

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

Kareem Hatam Nahavandi,<sup>1</sup> Amir Hossein Mahvi,<sup>2</sup> Mehdi Mohebali,<sup>1,3</sup> Hossein Keshavarz,<sup>1</sup> Sasan Rezaei,<sup>1</sup> Hamed Mirjalali,<sup>4,5</sup> Samira Elikaei,<sup>1</sup> and Mostafa Rezaeian<sup>1,5</sup>

<sup>1</sup>Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

<sup>2</sup>Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

<sup>3</sup>Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, IR Iran

<sup>4</sup>Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences (SBUMS), Tehran, IR Iran

<sup>5</sup>Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

\*Corresponding author: Mostafa Rezaeian, Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-2188973901, E-mail: rezaian@tums.ac.ir

**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 oocysts 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 phylogenetic 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.

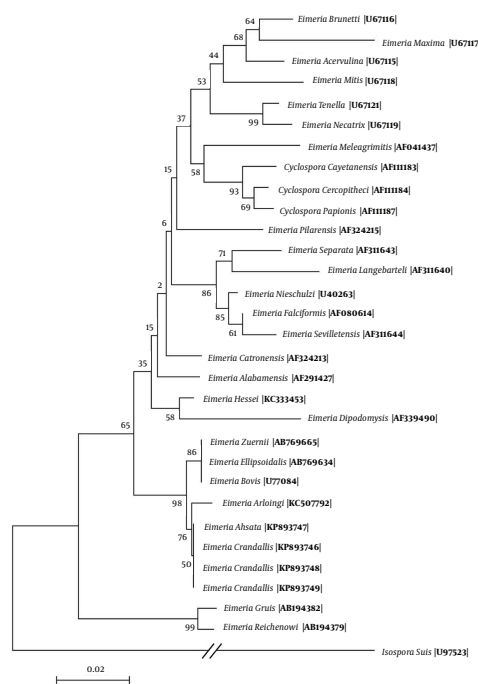
Twenty-four grab samples of untreated wastewater (5 L each) were collected from the two slaughterhouse treatment plants located in the southwest suburban area of Tehran, Iran. The animals slaughtered in these two slaughterhouses included cattle, sheep and goats. Water samples were concentrated by the centrifugal (water-ether) concentration procedure, as previously described (12). Oocysts were sporulated in a 2% (w/v) potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) solution at room temperature (25°C) for three weeks. The sporulated oocysts were partially identified as *E. ahsata* and *E. crandallis* based on the morphology of oocysts 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 oocysts 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 *Isospora suis* 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 Rezaei 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).

### References

- Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10 ed. London: W.B. Saunders; 2007.

2. Blake DP, Qin Z, Cai J, Smith AL. Development and validation of real-time polymerase chain reaction assays specific to four species of *Eimeria*. *Avian Pathol.* 2008;**37**(1):89-94. doi: [10.1080/03079450701802248](https://doi.org/10.1080/03079450701802248). [PubMed: [18202955](https://pubmed.ncbi.nlm.nih.gov/18202955/)].
3. Kheirandish R, Nourollahi-Fard SR, Eslah E. The prevalence and pathology of ovine coccidiosis in Kerman, Iran. *Eurasian J Vet Sci.* 2012;**28**(4):194-8.
4. Khodakaram-Tafti A, Hashemnia M, Razavi SM, Sharifiyazdi H, Nazifi S. Genetic characterization and phylogenetic analysis of *Eimeria arloingi* in Iranian native kids. *Parasitol Res.* 2013;**112**(9):3187-92. doi: [10.1007/s00436-013-3494-0](https://doi.org/10.1007/s00436-013-3494-0). [PubMed: [23779225](https://pubmed.ncbi.nlm.nih.gov/23779225/)].
5. Bahrami S, Alborzi AR. Prevalence of subclinical coccidiosis in river buffalo calves of southwest of Iran. *Acta Parasitol.* 2013;**58**(4):527-30. doi: [10.2478/s11686-013-0167-1](https://doi.org/10.2478/s11686-013-0167-1). [PubMed: [24338314](https://pubmed.ncbi.nlm.nih.gov/24338314/)].
6. Levine ND, Smith WN, Davis LR, Ivens V. A redescription of the oocysts of *Eimeria ahsata*, Honess, 1942, from the domestic Sheep. *Proc Helm Soc Wash.* 1962;**29**(1):87-90.
7. Kawahara F, Zhang G, Mingala CN, Tamura Y, Koiwa M, Onuma M, et al. Genetic analysis and development of species-specific PCR assays based on ITS-1 region of rRNA in bovine *Eimeria* parasites. *Vet Parasitol.* 2010;**174**(1):49-57. doi: [10.1016/j.vetpar.2010.08.001](https://doi.org/10.1016/j.vetpar.2010.08.001). [PubMed: [20817404](https://pubmed.ncbi.nlm.nih.gov/20817404/)].
8. Kaya G, Dale C, Maudlin I, Morgan K. A novel procedure for total nucleic acid extraction from small numbers of *Eimeria* species oocysts. *Turkiye Parazitoloj Derg.* 2007;**31**(3):180-3. [PubMed: [17918054](https://pubmed.ncbi.nlm.nih.gov/17918054/)].
9. Lopez G, Figuerola J, Soriguer R. Time of day, age and feeding habits influence coccidian oocyst shedding in wild passerines. *Int J Parasitol.* 2007;**37**(5):559-64. doi: [10.1016/j.ijpara.2006.12.014](https://doi.org/10.1016/j.ijpara.2006.12.014). [PubMed: [17289051](https://pubmed.ncbi.nlm.nih.gov/17289051/)].
10. Carvalho FS, Wenceslau AA, Teixeira M, Matos Carneiro JA, Melo AD, Albuquerque GR. Diagnosis of *Eimeria* species using traditional and molecular methods in field studies. *Vet Parasitol.* 2011;**176**(2-3):95-100. doi: [10.1016/j.vetpar.2010.11.015](https://doi.org/10.1016/j.vetpar.2010.11.015). [PubMed: [21167646](https://pubmed.ncbi.nlm.nih.gov/21167646/)].
11. Ghanem MM, Abd El-Raof YM. Clinical and Haemato-Biochemical studies on lamb Coccidiosis and changes following amprolium and sulphadimthoxine therapy. *Benha Vet Med J.* 2005;**16**(2):286-99.
12. Bertrand I, Schwartzbrod J. Detection and genotyping of *Giardia duodenalis* in wastewater: relation between assemblages and faecal contamination origin. *Water Res.* 2007;**41**(16):3675-82. doi: [10.1016/j.watres.2007.02.043](https://doi.org/10.1016/j.watres.2007.02.043). [PubMed: [17434561](https://pubmed.ncbi.nlm.nih.gov/17434561/)].
13. Soulsby EJJ. Helminths, arthropods and protozoa of domesticated animals. 7 ed. London: Bailliere Tindall; 1982.
14. Orlandi PA, Carter L, Brinker AM, da Silva AJ, Chu DM, Lampel KA, et al. Targeting single-nucleotide polymorphisms in the 18S rRNA gene to differentiate *Cyclospora* species from *Eimeria* species by multiplex PCR. *Appl Environ Microbiol.* 2003;**69**(8):4806-13. [PubMed: [12902274](https://pubmed.ncbi.nlm.nih.gov/12902274/)].
15. Ben Ayed L, Yang W, Widmer G, Cama V, Ortega Y, Xiao L. Survey and genetic characterization of wastewater in Tunisia for *Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bienersi*, *Cyclospora cayentanensis* and *Eimeria* spp. *J Water Health.* 2012;**10**(3):431-44. doi: [10.2166/wh.2012.204](https://doi.org/10.2166/wh.2012.204). [PubMed: [22960487](https://pubmed.ncbi.nlm.nih.gov/22960487/)].
16. Bush AO. Parasitism: the diversity and ecology of animal parasites. United Kingdom: Cambridge University Press; 2001.