Jundishapur J Microbiol. 2016 April; 9(4):e34140. doi: 10.5812/jjm.34140.

Published online 2016 April 23. **Letter**

Molecular Typing of *Eimeria ahsata* and *E. crandallis* Isolated From Slaughterhouse Wastewater

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Received 2015 October 28; **Revised** 2016 February 02; **Accepted** 2016 February 07.

Keywords: 18S rRNA Gene, Iran, Wastewater, *Eimeria ahsata*, *Eimeria crandallis*

Dear Editor,

The *Eimeria* species are host-specific protozoan parasites that cause the disease known as coccidiosis in a variety of animals including cattle, sheep, goats, pigs and poultry throughout the world (1). These parasites invade epithelial tissues of the intestine, causing severe damage in the host and result in significant economic losses (2). At least twelve species of bovine *Eimeria*, ten species of ovine *Eimeria* and nine species of caprine *Eimeria* occur in Iran (3-5). *Eimeria ahsata* and *E. crandallis* are two of a group of ovine coccidia of what might be called the arloingi type (6). Conventionally, microscopic examination of Eimerian oocytes is the only practical method to discriminate species of *Eimeria* (2, 7, 8). However, the particular morphology of a given species may vary considerably. Accordingly, microscopic differentiation is not reliable because several species have confusing features, along with the presence of intraspecies variation (7). Therefore, morphological observations should not be used as isolated criterion for differentiation of species (9).

Thus, molecular assays have proven useful for the identification of *Eimeria* spp. to overcome the limitations of these traditional procedures (7, 10). However, hematological and biochemical changes in blood serum, and histopathological lesions of ovine coccidiosis in sheep and lambs naturally infected by *E. crandallis* and *E. ahsata* were mentioned in previous studies (11), but no information is available about the genetic characterization and phylogenic positions of *E. crandallis* and *E. ahsata*. In the present study, the 18S rRNA gene was employed as a molecular genetic approach to investigate the phylogenetic analysis and DNA sequence variations of *E. crandallis* and *E. ahsata* compared with other *Eimeria* that exist in the GenBank database. Therefore, the present study was undertaken to identify genetic characteristics of *E. ahsata* and *E. crandallis* in slaughterhouse wastewater samples.

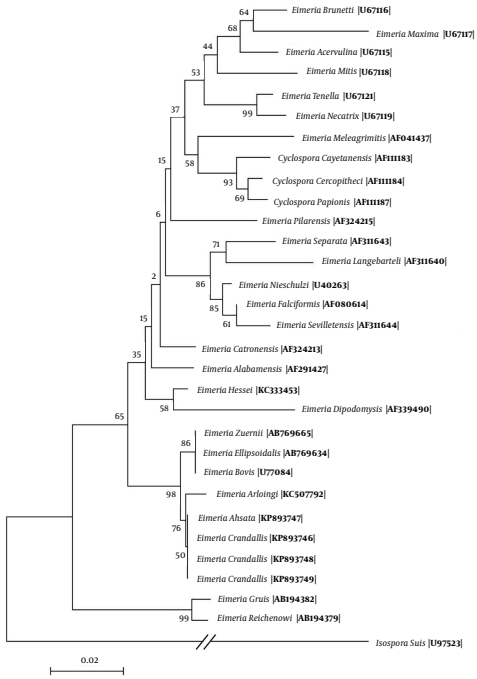
Twenty-four grab samples of untreated wastewater (5 L each) were collected from the two slaughterhouse treatment plants located in the southwest suburban area of Tehran, Iran. The animals slaughtered in these two slaughterhouses included cattle, sheep and goats. Water samples were concentrated by the centrifugal (water-ether) concentration procedure, as previously described (12). Oocytes were sporulated in a 2% (w/v) potassium dichromate ($K_2Cr_2O_7$) solution at room temperature (25°C) for three weeks. The sporulated oocytes were partially identified as *E. ahsata* and *E. crandallis* based on the morphology of oocytes at × 40 and × 1,000 magnifications (13). DNA was extracted using an AccuPrep® stool DNA extraction kit (Bioneer Corporation, Daejeon, South Korea) according to the manufacturer's protocol. An approximately 636-bp fragment of DNA extract was PCR amplified on the basis of the 18S rRNA gene, as previously described (14). All PCR amplicons were sequenced in both directions on an automated DNA analyzer (ABI 3730 XL, Bioneer, South Korea).

Unique nucleotide sequences described in this work were deposited in GenBank and accession numbers KP893746, KP893747, KP893748 and KP893749 were provided for Iranian *E. ahsata* and *E. crandallis* sequences of 18S rRNA.

Sporulated oocytes of *E. ahsata* and *E. crandallis* were

identified in all wastewater samples three weeks after their storage at 25°C. All samples that were found to be positive by microscopic evaluation were confirmed to be *Eimeria* by PCR. BLAST search of our 18S rRNA sequences against those previously published for other *Eimeria* spp. revealed the highest similarity (99% - 100% homology) with *E. ahsata* and *E. crandallis*, with differences observed at 2 - 4 nucleotides. Genetic distances among *Eimeria* species were in the range of 0.002 to 1.264 nucleotide substitutions per 100 bp. There was a minor difference in the sequence of 18S rRNA gene in *E. ahsata* and *E. crandallis* with *E. arloingi* available in GenBank. Phylogenetic analysis showed that one clade contained *E. ahsata*, *E. crandallis* and *E. arloingi* (the most pathogenic *Eimeria* in goats), and *E. ahsata* and *E. crandallis* grouped together in one clade (Figure 1).

Figure 1. Phylogenetic Tree Based on 18S rRNA Sequences, Constructed According to the NJ Method, Showing the Position of *E. ahsata*, *E. crandallis* and Other *Eimeria* Species *Isosporasuis* is Used as an Outgroup



The percentage of replicate tree in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches.

Prior to this work, *Eimeria* parasites had not been reported in domestic wastewater samples. However, in the study conducted by Ben Ayed et al. (15) in Tunisia, *E. alabamensis*, *E. ahsata* and *E. crandallis* were found in urban wastewater, which was probably the result of effluents from cattle slaughterhouses in the city or fertilizers of animal origin used in parks and boulevards. In the present study, the microscopic examination resulted in positive results for all water samples from both slaughterhouse treatment plants. The presence of positive samples for *E. ahsata* and *E. crandallis* was confirmed by PCR assay.

In the present study, 18S rRNA sequences of *E. crandallis* and *E. ahsata* had considerable homology with *E. arloingi*, *E. bovis* and *E. zuernii* (98% - 99%). Similarly, Khodakaram-Tafti et al. (4) reported a close relationship between *E. arloingi* and bovine (*E. bovis* and *E. zuernii*) and ovine (*E. ahsata* and *E. crandallis*) coccidia, based on the 18S sequence analyses. It has also been shown that there is a similarity between these two species (*E. crandallis* and *E. ahsata*) and *E. arloingi*, based on microscopic evaluation of their sporulated oocytes. Identically, *E. ahsata* and *E. crandallis* in sheep, *E. arloingi* in goats and *E. bovis* and *E. zuernii* in cattle are highly pathogenic and formed a monophyletic group in the position away from other members in spite of many different biological characteristics and the histopathological lesions. The phylogram based on the 18S rRNA sequences showed that one clade contained *E. ahsata*, *E. crandallis* and *E. arloingi*, and *E. ahsata* and *E. crandallis* grouped together in one clade. Similarly, Bush (16) reported a tendency provided by the phylogenetic analysis of avian *Eimeria* for *E. necatrix* and *E. tenella*, the most pathogenic *Eimeria* in chicken.

While the 18S rRNA sequence may not eventually prove to be useful to examine in greater detail the magnitude of population variation of a single *Eimeria* species, it can be used to identify Eimerian oocytes in environmental samples.

Acknowledgments

The authors thank Fatemeh Tarighi and Tahereh Rezaeian for technical assistance.

Footnotes

Authors' Contribution: The overall implementation of this study was the results of efforts of all authors. All authors read and approved the final manuscript.

Funding/Support: This work was supported by the institute for environmental research (IER) and center for research of endemic parasites of Iran (CREPI), Tehran University of Medical Sciences (TUMS), Iran (grant no. 92-02-160-23616).

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