Genotypic Characterization of *Enterocytozoon bieneusi* in Specimens from Pigs and Humans in a Pig Farm Community in Central Thailand\(^\dagger\)

Saovanee Leelayoova,\(^1\) Phunlerd Piyaraj,\(^1\) Ittisak Subrungruang,\(^1\) Wacharee Pagornrat,\(^1\) Tawee Naaglor,\(^1\) Sirowan Phumklan,\(^2\) Paanjit Taamasri,\(^1\) Jiraporn Suwanasri,\(^2\) and Mathirut Mungthin\(^1\)

Department of Parasitology, Phramongkutklao College of Medicine, 315 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand,\(^1\) and Nongpaklong Public Health Center, Nongpaklong District, Nakonprathom 73000, Thailand\(^2\)

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We determined that 15.7% of pigs and 1.4% of humans in a pig farm community in central Thailand harbored *Enterocytozoon bieneusi*. Genotyping of *E. bieneusi* from pigs showed genotypes O, E, and H. However, only genotype A was found in human subjects. This indicates nonzoonotic transmission of *E. bieneusi* in this community.

*Enterocytozoon bieneusi* is an opportunistic organism causing diarrhea in human immunodeficiency virus (HIV)-positive patients, in whom it has a prevalence of 2 to 50% (5). The infection not only has been reported to occur in immunocompromised hosts but also has been found in healthy individuals (14, 20). This organism can infect a broad range of animals (4, 12, 16, 18, 22, 23). Genotypes of *E. bieneusi* in humans and animals are differentiated using the polymorphisms of the internal transcribed spacer (ITS) sequence of the rRNA gene (4, 11). To date, at least 70 ITS genotypes have been reported to infect humans and animals (2, 6). The zoonotic nature of *E. bieneusi* was confirmed because ITS genotypes found in domestic and wild animals had been reported to occur in immunosuppressed hosts (22). In Thailand, we reported genotypes O, E, and *PigEBITS* 7, which have previously been identified in pigs (3, 4) and in Thai HIV-infected patients (9). This study aimed to identify the ITS genotypes of *E. bieneusi* in pigs and humans who worked in or lived near pig farms to investigate the transmission of *E. bieneusi* among these host species.

A cross-sectional study of *E. bieneusi* infection was conducted in a community in Nakorn Pathom Province, Central Thailand, January, 2005. This community is composed primarily of four pig farms, a residential area, and a school. The residential area, but not the school, was near the pig farms. Fecal specimens were collected from school children and those who were living in this community, including pig farmers. Fecal specimens were also collected from pigs of four farms and examined for *E. bieneusi*. The study was approved by the Ethics Committee of the Royal Thai Army Medical Department. Informed consent was obtained from each adult individual and from parents of school children before enrollment in the study.

Fecal specimens from pigs and humans were examined for microsporidian spores using gram-chromotrope staining under light microscopy (13). DNA was prepared from water-ethyl acetate-concentrated stool specimens using FTA filter paper (Whatman Bioscience, United Kingdom) as previously described (21). Amplification of the ITS region of the small-subunit rRNA gene was performed using primers under conditions described by Katzwinkel-Wladarsch et al. (8). For specimens with PCR-negative results, PCR amplification was repeated at least twice. DNA sequencing was conducted by Macrogen Inc., Seoul, Republic of Korea. Nucleotide sequences were determined using the Sequencher program (Gene Codes Corporation, Inc.), and multiple alignment was performed using Clustal X 1.83 for Windows (24). The genotype of each specimen was confirmed by determining the homology of the sequenced PCR product with the published sequence.

A total of 268 pig fecal samples were collected. Pigs aged between 21 days and 22 months were examined for *E. bieneusi* infection. Microsporidian spores were found in 0.7% of pig fecal samples using gram-chromotrope staining, while a greater prevalence of *E. bieneusi* infection, 15.7%, was detected by PCR. The prevalences of *E. bieneusi* infection among the four farms and different age groups are presented in Table 1. A significantly higher prevalence of *E. bieneusi* was found in pigs aged 2 to 3.9 months than in pigs of other age groups (chi-square test, \(P < 0.001\)). Multivariate analysis confirmed that pigs aged 2 to 3.9 months had a 5.3-times-greater risk of infection than pigs in other age groups (95% confidence interval, 2.6 to 10.6; \(P < 0.001\)). Of these 42 *E. bieneusi*-positive samples, 21 (50%) were successfully characterized by sequencing analysis, and the organism was identified as being of genotypes O (12 samples [57.1%]), E (8 samples [38.1%]), and H (1 sample [4.8%]).

To examine *E. bieneusi* infection in humans living near pig farms, we collected a total of 499 fecal specimens from school children (279, 55.9%), agricultural workers (53, 10.6%), wage earners (51, 10.2%), merchants (24, 4.8%), officers (17, 3.4%), pig farm workers (12, 3.4%), factory workers (6, 1.2%), and others (57, 11.4%). All these fecal specimens showed negative

\(^{\dagger}\) Corresponding author. Mailing address: Department of Parasitology, Phramongkutklao College of Medicine, Ratchawithi Rd., Bangkok 10400, Thailand. Phone and fax: 662 354 7761. E-mail: s_leelayoova@scientist.com.

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E. bieneusi was identified in seven individuals: five adults and two school children. Thus, the prevalence of human E. bieneusi infection in this community was 1.4%. All E. bieneusi samples found in these persons were of genotype A. No E. bieneusi organism was detected in the fecal specimens of pig farm workers.

In this study, the PCR method showed a higher sensitivity for the detection of E. bieneusi than gram-chromotrope staining. Spore shedding of E. bieneusi in asymptomatic humans and pigs was intermittent and sometimes too low to be detected under a microscope (1, 15). Therefore, assessing fecal samples by microscopic examination might underestimate the prevalence of E. bieneusi infection. The present study showed that the average prevalence of E. bieneusi infection in pigs ranged from 30.5% to 94% (1, 3, 19). The differences in prevalence among these studies may be due to diverse husbandry practices and geographical seasonal variation.

E. bieneusi is classified into human-specific genotypes, mainly genotypes A, B, and C and non-host-specific genotypes. The present study confirmed that pigs harbor some non-host-specific genotypes, i.e., E and O. However, these ITS genotypes were not found in pig farm workers or in healthy persons living near pig farms. In contrast, E. bieneusi genotype A was found in seven healthy individuals who had no gastrointestinal symptoms. The HIV status of all E. bieneusi-positive individuals was also negative. Since E. bieneusi genotype A is a human-specific genotype (1, 9, 17), human infection with E. bieneusi in this community was not zoonotic. The lack of zoonotic transmission identified in this population was possibly due to a few cases who were directly exposed to pig feces. The risk factors of E. bieneusi infection were determined using standardized questionnaires concerning demographic data and sanitary behavior. However, univariate and multivariate analyses could not identify significant risk factors since the number of positive cases was too small. Two E. bieneusi-positive adults lived in the same house, indicating person-to-person transmission. The other positive cases lived in the same neighborhood, where transmission by food or water cannot be ruled out.

In conclusion, the present study, as with previous studies in Thailand, indicates that non-host-specific and human-specific genotypes could infect HIV-infected patients (9, 10). In contrast, only human-specific genotypes infected healthy individuals.

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REFERENCES


<table>
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<tr>
<th>Source of specimens</th>
<th>No. of specimens (%) of total</th>
<th>E. bieneusi-positive specimens (%)</th>
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<tr>
<td>Farms:</td>
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<tr>
<td>1</td>
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<tr>
<td>4</td>
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<tr>
<td>&lt;1</td>
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<td>1 (3.4)</td>
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<tr>
<td>≥8</td>
<td>17 (6.3)</td>
<td>2 (11.8)</td>
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<td>Total</td>
<td>268 (100)</td>
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