Rev Iberoam Micol. 2017;xxx(xx):xxx–xxx

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Original article

Pigeons and their droppings as reservoirs of *Candida* and other zoonotic yeasts

Inmaculada Rosario Medina, Lorena Román Fuentes, Miguel Batista Arteaga, Fernando Real Valcárcel, Félix Acosta Arbelo, Daniel Padilla del Castillo, Soraya Déniz Suárez, Otilia Ferrer Quintana, Belinda Vega Gutiérrez, Freddy Silva Sergent, Begoña Acosta-Hernández*

Instituto Universitario de Sanidad Animal y Seguridad Alimentaria (IUSA), Universidad de Las Palmas de Gran Canaria, Las Palmas, Spain

ARTICLE INFO

Article history:
Received 9 November 2016
Accepted 9 March 2017
Available online xxx

Keywords:
Columba livia
Pigeons
Candida
Yeasts
Zoonotic yeasts

ABSTRACT

Background: The importance of pigeons as reservoirs and carriers of *Cryptococcus neoformans* and other species of this genus is well-known; however, less is known about their role as reservoirs and carriers of other yeasts that impact public health.
Aims: The present study was performed on Gran Canaria Island to define yeasts other than *Cryptococcus* spp. that have been reported to impact public health and which could be carried by pigeons.
Methods: Samples were obtained from 83 pigeon lofts (*Columba livia*); moreover, 331 crop samples, 331 cloacal samples and 174 dropping samples were collected. In addition, 17 dropping samples were taken from a total of 17 public squares. Samples were inoculated on Sabouraud dextrose agar with chloramphenicol.
Results: Different yeast species, i.e. *Candida guilliermondii* (24.36%), *Candida kefyr* (1.21%), *Saccharomyces cerevisiae* (2.43%), and *Trichosporon asahii* (1.21%) were isolated for the first time from the cloaca. The most frequently isolated yeast from the crop, cloaca and dropping samples from lofts was *C. guilliermondii* (30.46%, 24.36% and 49.37%, respectively). In addition, for the first time, *C. kefyr* (3.65%), *Candida pelliculosa* (2.43%), *Candida rugosa* (1.21%), *T. asahii* (3.65%), *Trichosporon mucoides* (3.65%) and *Prototheca wickerhamii* (1.21%) were obtained from crop samples; *Candida pelliculosa* (1.20%), *T. asahii* (9.63%) and *T. mucoides* (7.22%) were isolated from dropping samples in the lofts. *Candida albicans* was the most frequently isolated yeast in dropping samples collected in public squares.
Conclusions: It can be assumed that pigeons and their droppings act as carriers and reservoirs of *Candida* spp. and other zoonotic yeasts.

La paloma y sus excrementos como reservorios de *Candida* y otras levaduras zoonóticas

Palabras clave:
Columba livia
Palomas
Candida
Levaduras
Levaduras zoonóticas

RESUMEN

Antecedentes: Es bien conocido el papel que desempeña la paloma como reservorio y portadora de *Cryptococcus neoformans* y otras especies del género; sin embargo, se conoce poco sobre el papel que desempeña como reservorio y portadora de otras levaduras que repercuten en la salud pública.
Objetivos: El presente estudio fue realizado en la isla de Gran Canaria para determinar otras levaduras diferentes del género *Cryptococcus* que podrían portar las palomas y que repercuten en la salud pública.
Métodos: Se tomaron muestras en 83 palomares *(Columba livia):* 331 muestras de buche, 331 cloacales y 174 muestras de excrementos. También se tomaron 17 muestras de excrementos en 17 plazas públicas. Las distintas muestras se sembraron en agar glucosado de Sabouraud con cloranfenicol.

* Corresponding author.
E-mail address: bego.acosta@ulpgc.es (B. Acosta-Hernández).

http://dx.doi.org/10.1016/j.riam.2017.03.001
1130-1406/

Resultados: Se aislaron por primera vez de la cloaca diferentes especies de levaduras: *Candida guilliermondii* (24,36%), *Candida kefyr* (1,21%), *Saccharomyces cerevisiae* (2,43%), *Trichosporon asahii* (1,21%). La levadura más frecuentemente aislada de muestras de buche, cloaca y heces de palomares fue *C. guilliermondii* (30,46, 24,36 y 49,37%, respectivamente). A estas especies se suman otras aisladas por primera vez a partir de muestras de buche: *C. kefyr* (3,65%), *Candida pelliculosa* (2,43%), *Candida rugosa* (1,21%), *T. asahii* (3,65%), *Trichosporon mucoides* (3,65%) y *Prototheca wickerhamii* (1,21%). Se aislaron por primera vez de muestras de excrementos de palomares las especies *C. pelliculosa* (1,20%), *T. asahii* (9,63%) y *T. mucoides* (7,22%). *Candida albicans* fue la levadura más frecuentemente aislada de muestras de heces recogidas de plazas públicas.

Conclusiones: Por todo ello, concluimos que la paloma y sus excrementos actúan como portadores y reservorios de especies de *Candida* y otras levaduras zoonóticas.

The role of pigeons and other birds as carriers of various important public health pathogens such as protozoa, fungi, bacteria and viruses is well-known.[5] Pigeons and other birds play an important role in public health as carriers of *Cryptococcus* species,[21–23] especially *Cryptococcus neoformans*.[6] However, *Cryptococcus* is not the only yeast genus that impacts human public health, as there is currently a boom in pathologies involving other yeasts, especially *Candida* species.[25] In fact, the newly emerging human pathogenic yeast species include *Malassezia furfur*, *Trichosporon asahii*, species of *Rhodotorula*, *Wickerhamomyces anomalus* and different species of *Candida* such as *Candida albicans*. To a lesser extent, *Candida lusitaniae*, *Candida holmii*, *Candida norvegensis*, *Candida valida* and *Candida dubliniensis* appear to be of importance in public health.[4,31] Similarly, other agents that have previously been considered environmental pollutants or of industrial importance, such as *Candida utilis* and *Candida lipolytica*, are currently identified as agents of fungemia, onychomycosis and systemic disease.[7] For this reason, it is important to study the different yeast strains obtained from pigeon samples (crop, cloaca, droppings). The aim of this work was to define which genera of yeasts, other than *Cryptococcus*, could be carried by pigeons and their droppings.

Materials and methods

Sampling was performed in 83 pigeon lofts from 17 pigeon-breeding associations distributed throughout Gran Canaria Island (Spain). For this study, 331 samples from the crop and cloaca and 174 samples of pigeon droppings were analyzed. In addition, we also sampled 17 public squares, and a mixture of pigeon droppings from each park was taken.

Samples of the cloaca and crop were collected from 331 pigeons using sterile cotton swabs in a transport medium (Eurotubo®, Rubi, Barcelona, Spain). The swab was introduced and rotated in the cloaca after cleaning the area with antiseptic iodine solution (10%). For crop sampling, pigeons were restrained and after gently opening the beak of the pigeon, one swab was introduced into the crop. All samples were stored and kept at 4 °C until further processing in the laboratory. Dropping samples from public squares and pigeon lofts were collected using sterile wooden spatulas and then kept in sterile vials at 4 °C until further processing.

Fecal samples (2 g) were diluted in sterile saline solution (10 ml), homogenized by shaking and allowed to settle for 30 min. Then, 0.5 ml aliquots of each supernatant were used for inoculation on laboratory media agar. All samples (crop, cloaca and feces) were inoculated on Sabouraud dextrose agar and malt agar; chloramphenicol (50 mg/l) was added to the media to prevent bacterial growth. Plates were incubated at 30 °C and 37 °C (1–7 days) and inspected daily. All colonies with a microscopic morphology consistent with yeast were isolated and identified by auxanogram using API 20C Auxanogram strips and API ID 32C Auxanogram strips (bioMérieux®, Madrid, Spain).

For the strains identified as *Candida* spp., several complementary tests were performed. Pseudohyphae and blastoconidia production was analyzed using corn meal agar (Oxoid Limited, Basingstoke, England) with Tween 80 at a final concentration of 0.02% to reduce surface tension. The identification was performed using the technique described by Koneman et al.[13] and confirmed by using the ChromID® Candida (bioMérieux®) and bird-seed agar.[18]

Results

A total of 13 different yeast species belonging to four different genera (*Candida*, *Saccharomyces*, *Trichosporon*, *Rhodotorula*) and a strain of a unicellular alga belonging to the genus *Prototheca* were isolated. The relative incidence of each species detected in crop, cloaca and dropping samples is compiled in Table 1.

Candida guilliermondii and *Candida albicans* were isolated in a significant percentage in the three sampling locations. *C. guilliermondii* was the most frequently isolated yeast both in crop (30.46%) and cloaca (24.36%) samples. Regarding the yeasts isolated in dropping samples, *C. guilliermondii* was also isolated the most frequently (49.37%), followed by *C. albicans* (15.66%), *T. asahii* (9.63%), *Rhodotorula mucilaginosa* and *Trichosporon mucoides* (7.22%). In contrast, *Candida kefyr*, *Candida zeylanoides* and *Prototheca wickerhamii* were not isolated from any dropping sample.

C. albicans was the most frequently isolated yeast in dropping samples of public squares (29.4%, 5/17), followed by two other yeasts with a significant impact (*Candida krusei* and *Candida inconspicua*, 17.6%, 3/17). The less frequently isolated were *C. guilliermondii* and *Candida famata* (11.8%, 2/17), and finally,

Table 1
Incidence of yeast species isolated in crop, cloaca and dropping samples from pigeons.

Species	Crop $n = 82$ (%)	Cloaca $n = 82$ (%)	Dropping $n = 83$ (%)
Candida guilliermondii	25 (30.46)	20 (24.36)	41 (49.37)
Candida albicans	11(13.41)	3 (3.65)	13 (15.66)
Candida kefyr	3 (3.65)	1 (1.21)	0 (0)
Saccharomyces cerevisiae	3 (3.65)	2 (2.43)	1 (1.20)
Trichosporon asahii	3 (3.65)	1 (1.21)	8 (9.63)
Rhodotorula mucilaginosa	3 (3.65)	0 (0)	6 (7.22)
Trichosporon mucoides	3 (3.65)	0 (0)	6 (7.22)
Candida pelliculosa	2 (2.43)	0 (0)	1 (1.20)
Candida rugosa	1 (1.21)	0 (0)	1 (1.20)
Candida zeylanoides	1 (1.21)	0 (0)	0 (0)
Rhodotorula glutinis	1 (1.21)	0 (0)	1 (1.20)
Prototheca wickerhamii	1 (1.21)	0 (0)	0 (0)
Candida parapsilosis	0 (0)	0 (0)	1 (1.20)
Candida lusitaniae	0 (0)	0 (0)	1 (1.20)

Please cite this article in press as: Rosario Medina I, et al. Pigeons and their droppings as reservoirs of *Candida* and other zoonotic yeasts. Rev Iberoam Micol. 2017. http://dx.doi.org/10.1016/j.riam.2017.03.001