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Atypical presentations of cutaneous leishmaniasis: A systematic review

Camilla Barros Mendes, Luis Chaves Maia, Gustavo Guelfo Soares, Isara Parente Pimenta Teodoro, Maria do Socorro Vieira Gadelha, Cibelle DeMabona Lima da Silva, Marcus Antonio Feres da Silva

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**Frequency and risk factors analysis of Escherichia coli O157:H7 in Ball-cattle**

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#### Highlights

- The prevalence of E. coli O157:H7 on Ball-cattle covering 4 Sub districts i.e. Petang, Abatamareny, Mengen, and Kubiwat 8.30% (152/1845).
- Molecular analysis of 165 rRNA genes showed high similarity (>96.5%) of locally strains against reference.
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- Wolbachia infection reduces mosquito population growth at high larval density conditions

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<tr>
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<tbody>
<tr>
<td>Low density</td>
<td>Intra 90%</td>
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<tr>
<td></td>
<td>Inter 85%</td>
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<td>High density</td>
<td>Intra 70%</td>
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Frequency and risk-factors analysis of *Escherichia coli* O157:H7 in Bali-cattle

I Wayan Suardana, Dyah Ayu Widiasih, Widagdo Sri Nugroho, Michael Haryadi Wibowo, I Nyoman Suyasa

**A R T I C L E   I N F O**

**Keywords:**
Bali cattle
*E. coli* O157:H7
Molecular analysis
Risk factors

**A B S T R A C T**

Cattle are known as the main reservoir of zoonotic agents verocytotoxin-producing *Escherichia coli*. These bacteria are usually isolated from calves with diarrhea and/or mucus and blood. Tolerance of these agents to the environmental conditions will strengthen of their transmission among livestock. A total of 238 cattle fecal samples from four sub-districts in Badung, Bali were used in this study. Epidemiological data observed include cattle age, sex, cattle rearing system, the source of drinking water, weather, altitude, and type of cage floor, the cleanliness of cage floor, the slope of cage floor, and the level of cattle cleanliness. The study was initiated by culturing samples onto eosin methylene blue agar, then Gram stained, and tested for indole, methyl-red, voges proskauer, and citrate. Potential *E. coli* isolates were then cultured onto sorbitol MacConkey agar, and further tested using O157 latex agglutination test and H7 antisera. Molecular identification was performed by analysis of the 16S rRNA gene, and epidemiological data was analyzed using STATA 12.0 software. The results showed, the prevalence of *E. coli* O157:H7 in cattle at Badung regency was 6.30% (15/238) covering four sub districts i.e. Petang, Abiansemal, Mengwi, and Kuta which their prevalence was 8.62%(5/58), 10%(6/60), 3.33%(2/60), and 3.33(2/60)% respectively. The analysis of 16S rRNA gene confirmed of isolates as an *E. coli* O157:H7 strain with 99% similarities. Furthermore, the risk factors analysis showed that the slope of the cage floor has a highly significant effect (P < 0.05) to the distribution of infection. Consequently, implementing this factor must be concerned in order to decrease of infection.

1. Introduction

*Escherichia coli* O157:H7 caused multiple food and water-borne outbreaks worldwide and considered as serious threat to public health. The agent produce verocytotoxin which cause diarrhea and hemorrhagic colitis which may cause an adverse effects on the central nervous system, pancreas, lungs and heart with a case fatality rate ranging from 3 to 5% (Mohawk and O’Brien, 2011). Infection by these bacteria is often followed by life-threatening hemolytic uremic syndrome (HUS) and death especially in the elderly and young children (Mendonça et al., 2012). The transmission of bacteria to human is known usually by consumption of undercooked ground beef and unpasteurized milk (Rangel et al., 2005).

Verocytotoxin-producing *E. coli* has been isolated from a variety of animals, particularly ruminants, and cattle are regarded as the main reservoir (Karmali et al., 2010). The proportion of animals infected by this agent varied. The previous study found 52/257 (20%) were recovered from cows, 16/71 (23%) from calves (Blanco et al., 1996), 16/60 (27%) from cattle faeces, and 7/70(10%) from feedlot pens (Oot et al., 2007). The high prevalence was isolated in dairy cattle 144/198 (72.73%) (Ferreira et al., 2014).

Global studies consisting 220,427 cattle were included in the meta-analysis showed the estimated prevalence of *E.coli* O157 in cattle at the global level was 5.68%. The random effects pooled prevalence estimates in Africa, Northern America, Oceania, Europe, Asia and Latin America-Caribbean was 31.20%, 7.35%, 6.85%, 5.15%, 4.69%, and 1.65%, respectively (Islam et al., 2014).

In Indonesia, the study of *E. coli* from calves with diarrhea was initiated by the researchers at Veterinary Research Institute (Kusmiyati and Supar, 1998) who discovered alpha hemolytic *E. coli* isolates which some of them were verocytotoxigenic. The study of Drastini identified the prevalence of this agent in dairy cows was 1.3% (Drastini, 2007).

Furthermore, other researcher (Suardana et al., 2010) found as many as 4/80(5%) of cattle faeces, 2/78(2.6%) of beef, 2/80(2.5%) of chicken
faeces, 2/30 (6.7%) of non-clinically human faeces, and 12/76 (15.8%) of human suffering kidney failure were positive E. coli O157:H7. The study about the adaptation of this agent to environmental changes also conducted by previous researchers who found E. coli O157:H7 survived at 5 °C for 63–70 days, with the moisture content (74%) of faeces (Wang et al., 1996). These facts indicated that faeces as a potential vector for the transmission of this organism. In addition, the horizontal transmission of E. coli O157: H7 may occur during cattle housing. The transmission following ingestion of the pathogen at low levels and that animal hide as an important source of transmission (McGee et al., 2004). Previous study showed that the E. coli O157:H7 occurrence in faeces of feedlot cattle depending on the age of the animal (dominant in young cattle), changes of feed, transportation and hot conditions (Dargatz et al., 1997). Other researchers identified the high infection in cattle is caused by several factors including feed, stress, livestock density, geography, and season (Kudva et al., 1996). Study of risk-factors on bovine infection with verocytotoxigenic producing E. coli in Ontario found that calves > 2 weeks of age were at significantly greater risk of infection than those under 2 weeks (OR = 2.0) and farm-level calf infection was negatively associated with herd size, and the maintenance of a closed herd (Wilson et al., 1993). Based on these facts, identification of the frequency of local strains of E. coli O157:H7 and analyses of risk factors that contributed to the spread of these agents in Bali cattle need to be revealed as primary step of prevention.

2. Materials and methods

2.1. Samples and epidemiological data

Samples of study were collected aseptically by directly rectal palpation from communal cattle using sterile arm-length gloves. Approximately 100 g of fecal samples were collected from each cattle in each farm then immediately placed in sample cool box before transferred to the laboratory for analysis. In order to represent Badung regency, samples were stratified according to sub district with its characterization i.e. Petang, Aiansemal, Mengwi, and Kuta. Sample size that we used following formula n = 4PQL2, where “n” is the sample size, “P” is the assumption of the infection prevalence in the study area, “Q” is (1-P), and “L” is the desired error (Martin et al., 1987), wherein the prevalence infection was 3.5% (Suardana et al., 2014). The 16S rRNA gene was amplified using Platinum PCR Supermix kit (Invitrogen) on Thermocycler Eppendorf Mastercycler personal/PTC 100. The PCR program was carried out in 40 μl reaction volumes containing 2μL DNA template (200 ng/μL), 34 μL PCR Supermix 2x, and 2μL (20 pmol/μL) of each primer. The primers used in this Study i.e. 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and U1492R (5′-GTTACCTGTAGCAGCTT-3′). The PCR amplification was programmed refered to previously with initial DNA denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min. At the end of cycles, it was followed by a final extension at 72 °C for 5 min 5 μL of PCR products were analyzed by electrophoresis (Bio-Rad) in 1% agarose (Gibco BRL) gel, at 90 V for 45 min. The gel was stained with 1% solution of ethidium bromide (50 μL/L) and distained with TBE 1 x for 10 min. Gel was visualized by UV transillumination and recorded by digital camera FE-270 7.1 megapixels (Suardana, 2014).

2.2. Isolation and identification of Escherichia coli

The isolation started by dissolving of 10 g of the fecal samples derived from a mixture of 100 g of each sample with 90 ml of buffered peptone water (BPW). Furthermore, a tenfold series dilution was performed using sterile distilled water and aliquots (100 μL) of each dilution were plated onto eosin methylene blue agar (EMBA), and the plates were incubated at 37 °C for 24 h. A positive result characterized by distinctive metallic green colony, confirmed using Gram staining, and tested for indole, methyl red, voges-proskauer, and citrate (IMViC) medium to ensure as group of fecal coli (Lodish, 2013). The positive results subsequently inoculated onto nutrient agar medium for further investigation.

2.3. Identification of Escherichia coli O157:H7 serotype

Identification of E. coli O157:H7 was carried out by inoculating of potential E. coli isolates onto sorbitol MacConkey agar (SMAC) medium and incubated at 37 °C for 24 h. Positive result on SMAC medium was characterized by colourless colony. Further test was done by reacting of all positive isolates on SMAC medium against E. coli O157 latex agglutination test in order to ensure the isolate was E. coli O157 strain. The identification was ended by testing of isolates with H7 serotype test which were characterized by the precipitation form on the bottom of plate (Suardana et al., 2015).

2.4. Analysis of 16S rRNA gene

2.4.1. Extraction of DNA and PCR

Bacterial DNA was extracted using QIAamp DNA Mini Kits (Qiagen) according to manufacturer’s instructions with slightly modification (Suardana, 2014). The 16S rRNA gene was amplified using Platinum PCR Supermix kit (Invitrogen) on Thermocycler Eppendorf Mastercycler personal/PTC 100. The PCR program was carried out in 40 μl reaction volumes containing 2μL DNA template (200 ng/μL), 34 μL PCR Supermix 2x, and 2μL (20 pmol/μL) of each primer. The primers used in this Study i.e. 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and U1492R (5′-GTTACCTGTAGCAGCTT-3′). The PCR amplification was programmed refered to previously with initial DNA denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min. At the end of cycles, it was followed by a final extension at 72 °C for 5 min 5 μL of PCR products were analyzed by electrophoresis (Bio-Rad) in 1% agarose (Gibco BRL) gel, at 90 V for 45 min. The gel was stained with 1% solution of ethidium bromide (50 μL/L) and distained with TBE 1 x for 10 min. Gel was visualized by UV transillumination and recorded by digital camera FE-270 7.1 megapixels (Suardana, 2014).

2.4.2. Sequencing and phylogenetic analysis

The sequencing of 16S rRNA gene was conducted using genetic analyzer (ABI Prism 3130 and 3130 xl Genetic Analyzer) at Eijkman Institute for Molecular Biology, Jakarta. The sequencing used both primers; Stx2 (F) and Stx2 (R). The sequences were edited using MEGA 5.2 version software. The nucleotide sequence of 16S rRNA gene of E. coli O157:H7 strains that available in the genBank such as E.coli 933W (AE 005174), SM-25(1) (KF768068), KL-48(2) (KF768069) as reference and one nucleotide sequence E. coli O111:H11 (NZ_AKAX01000438) as an out group were used in this study. The sequences were aligned using Clustal W and the phylogenetic analysis was constructed using neighbor joining algorithm (Saitou and Nei, 1987; Tamura et al., 2007). Criteria for species identification is 99% sequence similarity or higher for species assignment and 95% sequence similarity or higher for genus assignment (Bosshard et al., 2003) or minimum 99% sequence similarity and ideally 99.5% sequence similarity or < 1% divergence for species assignment (Janda and Abbott, 2007).

2.5. Data analysis

The positive and negative result per sample which was showed by biochemical test complete with molecular analysis and questioner was analyzed descriptively. The association of infection against risk factors was tested by Chi-Square test (Steel and Torrie, 1996) and Odds Ratio test to determine the strength of association (Martin et al., 1987). All of the data were analyzed by using STATA 12.0 software.

3. Results and discussion

3.1. Descriptive analysis

Fifteen out of 238 samples which were stratified according to four sub districts were positive detected E.coli O157:H7 in survey that was conducted from Mart to August 2013. All of positive isolates characterized by a distinctive metallic green sheen on eosin methylene blue agar.
EMBA) medium, and showed positive reaction on indole and methyl red, but negative reaction on Voges proskauer and citrate test. Furthermore, these isolates formed colourless colonies on selective medium sorbitol MacConkey (SMAC) agar. They were also demonstrated slightly agglutination on E. coli O157 Latex Test Kit, and fluid moderately cloudy of supernatant on tested by H7 antisera. The infection of E. coli O157:H7 in this study was variously in each sub district with the lower prevalence in Mengwi and Kuta but the higher case was found in Petang and Abiansemal sub districts (Table 1).

According to Table 1, the percentage of E. coli O157:H7 positive in Bali cattle was 6.30% distributed in four sub districts i.e. Petang, Abiansemal, Mengwi, and Kuta with prevalence of 8.62% (n = 5/58); 10.0% (n = 6/60); 3.33% (n = 2/60), and 3.33% (n = 2/60), respectively. These results were in accordance with the previous study where the occurrence was 5% (Suardana et al., 2010). On the other hands, the prevalence was higher (27.4%) in dairy cattle in Central Java and Yogyakarta (Sumiarto, 2004).

### 3.2. Molecular analysis of 16S rRNA gene

The molecular analysis of 16S rRNA gene of local strain E. coli O157:H7 has been successfully conducted. Spatial sequences (1351 out of 1500 bp) originated from 10 strains as a representation of this study were alignmed with some database sequences in genBank i.e. E.coli EDL 933 W (AE 005174), E.coli SM 25(1) (KF 768068), and E. coli KL-48(2) (KF768069). On the other hand, all strains also aligned against E. coli O111:H11(NZ_AKAX01000438) as an out group. The result of the analysis in the form of the phylogenetic tree is showed in Fig. 1, and genetically distances among isolates were summarized in Table 2.

### 3.3. Risk factors analysis

As many as ten variables which were subjected in the interview of 238 cattle’ owners, and direct field investigation of 238 fecal samples were analyzed in this study. Cattle’s owners were interviewed using a standardized questionnaire to obtain the information on farm and individual animal-level characteristics and management practices. Most of respondents were women and primary school education, and they were cooperate to share their cattle’s status. The analysis of risk factors showed the infection level of E. coli O157:H7 was frequently correlated with cattle age of > 1 year, male, cattle rearing with housing system, non-tap water as a source of drinking water, and cattle with lower level cleanliness. The infection also showed frequent occurrence in the highland at dry weather. The descriptive analysis of each variable was summarized in Table 3, and the single variable analysis is showed in Table 4.

The study that was performed base on biochemical and molecular analysis denoted 15 out of 238 (6.30%) samples positive E. coli O157:H7. The E. coli O157:H7 differs from other strains of E. coli in being unable to ferment sorbitol. In sorbitol MacConkey agar (SMAC), lactose is replaced by sorbitol, and pathogenic E. coli cannot ferment sorbitol, so this strain uses peptone to grow. This raises the pH of the medium, allowing the pathogenic strain to be differentiated from other non-pathogenic E.coli strains. Detection of E. coli O157:H7 by using SMAC medium has a sensitivity of 100%, specificity of 85%, and accuracy of 86% (March and Ratnam, 1986). Prompt identification was performed after all isolates were positive on the O157 latex test which was known as a simple, efficient and reliable method in detecting of E. coli O157:H7 with a 100% sensitivity and specificity (Farmer and Davis, 1985).

<table>
<thead>
<tr>
<th>Sub districts</th>
<th>Numbers of Fecal-samples</th>
<th>E. coli O157:H7 positive</th>
</tr>
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<tbody>
<tr>
<td>Petang</td>
<td>58</td>
<td>5 (8.62%)</td>
</tr>
<tr>
<td>Abiansemal</td>
<td>60</td>
<td>6 (10.0%)</td>
</tr>
<tr>
<td>Mengwi</td>
<td>60</td>
<td>2 (3.33%)</td>
</tr>
<tr>
<td>Kuta</td>
<td>60</td>
<td>2 (3.33%)</td>
</tr>
<tr>
<td>Total</td>
<td>238</td>
<td>15 (6.30%)</td>
</tr>
</tbody>
</table>

Fig. 1. Phylogenetic tree of local strains of E. coli O157:H7 against some database sequences in genBank. The phylogenetic tree was constructed using Neighbor Joining algorithm of 1351 nucleotides sequence of 16S rRNA gene. The number in the branch of phylogram indicated bootstrap value(%) by 1000 replication multiple, and scale indicated one per 1000 substitution of nucleotide sequences.
According to data in Table 1, the occurrence of \textit{E. coli} O157:H7 in each sub district showed that Petang and Abiansemal sub districts were higher than Mengwi and Kuta sub districts. This fact was supported by the ideal location of both sub districts at northern area of Badung regency as a land field for growing and multiplication of the agent. According to the statistical data of Badung regency, each of the sub districts has an altitude > 350 m above sea levels, and known as an agricultural land. Whereas Mengwi and Kuta sub districts each has an altitude lower than of those, and famously are known as an area of tourism and business. Livelihoods of people in Petang and Abiansemal sub districts are generally as traditional farmers, as well as their activity in the management of livestock (cattle). Furthermore, the geographical condition such as the rainfall, humidity and temperature around 24 °C also support for survival and maintenance of agents.

Molecular analysis of 16S rRNA gene as a new gold standard for specification of bacteria was also performed as a deep confirmation of 15 out of 238 isolates as an \textit{E. coli} O157:H7 strains (Janda and Abbott, 2007). This method has many advantages i.e. by using of 16S rRNA sequences, numerous of bacterial genera and species have been reclassified and renamed, phylogenetic relationships have been determined, and the discovery and classification of novel bacterial species have been facilitated (Bosshard et al., 2003).

The phylogenetic tree of the local strain in Fig. 1 showed that 10 out of 15 strains of \textit{E. coli} O157: H7 as a representation of this study lies in the same clade with positive control ATCC 43894, \textit{E. coli} EDL 933 W (AE 005 174), \textit{E. coli} SM 25 (1) (KF 768 068), and \textit{E. coli} KL-48 (2) (KF768069), but separated by \textit{E. coli} O111: H11 (NZ_AKAX01000438) as an out group. Phylogram in Fig. 1 showed highly probability of local strain genetically linked to the strain of \textit{E. coli} ATCC 43894, as well as other reference strain.

This conclusion was supported by the data in Table 2 which showed all of local strains only had nucleotide differing ranging from 1 to 12 nucleotides against ATCC 43894 control, and \textit{E. coli} nucleotide database that available in the genBank. The conclusion refers to the concept of similarity or nucleotides differences which was proposed previously by some researchers. There were recommended when the similarity of 16S rRNA gene was more than 95% or the nucleotides different less than 1% (15 out of 1500 bp), the query nucleotides should be categorized as the same species (Janda and Abbott, 2007). Those results were well add to the collection of local strain of \textit{E. coli} O157: H7 that have been confirmed molecularly, and have been deposited the 16S sequences in Genbank, namely \textit{E. coli} SM 25 (1) (KF 768 068), and \textit{E. coli} KL-48 (2) (KF768069) derived from cattle and human faeces, respectively.

Furthermore, the risk factors of \textit{E. coli} O157:H7 infection in Bali cattle (Table 2) in Badung regency were more associated to variables of female, age < 1 year, and cattle reared in the cages with their percentages were 6.55, 6.67, and 7.69%, respectively, although all of those factors were not yet significantly affect (p < 0.05) statistically.

The contribution of those factors, especially for the cattle with age < 1 year was resulted by the calves at this age were found kept in their cages and rarely rearing in the field. The observations also showed that the calves in this age usually found in less clean condition as a result of a lot of movement in a narrow space. Moreover, the farmers hardly bathe their calves as results of they were raised without straps. These results also supported by previous study which found a strong effect in young animals (calfes within 2–6-month) were as the high-risk age group (8.6% positive) in contrast to older animals (2.4%). However, there was a tendency of non-significant effect in male calves to have a higher prevalence than heifers within the same age (Nielsen et al., 2002).

On the other hands, several factors like cemented cage-floor, slope of cage floor and cleanliness of cage floor had a contradictory effect to the incidence of \textit{E. coli} O157:H7 infection (Table 3 and 4). These results were different with the previous study which found the occurrence of \textit{E. coli} O157:H7 infection correlated to unclean of cage. The facts
The long contact of cattle with a conventional management especially that reared in the cage resulting in more opportunity of the transmission of agent from manure. Furthermore, the researchers mentioned, environmental adaptations of E. coli play an important role in the persistence and dissemination of this microorganism on farms and increasing transfer of agent from cattle to others (Maule, 2000).

The infection of cattle by E. coli O157, also a substantial contribution to the prevalence of O157:H7 infection in Bali cattle at Badung Regency. E. coli O157:H7 infection in Bali cattle at Badung Regency.

<table>
<thead>
<tr>
<th>No</th>
<th>Variable</th>
<th>Category</th>
<th>Positive</th>
<th>Negative</th>
<th>p-value</th>
<th>OR (95%, CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age of cattle</td>
<td>&gt; 1 year</td>
<td>11</td>
<td>167</td>
<td>0.893</td>
<td>1.08 (0.35 – 3.26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 1 year</td>
<td>4</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sex</td>
<td>Male</td>
<td>4</td>
<td>66</td>
<td>0.810</td>
<td>1.15 (0.38 – 3.48)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>11</td>
<td>157</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rearing system</td>
<td>In the cage</td>
<td>13</td>
<td>156</td>
<td>0.167</td>
<td>0.38 (0.09–1.63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not in the cage</td>
<td>2</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Drinking water source</td>
<td>Non-tap water</td>
<td>11</td>
<td>115</td>
<td>0.102</td>
<td>2.44 (0.80 – 7.46)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tap water</td>
<td>4</td>
<td>108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Weather</td>
<td>Dry weather</td>
<td>14</td>
<td>196</td>
<td>0.527</td>
<td>0.54 (0.07 – 3.92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rainy weather</td>
<td>1</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Altitude</td>
<td>Highland</td>
<td>6</td>
<td>92</td>
<td>0.924</td>
<td>0.96 (0.35 – 2.59)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lowland</td>
<td>9</td>
<td>131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Type of cage floor</td>
<td>Cemented</td>
<td>11</td>
<td>121</td>
<td>0.150</td>
<td>0.45 (0.15–1.38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-cemented</td>
<td>4</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Cleanliness of cage floor</td>
<td>Clean</td>
<td>7</td>
<td>80</td>
<td>0.400</td>
<td>0.66 (0.25–1.75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dirty</td>
<td>8</td>
<td>143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>The slope of the cage floor</td>
<td>Flat</td>
<td>8</td>
<td>177</td>
<td>0.0196</td>
<td>0.33 (0.12–0.86)</td>
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<tr>
<td></td>
<td></td>
<td>Sloping</td>
<td>7</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Cattle cleanliness</td>
<td>Dirty</td>
<td>6</td>
<td>63</td>
<td>0.332</td>
<td>1.63 (0.60–4.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clean</td>
<td>9</td>
<td>160</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: OR: Odds ratio, CI: Confident Interval. *: significant difference (P < 0.05).

generated by the cattle’s owners in the area of study only wipe of the cattle faeces from the cage floor, but it was still a pile up around the cage for a long time. So that, although the cage-floor showed cleanliness, cemented, and sloping floor, there were not guaranteed to prevent the transmission of agent from manure. Furthermore, the researchers mentioned, environmental adaptations of E. coli O157:H7 play an important role in the persistence and dissemination of this microorganism on farms and increasing transfer of agent from cattle to others (Maule, 2000).

Several previous study showed animals that carrying VTEC O157 do not show clinical signs of illness following infection with this organism and shedding is intermittent and transient (Keen et al., 2006; Wang et al., 1996). Shedding has also been shown to be seasonal, with excretion rates peaking in the summer and early autumn (Syngge, 2000). The distribution of this agent also supported by several factors. Irrigations are known have an important contribution to the occurrence of E. coli O157:H7. Irrigations such as irrigation water, swimming water (pools, beaches, and lakes), surface water runoff, and municipal water contaminated with faeces are some of the reservoirs of E. coli O157:H7 (Islam et al., 2004a; Islam et al., 2004b).

Considering all of the risk factors which were observed, the study found the slope of the cage floor of Bali-cattle had a significant effect (p < 0.05) to the transmission of E. coli O157:H7 infection with odds ratio 0.33. This fact indicated this condition as a dominant risk factor in the area of study which must be thoroughly monitored in order to decrease the transmission of E. coli O157:H7 infection.
4. Conclusion

This study demonstrates the occurrence of *Escherichia coli* O157:H7 in Bali-cattle at Badung regency was 6.30% which was distributed in the 4 sub districts i.e. Petang, Abiansemal, Mengwi, and Kuta with prevalence rates of 8.62; 10.0; 3.33; and 3.33%, respectively. Molecular analysis of 16S rRNA gene showed that local strains have high similarity (> 99%) against *E. coli* ATCC 43894 and several nucleotide sequences as a reference strain. The analysis of risk factors indicated that the slope of the cage floor has a significant contribution to the infection.

Conflict of interest

The authors declare that we have no conflict of interest that might inappropriately influence the reported work.

Acknowledgements

The authors would like thank Prof. Dr. Supar, MS for his kindness to supply *E. coli* ATCC 43894 control isolate, Dr. Aida L.T. Rompis for her English correction, and Minister of Agriculture for their support in the form of KKP3N grant contract no.795/LB. 620/I.1/2/2013, dated February 25th, 2013

References


