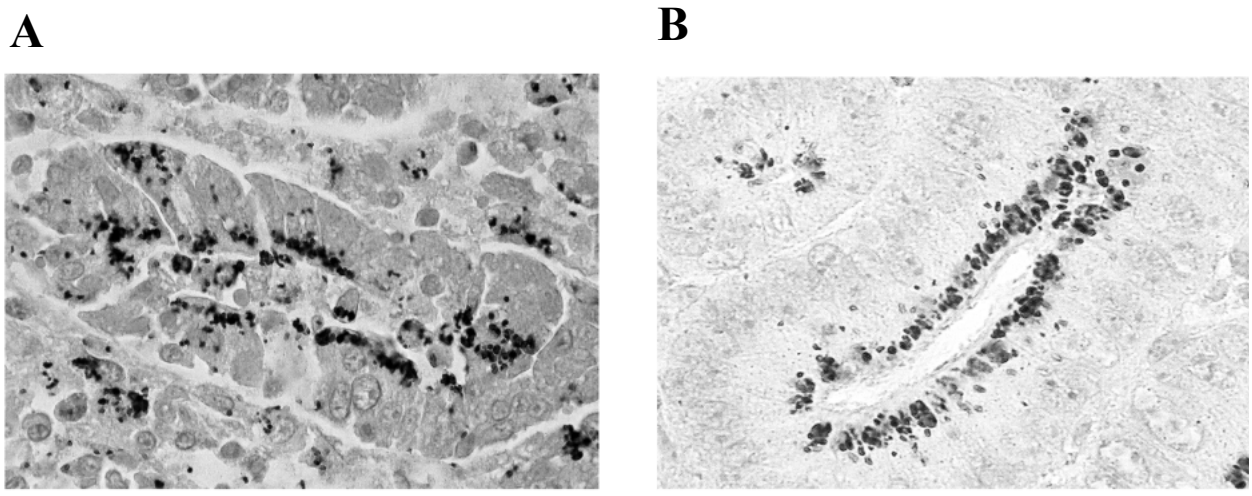

Fig. 2. Identification of *L. intracellularis* in the crypt cells of the ileum. (A) The ileum of a pig and (B) the ileum of a hamster experimentally infected with *L. intracellularis*. The presence of *L. intracellularis* was confirmed by immunohistochemistry with *L. intracellularis*-specific monoclonal antibodies. Magnification, × 400.

(Fig. 1B). The isolate of *L. intracellularis* (PHE/KK421) has been deposited in the Korean Collection for Type Culture (KCTC 10686BP).

The clinical signs, including diarrhea, depression and weight loss, were observed in all pigs infected with *L. intracellularis* from day 11 to day 21 PI. Gross and histological lesions of PE were observed in all infected pigs by autopsy after sacrifice on day 21 PI. A severely thickened mucosa was identified along the middle to terminal ileum, as has been reported in other studies (data not shown). A lot of *L. intracellularis* was identified in the ileal tissue by immunohistochemistry with the *L. intracellularis*-specific monoclonal antibodies (Fig. 2A). Pigs that had been infected with the ileal homogenates prepared from a pig with an acute hemorrhagic PE also produced typical clinical signs and PE lesions (data not shown). However, *L. intracellularis* was not detected in the mesenteric lymph nodes of the infected pigs. Similar pathological changes and the presence of *L. intracellularis* were observed in the ileum of the hamsters infected with the pure bacteria (Fig. 2B). The phenotypic study indicated that the Korean isolate was almost identical with other European and North American isolates of *L. intracellularis* (data not shown) [6, 11, 12]. In conclusion, this study reports the first isolation of *L. intracellularis* in Korea and describes the experimental reproductive infections produced in pigs and hamsters with pure culture of the bacterium.

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REFERENCES

1. Elder, R. O., Duhamel, G. E., Mathiesen, M. R., Erickson, E. D., Gebhart, C. J. and Oberst, R. D. 1997. *J. Vet. Diagn. Invest.* **9**: 281–286.
2. Guedes, R. M. C. and Gebhart, C. J. 2003. *J. Vet. Diagn. Invest.* **15**: 438–446.
3. Guedes, R. M. C, Winkelman, N. L. and Gebhart, C. J. 2003. *Vet. Rec.* **153**: 432–433.
4. Jasni, S., McOrist, S. and Lawson, G. H. K. 1994. *Vet. Microbiol.* **41**: 1–9.
5. Jones, G. F., Ward, G. E., Murtaugh, M. P., Lin, G. and Gebhart, C. J. 1993. *J. Clin. Microbiol.* 31: 2611–2615.
6. Knittel, J., Larson, D. I., Harris, D. L., Roof, M. B. and McOrist, S. 1996. *J. Swine Health Prod.* **4**: 119–122.
7. Lawson, G. H. K., McOrist, S., Jasni, S. and Mackie, R. A. 1993. *J. Clin. Microbiol.* **31**: 1136–1142.
8. Lee, S. W., Kim, T. J., Park, S. Y., Song, C. S., Chang, H. K., Yeh, J. K., Park, H. Y. and Lee, J. B. 2001. *Kor. J. Vet. Sci.* **2**: 209–212.
9. McOrist, S., Barcellos, D. E. and Wilson, R. J. 2003. *Pig J.* **51**: 26–35.
10. McOrist, S., Boid, R., Lawson, G. H. K. and McConell, I. 1987. *Vet. Rec*. **121**: 421–422.
11. McOrist, S., Gebhart, C. J., Boid, R. and Barns, S. M. 1995. *Int. J. Syst. Bacteriol.* **45**: 820–825.
12. McOrist, S. and Gebhart, C. J. 2004. *In*: Bergey's Manual of Systematic Bacteriology, 2nd ed. (Garrity, G. M. ed.), Williams and Wilkins, Baltimore.
13. McOrist, S., Jasni, S., Mackie, R. A., MacIntyre, N., Neef, N. and Lawson, G. H. K. 1993. *Infect. Immun.* **61**: 4286–4292.