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Isolation of *Cronobacter* spp. (formerly *Enterobacter sakazakii*) from Nostrils of Healthy Stable Horse – short communication

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SUMMARY

***Cronobacter* spp. belongs to the family Enterobacteriaceae. It is a motile (peritricha) Gram-negative non-spore forming bacterium. At present, *Enterobacter sakazakii* is reported as a *Cronobacter* spp. species with 16 biogroups. It is a ubiquitous organism whose isolation used to be associated with a contaminated powdered infant formula and feed for neonates and infants. Information about the *Cronobacter* spp. species incidence in the environment, its potential dissemination and its vectors, is very limited. The authors have documented incidence of *Cronobacter* spp. in the nostril mucous membrane of a healthy stabled horse. The above points out at the absolutely insufficient and unsystematic information about the dissemination of the *Cronobacter* spp. strain in the environment of animals and the people who are in contact with them.**

***Keywords: Cronobacter* spp. (*Enterobacter sakazakii*) – powdered infant formula – vehicle and reservoir in the environment**

SOUHRN

Holý O., Matoušková I., Holý V., Koukalová D., Chmelař D.: Izolace *Cronobacter* spp. (dříve *Enterobacter sakazakii*) z nozder zdravého ustájeného koně – krátké sdělení

Rod *Cronobacter* spp. patří do čeledi Enterobacteriaceae. Jedná se o pohyblivou (peritricha) gramnegativní bakterii, která netvoří spóry. V současnosti je *Enterobacter sakazakii* uváděn jako rod *Cronobacter* spp., který zahrnuje 16 bioskupin. Jedná se o ubikvitérní organismus, jehož izolace byla spojována s kontaminovanou sušenou počáteční kojeneckou výživou a nápoji pro novorozence a malé děti. Velmi omezené jsou informace o výskytu rodu *Cronobacter* spp. v životním prostředí, možném šíření a vektorech, které se mohou uplatňovat. Autoři sdělení prokázali výskyt *Cronobacter* spp. na sliznici nozder zdravého ustájeného koně. Tímto sdělením chtějí upozornit na zcela kusé a nesystematické informace o rozšíření kmene *Cronobacter* spp. v životním prostředí zvířat a lidí, kteří jsou s nimi v kontaktu.

***Klíčová slova: Cronobacter* spp. (*Enterobacter sakazakii*) – počáteční sušená kojenecká výživa – vehikulum a rezervoár v životním prostředí**

Until 1980, *Enterobacter sakazakii* was known as yellow pigmented *Enterobacter cloacae*. It was named in honour of the eminent Japanese microbiologist Riichi Sakazakii. In 2007 Iversen reported *E. sakazakii* as a *Cronobacter* spp. species with 16 biogroups and finally in 2008, *E. sakazakii* was reclassified as a new genus [1, 2].

This opportunistic bacterium induces life-threatening infections in neonates and infants and is associated with contaminated powder infant formula and feed [3]. During the implementation of the insulated tray system in the kitchen of University Hospital, Olomouc, *Cronobacter* spp. was isolated from a swab of the tray bottom. An epidemiologic study showed the tray had been used at the Department of Obstetrics & Gynaecology, where a patient with a vaginal infection caused by *Cronobacter* spp. was hos-

proLékaře.cz | 14.2.2026

pitalized at the same time [4]. In 2002 ICMSF (International Commission on Microbiological Specification for Food) labelled *Cronobacter* spp. *(E. sakazakii)* as a highly dangerous infectious agent for a certain part of population. The species was placed to a group of dangerous food pathogens, together with *Listeria monocytogenes* or *Clostridium botulinum* [5].

Information about the *Cronobacter* spp. incidence in the environment, its potential dissemination and its vectors, is very limited. In 2003 isolation of the *Cronobacter* spp. strain from the intestine of *Stomoxys calcitrans* stable fly larvae was reported. The authors believe these larvae might be a possible natural reservoir of the *E. sakazakii* strain [6].

During the course of study on incidence of MRSA (Methicillin-resistant *Staphylococcus aureus*) in horses we have looked also for the presence of *Cronobacter* spp. The paper describes the first isolation of *Cronobacter* spp. from the horse nostrils.

The microorganism is thermotolerant, its thermal tolerance in the powdered infant formula is well documented. Likewise, it proves to be able to generate biofilm on various surfaces and has an increased resistance to cleaning agents and disinfectants [7].

In the early morning, nasal swabs were taken from 21 sports and breeding horses of the age ranging from 1 month to 22 years. Both nostrils were swabbed with a COPAN sterile swab, soaked in a sterile physiological solution prior to use. The applicator was then shortened and inserted in a plastic tube with 5 ml of thioglycollate broth and transported immediately into the laboratory. Primary cultivation was commenced at 37 °C for 24 hours. Subsequently, blood agar was inoculated. The blood agars were incubated at 37 °C for 24 hours under aerobic conditions. In one case the cultivation verified yellow pigmented colonies, which were subsequently isolated in blood agar and Endo agar. Cultivation media manufactured by TRIOS (Czech Republic) were used for the cultivation. Biochemical identification of the yellow pigmented colonies was performed using the BD Phoenix TM automated microbiological system (Becton, Dickinson and Company, USA). The standard microdilution method (CLSI 2009) was used [8]; the susceptibility was assessed in Mueller-Hinton broth. To establish antibiotic sensitivity, a set for determining minimal inhibitory concentrations (MIC) by means of a standard micro method in a microtiter plate was used. A set of antibiotics for Gram-negative bacteria (ATB I and II line), from TRIOS (Czech Republic), was used. The results of biochemical tests from Phoenix ID panels verified the *Enterobacter sakazakii* (*Cronobacter* spp.) strain to 99 %. Substrates for biochemical tests: sorbitol, methyl-beta-glucoside and esculin were positive, while adonitol was negative. The above are main biochemical tests distinguishing *E. sakazakii* strain from *E. cloacae* strain [9]. The yellow pigment generation was also positive, pointing out as the first one to the possibility of *E. sakazakii* cultivation verification from the horse nasal swab. Establishing antibiotic sensitivity using the MIC method verified resistance to ampicillin and first and second generation cephalosporin.

Discussion

The majority of current articles on the *Cronobacter* spp. strain focuses on rapid verification and prevention of the incidence of this infectious agent in powdered infant formula and in powdered dietary foods for special medical purposes designed for infants up to the age of six months. The European Commission adopted regulation measure No. 1441/2007, pursuant to which *Cronobacter* spp. must not be present in 10 g of a sample of the above foods [10]. High resistance to the outer environment effects is one of the key factors responsible for this agent's persistence in powdered infant formula. The high neonate mortality rate raises concern for public health. The fact that the infection dosage has not been determined yet remains one of the big problems as well as the possibility of biofilm generation, and the natural resistance to antimicrobial substances.

In relation to animal (horse, cattle or pigs) stabling, incidence of stable fly *Stomoxys calcitrans* is reported, which could function as the vector of the bacterium *Cronobacter* spp. dissemination and the intestine of its larvae as a suitable vehicle for the bacterium persistence in the environment [6]. The *Cronobacter* spp. incidence in the nostril mucous membrane of a stabled horse that we documented could fall into this part of discussion. In order to find the source or contaminated vehicle of this bacterium, an epidemiologic study would need to be carried out both in feeding (grain, hay) samples and directly in the stables or potential contamination of the stable fly would need to be verified. The above draws attention to the absolutely insufficient and unsystematic information about the dissemination of *Cronobacter* spp. in the environment of animals and the people who are in contact with them.

Epidemiologie 4-011 22.12.2011 14:18 Str. 169
proLékaře.cz | 14.2.2026

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Abbreviations:
ICMSF – International Commission on Microbiological Specification for Food
MRSA – Methicillin-resistant Staphylococcus aureus

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