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THE FIRST MOLECULAR INVESTIGATION OF *Lawsonia intracellularis* IN DROMEDARY CAMELS

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ABSTRACT

Lawsonia intracellularis is an obligate intracellular bacterium that causes proliferative enteropathy in pigs and horses. Although infection with prompt clinical findings is only reported in few species, the broad range of hosts has been documented for this organism. The aim of the present research was to investigate the possible presence, and occurrence of *L. intracellularis* in dromedary camels. Crude DNA was extracted from 95 faecal samples and was subsequently subjected to a specific and sensitive nested-PCR assay. Overall, three samples (3.1%) were shown to carry *L. intracellularis*. This represents the first report of *L. intracellularis* infection in dromedary camels. The result of this study indicates that this organism has been evolved to infect wide range of hosts, but the host preferences, pathogenic capacities and species-specificity of this organism need to be explored in different animal species.

Key words: Iran, *Lawsonia intracellularis*, nested-PCR, one-humped camel, proliferative enteropathy

Lawsonia intracellularis is a curved Gram-negative obligate intracellular bacterium; the extracellular form has single, long polar flagellum and darting motility. It is the only species in the genus; possesses a small genome of 1.45Mb and harbors three plasmids (Gebhart and Guedes, 2010). *L. intracellularis* cause an infectious intestinal hyperplastic disease named proliferative enteropathy (PE). PE is an economically important disease in pigs, but concerns are also increasing on the significance of the disease in horses in recent years. The disease in pigs has two forms: the sporadic proliferative haemorrhagic enteropathy, and a common form, which is characterised by mucosal hyperplasia, mild diarrhoea, and reduced performance. Infection has been reported in wide variety of wild and domestic animals including pigs, horses, hamsters, sheep, deer, guinea pigs, foxes, dogs, rabbits, ferrets, rats, ostriches and non-human primates. Although the wide range of hosts has been observed, the natural infection is best understood in pigs, horses and to a lesser extent in hamsters (Guedes, 2008; Pusterla and Gebhart, 2013).

Understanding the epidemiology of disease is intriguing due to some unique features of the etiological agent. In fact, in spite of broad range of hosts, *L. intracellularis* shows very limited genetic diversity and phenotypic differences (Guedes, 2008). On the other hand, new research show species-specific preference in isolates from pigs and horses. Finding the free-living natural reservoirs is particularly important in epidemiology of PE because it can help to clarify the unique properties of this organism in affecting broad range of hosts. The aim of the present study was to investigate the presence of *L. intracellularis* in camels by the previously validated sensitive and highly specific nested-PCR method (Jones *et al,* 1993; McCormick *et al,* 1995; Nascimento Chiriboga *et al,* 1999).

Materials and Methods

Samples were collected using sterile cotton swabs from the rectum of 95 three to six months old one-humped dromedary camel calves in Golestan province in northeast of Iran. All cases were apparently healthy, except four calves suffering from mild diarrhoea. The samples were transported to the laboratory within 24 h of collection in ice buckets. DNA was extracted from faecal swabs using a commercial DNA extraction kit (Cinnagen, Iran). In brief, swab tips were soaked in 500 µl PBS for one hour at room temperature, after vigorous vortex the tips were discarded. Then, the faecal suspension centrifuged at 1500 rpm for 5 min to settle down the large faecal materials; 100 µl of the supernatant was transferred to a new tube and DNA was extracted according to the manufacturer's instruction. Nested-PCR was conducted in two separate steps using two sets of primer pairs as described previously (Jones *et al,* 1993). The first PCR

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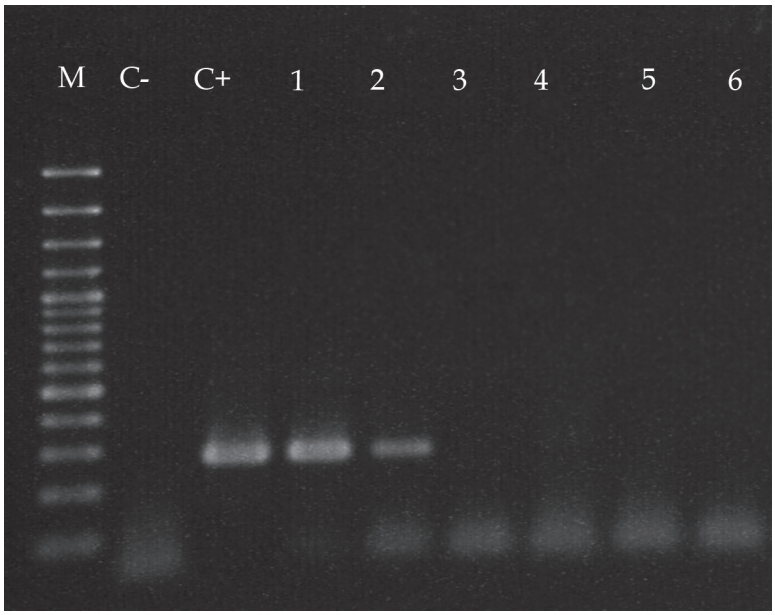

Fig 1. Specific amplicon (270 bp) of *Lawsononia intracellularis* in second step of nested-PCR showing two positive samples from camels (Lanes 1, 2), M, DNA marker 100bp-plus C-, Negative control, C+, Positive control (OS-223).

carried out using LIA (5´- tatggctgtcaaacactcc -3´) and LIB (5´- tgaaggtattggtattctc -3´) primer pairs. One microliter of the first step PCR was used as template in the second PCR using a pair of internal primers including LIC (5´- ttacaggtgaagttattgg -3´) and LID (5´- ctttctcatgtcccataagc -3´). PCR conditions were similar to the described values in previous study (Tomanova *et al,* 2003). The PCR products were electrophoresed in 1.5% agarose gels and visualised after staining with ethidium bromide. Positive and negative controls were included in each PCR reaction. The positive control was *L. intracellularis* that was previously identified in an ostrich chick in our laboratory (OS-223; Gene Bank accession number KF199338.1).

Results and Discussion

The results showed that three faecal samples (3.1%) carried the target organism. Fig 1 shows two positive samples obtained from camels in the present study. To the best of our knowledge this represents the first evidence that shows dromedary camel can be the reservoir for *L. intracellularis*. Considering the results of the present study, the known host range for this intracellular bacterium has been increased again. The low prevalence of fecal shedding in young camels indicates that this species is most probably the accidental host for the organism. Pigs have never been raised commercially in Iran, but wild boars inhabit some parts of the studied area. In addition, northeast of Iran is the most popular region for raising, training and racing of horses.

Interestingly, two of the positive samples were from two diarrhoeic camel calves, and the other one was obtained from an asymptomatic animal. Although the natural disease with overt clinical findings has not yet reported in many species, the possibility of mild gastrointestinal disorder needs further evaluation. Serological assays are preferred over molecular methods for studying the epidemiology of infection in unknown hosts, because they also reveal the previous contacts with the organism. For better understanding, the combinations of molecular, serological and pathological findings are required.

The challenging aspect in epidemiology of PE is the remarkable difference in species-specificity of different *L. intracellularis* isolates as shown in some brilliant research recently. Vannucci *et al* (2012) showed the host specificity of porcine and horse isolates, resulting in longer fecal shedding, prompt seroconversion, and pathological changes in animals challenged with the strains of the same species origin. Sampieri *et al* (2013) by studying the laboratory animal models, showed the host specificity of porcine and horse isolates in hamsters and rabbits, respectively. This apparent host specific preference occurs in spite of the modest genotypic and phenotypic differences. Isolation of Lawsonia needs strict environmental conditions and dividing cells; therefore, there are only few characterised isolates available in the reference laboratories. Isolation and genomic comparison of this organism from different hosts could potentially help unraveling the unique evolutionary mechanism of *L. intracellularis*.

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