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Faecal flora of captive European brown hares (*Lepus europaeus*)

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Abstract

To understand changes in the faecal flora, one has to know the physiological flora first. The aim of this paper is to give insight into the changes of the culturable faecal flora of European brown hares *(Lepus europaeus*) in a captive setting. Therefore faecal samples were collected from three juvenile hares as soon as possible after birth until their death, and from two adult female hares for almost one year. Samples were collected once a week and further processed as soon as possible. A routine bacteriological investigation was performed, as well as selective isolation of III. generation cephalosporin-resistant and fluoroquinolone-resistant enterobacterial isolates. The juvenile hares showed a somewhat more variable flora, the adult hares a rather stable composition. Only seldom potential pathogenic bacteria (i.e. Escherichia coli and Clostridium perfringens) were isolated from the adult hares. These pathogens were found after moments of stress. The more variable flora in the juveniles is seen as a result of the not yet completely developed immune system, and due to the stress of weaning. One juvenile showed a severe shift of the flora due to a severe typhlocolitis. Our results give insight into the normal faecal flora of European brown hares. These results can act as baseline information and help interpreting results gathered in a clinical setting, as well as during field work.

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*Keywords:*Bacteriology, culturable, European brown hare, faecal flora

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1. Introduction

The bacterial flora, the microbiome, of the intestine has been one of the most important subjects of research in the last years. The composition of the bacteria and what this means in regard to the overall health and the immune function of the animal has been discussed in various articles. Even members of the order *Lagomorpha*, mainly rabbits (*Oryctolaguscuniculus*), have been investigated (Combes et al., 2011; Eshar and Weese, 2014; Kušar and Avguštin 2010; Lelkes 1987; Straw 1988). The *Leporidae* are known to have a very sensitive intestinal tract in regard to food stuff, changes thereof, and treatment with antibiotics. Commonly reported is that certain bacteria, which are common in other species, are not found in their intestinal tract; i.e. *Escherichia* (*E.*) *coli*.

Changes of the normal microflora have been shown to lead to diseases, as well as being the result of diseases (e.g. Frank et al. 2007; Mazmanian et al. 2008; Round and Mazmanian 2009; Hoffmann et al. 2016, Suchodolsky et al. 2012, Cohn and Bornside 1965). Keeping this in mind, it is important to realize that simple things, such as e.g. dietary changes can have a severe impact on the intestinal flora (Bennegadi et al. 2001, Maslowski a. Mackay, 2011, Michelland et al., 2011), and thereby lead to dysbiosis, poor health, and subsequently even death. We have investigated several free-ranging European brown hares (*Lepus europaeus*) that showed what seems to be a highly unphysiological intestinal flora (Posautz et al., 2015), and due to the lack of literature regarding the European brown hair, this study aims to investigate the culturablefaecal flora with special consideration of *Enterobacteriaceae* of hares in a captive setting. This will help to fill the gap in the literature and provide base-line information on this subject.

2. Materials and methods

All five animals were born and raised at the institute. ID 1 was born on April 9th 2014, is female and belongs to the breeding colony. As she does not get along with the other females, she is kept for herself in a cage. ID 2, also female, was born on April 13th 2004 and had been living at the institute since, and due to her age was kept in floor penn by herself. Both animals were tried to sample every week, but due to management reasons this was not always possible. ID 3-5 were all born on August 28th.

Due to management reasons they were put in separate cages as soon as possible. These three animals were used in a trial, where they had to be sacrificed in the end. Necropsy was performed using standard procedures, during which a sample of small intestine, and in one case (ID 4) also faeceswerecollected.

Faecal samples were collected by the head-keeper in the morning (approximately 7am), placed in small separate containers and transported to the diagnostic laboratory for bacteriological and mycological examination. Each faecal sample was cultivated on two BBL™ Columbia Agar with 5% Sheep Blood (BA), BD MacConkey II Agar (MC), BD™ Campylobacter Bloodfree Selective Medium (CCDA), and BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin (SAB) (all from Becton, Dickinson and Company (BD), Heidelberg, Germany). One BA and MC were incubated under aerobic, and another BA under anaerobic atmosphere and at 37°C, CCDA was incubated under at 42°C under microaerobicatmosphere, SAB was incubated aerobically at 28°C.

Each fecal sample was also suspended in 1.5 ml of 0,9% sterile saline. 200 µl of suspended feces was precultured at 42°C overnight in Rappaport-Vassiliadis-Broth (RV) (Oxoid, Basingstoke, UK), in Selenit - Broth (Merck, Darmstadt, Germany) and then cultivated at 37°C on BD™ XLD Agar (Xylose-Lysine-Desoxycholate Agar) (BD, Heidelberg, Germany) for selective isolation of *Salmonella* sp. Besides routine bacteriological examination, selective isolation of III.generation cephalosporin-resistant and fluoroquinolone-resistant enterobacterial isolates was conducted.

For this, 200 µl of suspended feces was precultured at 37°C overnight in buffered peptone water (BPW) (Merck, Darmstadt, Germany) supplemented with cefotaxime (1 mg/L) and then cultivated at 37°C overnight on MacConkey agar (MCACTX) (Oxoid, Basingstoke, United Kingdom) supplemented with cefotaxime (1 mg/L). In parallel, each specimen was enriched at 37°C overnight in BD™ MacConkey Broth (BD, Heidelberg, Germany) and subcultivated on MacConkey agar supplemented with ciprofloxacin (0.06 mg/L) (MCACIP) to isolate fluoroquinolone-resistant *Enterobacteriaceae*. BD™ MacConkey Broth was also subcultivated on MC.All bacteria were identified by classical bacteriological and mycological methods.