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No contact transmission of avian bornavirus in experimentally infected cockatiels (*Nymphicus hollandicus*) and domestic canaries (*Serinus canaria* forma domestica)

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ABSTRACT

Avian bornaviruses (ABV) are the causative agents of proventricular dilatation disease (PDD), a widely distributed disease of parrots. Distinct ABV lineages were also found in various non-psittacine avian species, such as canaries, but the pathogenic role of ABV in these species is less clear. Despite the wide distribution of ABV in captive parrots and canaries, its mode of transmission is poorly understood: both horizontal transmission via the urofaecal-oral route and vertical transmission are discussed to play a role.

In this study we investigated pathology and horizontal transmission of ABV in domestic canaries (*Serinus canaria* forma domestica) and cockatiels (*Nymphicus hollandicus*), two natural host species commonly used for experimental ABV infections. ABV inoculation resulted in persistent infection of all inoculated animals from both species. ABV-infected cockatiels exhibited PDD-like symptoms, such as neurologic signs or shedding of undigested seeds. In contrast, infected domestic canaries did not develop clinical disease. Interestingly, we did not detect viral RNA in cloacal swabs and organ samples or ABV-specific antibodies in serum samples of contact-exposed sentinel birds from either species at any time during a four months observation period. Our results strongly indicate that horizontal transmission of ABV by direct contact is inefficient in immunocompetent fully fledged domestic canaries and cockatiels.

1. Introduction

Avian bornaviruses (ABV) are single-stranded negative-sense RNA viruses of the family of *Bornaviridae*. Since their first description in 2008 at least 13 different ABV genotypes have been detected in parrots and their relatives (order Psittaciformes) (Honkavuori et al., 2008; Kistler et al., 2008; Rubbenstroth et al., 2012; Weissenbock et al., 2009a), songbirds (order Passeriformes) (Rubbenstroth et al., 2013, 2014; Weissenbock et al., 2009b) and waterfowl (Guo et al., 2012; Payne et al., 2011a).

In psittacine birds, ABV is the causative agent of proventricular dilatation disease (PDD), which is associated with central nervous symptoms, impaired motility of the gastrointestinal tract, shedding of undigested seeds and impaction of the proventriculus. Infiltration of lymphoid cells into the peripheral ganglia and the central

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nervous system are microscopic hallmarks of PDD (reviewed in Staeheli et al., 2010). Disease development was reproduced by parenteral inoculation of psittacine birds, such as cockatiels (*Nymphicus hollandicus*) or Patagonian conures (*Cyanoliseus patagonus*), with genotypes ABV-2 and ABV-4 (Gancz et al., 2009; Gray et al., 2010; Mirhosseini et al., 2011; Payne et al., 2011b; Piepenbring et al., 2012). However, ABV-infected birds may also remain apparently healthy for several years (De Kloet and Dorrestein, 2009; Gancz et al., 2009; Heffels-Redmann et al., 2011; Piepenbring et al., 2012). Few cases of PDD-like clinical disease in association with natural ABV infection have been observed also in the passerine species domestic canary (*Serinus canaria* forma domestica; further called "canary") (Rubbenstroth et al., 2013; Weissenbock et al., 2009b). However, after experimental infection of canaries with an ABV genotype C2 strain, which had been isolated from a diseased canary, the inoculated animals remained healthy for at least five months (Rubbenstroth et al., 2013).

ABV is widely distributed in captive psittacine birds, with up to 20% of all tested individuals being positive (Heffels-Redmann et al., 2011). Similarly, a high infection rate was observed in captive canaries in Germany (Rubbenstroth et al., 2013), as well as in certain populations of wild waterfowl in North America (Payne et al., 2011a). The mechanism by which ABV is spreading in these populations is poorly understood. The urofaecal-oral route is assumed to be important for horizontal transmission and also respiratory routes have been discussed, but so far supporting experimental evidence is rather poor (Heatley and Villalobos, 2012; Hoppes et al., 2010; Kistler et al., 2010; Rubbenstroth et al., 2012).

In a previous study we successfully infected canaries with genotype ABV-C2 by combined peroral (p.o.) and oculonasal (o.n.) inoculation, and observed that the virus was transmitted to four out of five co-housed sentinel birds. However, the viral load in most of these contact-exposed birds was low (Rubbenstroth et al., 2013). Piepenbring et al. (2012) reported successful ABV transmission to one cockatiel placed in contact with a group of cockatiels that were experimentally infected with ABV-4. Vertical transmission has been discussed as another possible route of infection, because several studies found eggs from infected hens to be ABV-positive by PCR assays. Confirmation of a productive infection of embryos by either immunohistostaining or virus isolation has to date not been successful (Kerski et al., 2012; Lierz et al., 2011; Monaco et al., 2012; Rubbenstroth et al., 2013).

This study was designed to investigate pathology and horizontal transmission of ABV under experimental conditions in the natural host species cockatiel and canary.

2. Material & methods

2.1. Viruses and production of virus stocks

ABV strains were isolated on CEC-32 quail fibroblast cells or QM7 quail smooth muscle cells as described previously (Rubbenstroth et al., 2012). Infected cultures were passaged twice weekly for five to eleven passages until all cells were infected, before viral stocks for inoculation of birds were produced. For stock production cells were harvested in PBS and disrupted by three freeze–thaw-cycles and ultra-sonication. Subsequently, cell debris was removed by centrifugation and stocks were stored at −80 °C. Isolate ABV-4 #6758 (GenBank accession number FJ603685) was isolated from a blue-and-yellow macaw (*Ara ararauna*) suffering from PDD (Rinder et al., 2009; Rubbenstroth et al., 2012) and stocks were produced after five passages of persistently infected CEC-32 cells. Isolates ABV-C1 AS-19 (KC464472) and AS-20 (KC464473) originated from two canaries from a common flock. Several birds of this flock had suffered from neurologic symptoms, such as seizures and circle movements, and the morbidity was about 20% according to the owner' information (Rubbenstroth et al., 2013). Stocks of AS-19 and AS-20 were produced after eleven passages of persistently infected QM7 cells or nine passages of persistently infected CEC-32 cells, respectively, and pooled subsequently.

In addition, stocks of purified viral particles were produced for use in virus neutralization tests (VNT) following previously described procedures (Briese et al., 1992). Briefly, CEC-32 cells persistently infected with ABV-4 #6758 or primary duck embryo fibroblasts (DEF) infected with ABV-C1 AS-20 were incubated for 90 min with 20 mM Hepes buffer supplemented with 250 mM $MgCl_2$ to release viral particles from the cells. Thereafter, cell debris was removed by centrifugation at $2{,}500 \times g$ for 10 min before viral particles were pelleted by ultracentrifugation at $100{,}000 \times g$ for 1 h through a 20% sucrose fraction and pellets were resuspended in PBS.

Isolate ABV-C2 #15864 (KC464478) was isolated from a canary (Rubbenstroth et al., 2013) and used for antibody detection by indirect immunofluorescence tests (iIFT) during this study. VERO cells were inoculated with a QM7-derived stock of the virus and the culture reached 100% infected cells after 20 cell passages.

2.2. Experimental animals

Eleven cockatiels (*N. hollandicus*) and 16 canaries (*S. canaria* f. dom.) of the breed "lizard" were used during this study. Cockatiels were two to seven months old at the beginning of the experiment and originated from our own breeding flock. The flock is confirmed to be free of ABV and further psittacine pathogens, including circovirus, polyomavirus, herpesvirus, adenovirus and reovirus, by regular monitoring. Canaries were seven to eleven months old at the start of the experiment and originated from a flock from which brain samples of 13 birds had been received and tested ABV-negative during a previous study (Rubbenstroth et al., 2013). All birds were clinically healthy and confirmed to be ABV-negative by serological methods and reverse transcription polymerase chain reaction (RT-PCR) from swab samples prior to the experiments. Birds were provided with commercial conure or canary feed, fruits and water ad libitum. Birds were housed in an aviary with a solid floor covered with paper. Feed and water were provided in metal bowls and exchanged daily. Faeces and feed dropped to the ground was removed at weekly intervals, when also papers were exchanged.