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PAGES: 45-52

ORIGINAL PDF URL: <https://dergipark.org.tr/tr/download/article-file/440118>

**THE BACTERIAL SPECIES FOUND IN THE FECAL MATTER OF EUROPEAN BROWN RABBIT****(*Lepus europaeus* Pallas,1778)**Ahmet Selçuk ÖZEN<sup>1\*</sup>      Zeynep Maide ERCAN-BARIŞ<sup>2</sup>      Ayhan YILMAZ<sup>3</sup><sup>1</sup> Dumlupınar University, Biology Department, Kütahya, TURKEY. \*aselcuk.ozen@dpu.edu.tr<sup>2</sup> Dumlupınar University, Evliya Çelebi Research and Education Hospital, Microbiology Laboratory.<sup>3</sup> Dumlupınar University, Biology Department, Kütahya, TURKEY.*Geliş Tarihi:09.02.2017**Kabul Tarihi:09.05.2017***ABSTRACT**

This study is based on the detection of bacterial species found in the fecal matter of European Brown rabbit (*Lepus europaeus*). The laboratory analysis of fecal matters are performed in two different periods. As a result, total nine bacterial species as Gram positive (+); *Kocuira rosea*, *Aerococcus viridans*, *Staphylococcus warneri*, *Staphylococcus saprophyticus* and *Bacillus cereus* and as Gram negative (-); *Rhizobium radiobacter*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Escherichia coli* were detected. *Bacillus cereus*, among these nine species, was the only one forming endospores. This study states that the detected eight bacterial species was noticed for the relationship between rabbit and human because of their shared opportunistic behaviors both in rabbits and humans.

**Keywords:** *Lepus europaeus* , European brown rabbit, fecal matter, bacteria.**AVRUPA KAHVERENGİ TAVŞANI (*Lepus europaeus* Pallas,1778)'NIN DIŞKISINDA BULUNAN  
BAKTERİ TÜRLERİ****ÖZ**

Bu çalışma, Avrupa Kahverengi tavşanının (*Lepus europaeus*) dışkısında bulunan bakteri türlerinin tespitine dayanmaktadır. Dışkıların laboratuvar analizi iki farklı periyotta gerçekleştirilmiştir. Sonuçta, Gram positifler (+) olarak *Kocuira rosea*, *Aerococcus viridans*, *Staphylococcus warneri*, *Staphylococcus saprophyticus* ile *Bacillus cereus* ve Gram negatif (-) olarak *Rhizobium radiobacter*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* ve *Escherichia coli* olarak toplam dokuz bakteri türü tespit edilmiştir. Bu dokuz tür arasında *Bacillus cereus*, endosporlar oluşturan tek türdür. Bu çalışma, tavşanlar ile insanların ortak olarak sahip oldukları fırsatçı davranışlarından dolayı tavşan ve insan arasındaki ilişkiye dikkat çeken sekiz bakteri türünün tespit edildiğini belirtmektedir.

**Anahtar Kelimeler:** *Lepus europaeus*, Avrupa kahverengi tavşanı, dışkı, bakteriler.

## 1. INTRODUCTION

*Lepus europaeus* (European Brown rabbit) belonging to Leporidae (Mammalia:Lagomorpha) family lives in the paleoarctic region where Turkey also belongs [1,2]. 32 species of Lepus genus in the World, only *Lepus europaeus* species in Turkey have large distribution areas [3,4]. The ecology, distribution, cariology and parasitology studies of this species gained a momentum in Turkey [5-9]. It is stated that wild rabbit species has important tasks in plant spreading by okori mechanism [10]. This species is the “least concerned (LC)” in the red list of IUCN and it is placed into the protected species category in the Bern Agreement Add-III list because of its european population decline [11]. The hunting of this species is allowed only in certain periods by Turkish Central Hunting Commission desicion [12]. Its population structure is getting shrunk because of over-hunting in Turkey [6]. Its biology is not known well. In the feces flora of *Lepus europaeus*, *E. coli*, *Clostridium perfringens* and *Klebsiella sp.* recorded [13].

The main aim of this study is to investigate the fecal matter of European Brown rabbit bacteriologically and get rid off information deficiency about this subject. The second aim of this study is to enlighten the future large investigation about this subject and help to solve health problems originating from rabbit-human relationships may develop in the future.

## 2. MATERIAL AND METHODS

This investigation is performed on the fecal matters of an adult female *Lepus europaeus* (European Brown rabbit) in 2014. The sample is obtained in Piracik area, Göynicek village, Bozüyük town, Bilecik city. The animal is calmed down and adapted to the laboratory by psychotherapy and fed by different plant species with the addition of wheat bread.

The fecal matters are taken with sterile care and placed into sterile laboratory wares made out of PVC. The sterile serum physiologic with 1/10 dilution is added to fecal matters in the laboratory. The diluted fecal matters are inoculated serially into EMB ( Eosin Methylene Blue), Hectoene, Bloody Agar and Chocolate Agar media cultures. After serial inoculations, the special identifications were performed in BD Phonex 100 identification and antibiogram machines. Incubation time and temperature were 48 h at 37 °C. To increase the reliability and validity of identification, the analysis is repeated two times at different dates and fecal matters. The facultative aerobe and anaerobe bacterial species are identified in the intestinal flora of *Lepus europaeus* by this method.

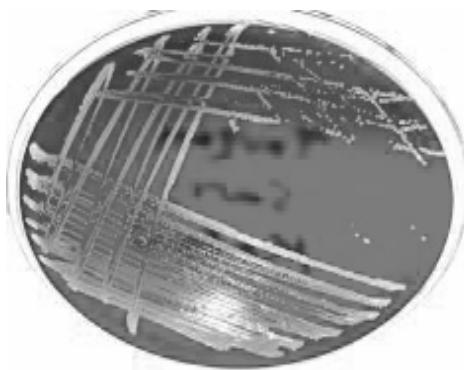
### 3. FINDINGS

Five gram positive and four gram negative bacterial species are identified by the investigation of fecal matters.

#### 3.1. Gram positive (+) species

***Kocuria rosea* (Flügge 1886) Stackebrandt et al., 1995.**

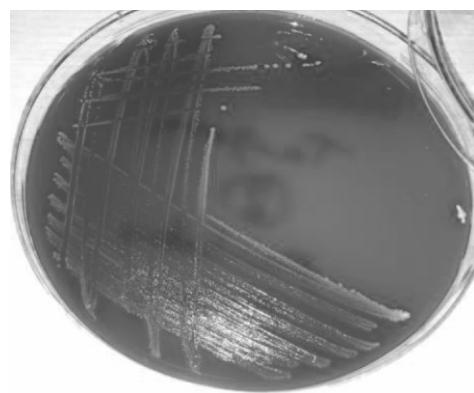
Coccal type cells of this facultative aerobe forms quadric groups. Isolated colonies look pale pink and somewhat convex with small circular structure. The optimum reproduction temperature is between 25-27 °C ( Figure 1).



**Figure 1.** *Kocuria rosea* colonies in bloody agar.

***Aerococcus viridans* Williams et al., 1953.**

Microaerophilic still cells form diadic and quadric groups. However, they weakly reproduce at anaerobic conditions. They hemolyse bloody agar producing green color. The optimum reproduction temperature is 37 °C ( Figure 2).



**Figure 2.** *Aerococcus viridans* colonies in bloody agar.

***Staphylococcus warneri* Kloas et Schleifer 1975.**

It is facultative anaerob, still species and form singlet, diadic and quadric groups. It forms yellowish color after 24 hour and grey-white color colonies after 48 hour in culture media. The optimal reproductive temperature is between 30–40 °C and colonies have 2–4 mm diameters.

***Staphylococcus saprophyticus* (Fairbroter 1940) Shaw et al., 1951.**

It is facultative anaerobe. Colonies are circular but somewhat convex, color changes from shiny white to cream and with the diameters of 4–9 mm. The optimal reproduction temperature is between 28–35 °C (Figure 3).



**Figure 3.** *Staphylococcus saprophyticus* colonies in bloody agar.

***Bacillus cereus* Frankland et Frankland 1887.**

It is facultative aerob, moving and spore forming species. Colonies are grey-colored, opaque and granular with amorph peripheral boundaries. The optimal reproduction temperature is between 28–35 °C.

**3.2. Gram negative (-) species**

***Rhizobium radiobacter* (Beijerinck and van Delden 1902) Young et al., 2001.**

It is facultative aerobic, saprophytic rod shaped species. It forms two type colonies; one after 24 hour incubation with straight, shiny and definite peripheral lines and the other after 48 hour incubation with a big coccoid, opaque and white color and a small, coccoid, less dense and transparent. The optimal reproduction temperature is between 25–28 °C (Figure 4).



**Figure 4.** *Rhizobium radiobacter* colonies reproducing in EBM

***Klebsiella pneumoniae* (Schroeter 1886) Trevisan 1887.**

It is facultative anaerobe rod shaped species. It ferments lactose in EMB culture media. Colonies have mucoid structure with definite mold smell. The optimal reproduction temperature is 37 °C ( Figure 5 ).



**Figure 5.** *Klebsiella pneumoniae* colonies reproducing in EBM.

***Klebsiella oxytoca* (Flügge 1886) Lautrop 1956.**

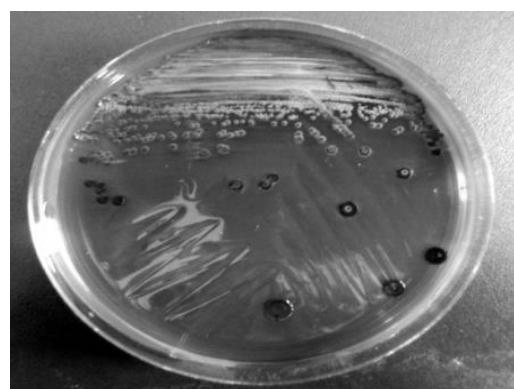
It is aerobic and facultative anaerobic rod shaped species. Colonies are dome shaped with shiny white and pink colors. The optimal reproduction temperature is 37 °C ( Figure 6 ).



**Figure 6.** *Klebsiella oxytoca* colonies reproducing in EBM.

***Echerichia coli* (Migula 1895) Castellani and Chalmers 1919.**

It is facultative anaerobic rod shaped species. Colonies have shiny typical metallic green color. The optimal reproduction temperature is 37 °C ( Figure 7 ).



**Figure 7.** *Echerichia coli* colonies reproducing in EBM.

#### 4. RESULTS AND DISCUSSION

The nine bacterial species detected in the fecal matter of European Brown rabbit are also found in human fecal matter. We could not compare the numbers, names and other properties of bacterial species detected in this study, because there is no previous studies made on the fecal matter bacterial species of *Lepus europaeus*.

*Kocuria rosea* found in fecal matter cause peritonitis in human [14,15] and *Aerococcus viridans* cause a deadly disease called gaffkemia in American lobster (*Homarus americanus*) and European lobster (*Homarus vulgaris*) [16,17]. *Staphylococcus warneri* is a causative agent in human bacteremia and endocarditis, in dog meningoencephalitis and in cow abortus [18-21]. *Staphylococcus saprophyticus* cause urinary system infections in human especially in older women [22,23] and *Bacillus cereus* cause food infections, endophtalmia and bacteremia related to immune system deficiency [24-27]. *Rhizobium radiobacter* cause human blood circulation infections related to catheter usage and bacteremia in pediatric patients [28,29]. *Klebsiella pneumoniae* cause human prulan mucus, inflamation, cell deaths destroying harms in lungs because of hemorargy when taken by respiration channels [30,31].

*Klebsiella oxytoca* is a causative agent of urinary tract and large intestine infections in immune repressed people [32,33]. *Echerichia coli* cause human urinary system infections and peritonitis in chicken, rat and human [34,35]. *Klebsiella sp.* and *Echerichia coli* were also recorded in this study, except for *Clostridium perfringens*, which was recorded by other investigators [13].

The eight out of nine bacterial species detected in fecal matter are opportunistic patogens and cause different type infections in animals and human. The human urinary system infective agents: *Staphylococcus saprophyticus*, *Klebsiella oxytoca* and *Echerichia coli*. However, *Aerococcus viridans* have no any records of causing human infections. By looking at species specific behavioral motifs, we tried to detect the diseases caused by infections or any other agents in sample with obtained fecal matter. We could not observe any negative behavioral performance such as feeding and attacking or running away from human during interaction. Therefore, no any problem is detected about the animal health during observation period. This situation can explain the biological property adaptation with bacterial species in fecal matter.

Therefore, the opportunistic patogenic bacteria detected in fecal matter of *Lepus europaeus* must always be considered serious in relation to rabbit-human interaction. However, we suggest that especially immune system repressed people must be carefull handling the rabbit fecal matter during the free rabbit hunting time zones when their interactive frequency increases.

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