A CLINICAL OUTBREAK OF POLYOMAVIRUS INFECTION IN SHAMAS (COPSYCHUS MALABARICUS)

Een klinische uitbraak van Polyomavirus infectie bij Shama's

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ABSTRACT

The present article describes for the first time a clinical outbreak of a polyomavirus infection in shamas (*Copsychus malabaricus*). Three out of four shamas became ill and died within 14 days after introduction in a passerine aviary. Other birds in the aviary remained clinically healthy. The diagnosis of polyomavirus infection was made by electron microscopic examination of the liver of a bird that died.

SAMENVATTING

Dit artikel beschrijft voor het eerst een klinische uitbraak van een Polyomavirus infectie in shama's (*Copsychus malabaricus).* Drie van de 4 shama's werden ziek en stierven 14 dagen na introductie in een volière met passeriforme vogels. De andere vogels in de volière bleven klinisch gezond. De diagnose van polyomavirus werd gesteld door middel van elektronenmicroscopisch onderzoek van de lever van een gestorven vogel.

Keywords: Shama - Passerine - Polyomavirus

INTRODUCTION

Polyomavirosis is a well-known disease entity in psittacine birds, with probably all species being susceptible to infection (Ritchie *et al.*, 1994; Ritchie *et al.*, 1996). Polyomavirus infections in passerine birds are less prevalent and have only been described in the superfamily *Fringilloidae* (Ritchie *et al.*, 1994; Ritchie *et al.*, 1996; Sandmeier *et al.*, 1999). The present article reports an outbreak of a polyomavirus infection in a passerine aviary. Of all species present, only shamas developed clinical signs and death. Shamas belong to the superfamily *Muscicapoidae*, in which polyomavirus infections have not been described so far.

MATERIALS AND METHODS

Case history

During the month of April 1997, two pairs of adult shamas were purchased from the same local pet shop with a one week interval. These birds were introduced into a closed aviary with ten shamas, two dhyal thrush (*Copsychus saularis*), four rufous-bellied niltavas (*Niltava sundara*), one green singing finch (*Serinus mozambicus*), one canary (*Serinus canarius*) and two Pekin nightingales (*Leiothrix lutea*). In this aviary, there had been no history of clinical disease or mortality. The birds were all housed individually or in couples, in separate cages with wire floors. The droppings were removed every two weeks. Drinking water was supplied through drinking nipples which were

cleaned every week but never disinfected. Thus the hygiene was rather poor.

On the 14th day after arrival, one shama of the first pair developed clinical signs of ruffled feathers and poor general condition. The bird died after two days. Ten days later, both shamas of the second pair also displayed a poor general condition and died two days after the onset of the first signs of illness. All the other birds in the aviary, including the female of the first shama pair, remained clinically healthy. During the outbreak of disease, treatment was not initiated.

Postmortem examinations

The three shamas that died were necropsied. Gross pathological examinations were performed by standard procedures. Impression smears of the lungs, liver, spleen, kidneys, proventriculus and intestinal tract mucosa were stained with the Hemacolor (Merck, Darmstadt, Germany) staining reagents and examined microscopically at a magnification of x 1000. Impression smears of conjunctiva, cloaca, lungs, liver and spleen were stained with the modified Giménez stain (Vanrompay *et al.*, 1992) and examined for the presence of *Chlamydophila*. Samples for bacteriological and/or histological examination were taken from organs showing macroscopic or cytologic lesions. One liver displaying nuclear inclusion bodies upon microscopic examination, was further processed for electron microscopic examination.

Formaldehyde-fixed paraffin-embedded liver and kidney tissues which contained intranuclear viral inclusion bodies were examined by PCR to detect DNA from polyomavirus and/or adenovirus.

Samples for histological examinations were fixed in 4 % phosphate buffered formaldehyde solution, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin.

Organ samples were inoculated on Columbia agar (Gibco, Paisley, Scotland) with 5 % bovine blood, Brilliant Green agar (LabM, Bury, England) and Slanetz & Bartley agar (Oxoid, Basingstoke, England). Intestinal contents were cultivated on Brilliant Green agar. Incubation was done at 37 °C under aerobic circumstances. For the incubation of Columbia agar and Slanetz & Bartley agar plates, the environment was enriched with 5 % CO_2.

The liver sample that was processed for transmission electron microscopy was fixed in 0.1 M cacodylate buffer pH 7.3 containing 2.5 % glutaraldehyde and 2 % paraformaldehyde and postfixed in 1 % (w/v) osmiumtetroxide in distilled water. The sample was block stained with 2 % (w/v) uranyl acetate in distilled water, dehydrated in ethanol and embedded in Epon/Spurr's (1/1) medium. Ultra thin sections of the organ were stained with lead citrate and examined with a Philips 201 transmission electron microscope.

For the PCR detection of polyomavirus, DNA was extracted using the Puregene DNA isolation kit (Gentra systems, Minneapolis). PCR analysis was performed as described by Phalen *et al.* (1991), using a primer complementary to sequences located in the putative coding region for the budgerigar fledgling disease virus (BFDV) VP 1 gene. For the detection of adenovirus by PCR, DNA was extracted using the DNeasy (TM) Tissue Kit (Qiagen, Hilden, Germany) and subsequently examined as described by Raue and Hess (1998) and Xie *et al.* (1999).

RESULTS

Necropsy revealed pale swollen livers and kidneys in all three shamas. In one shama, enlargement of the spleen was noted.

Cytology demonstrated clear intranuclear inclusion bodies in the livers (Figure 1) and kidneys of all birds. The infected cells were extremely swollen. One shama was infected with *Atoxoplasma* in the lungs and kidneys. *Chlamydophila* was not observed.

Histological examination of the liver showed that the majority of the hepatocytes contained large pale intranuclear inclusions (Figure 2). The swollen nuclei also showed condensed chromatin clumps at their periphery. They occupied much of the cellular space. Hepatocytes containing an intranuclear inclusion had more eosinophylic cytoplasm. Inflammatory cells were absent. In Disse's space and in the capillaries nu-

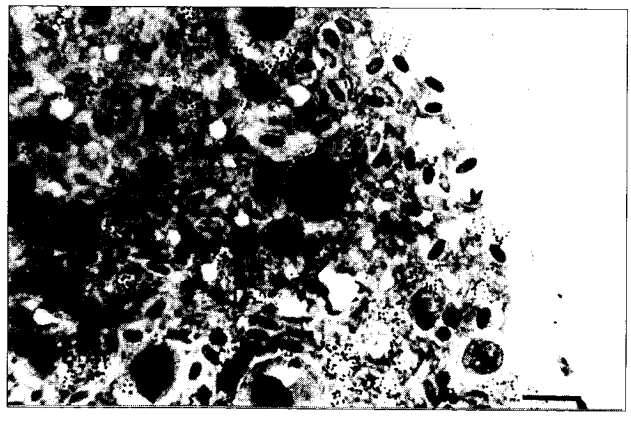

Figure 1. Impression smear of the liver of a shama. The liver shows swollen hepatocytes with clear intranuclear inclusion bodies (arrow) as well as numerous coc-shaped bacteria (open arrow). Haemacolor staining; bar = 20 µm.

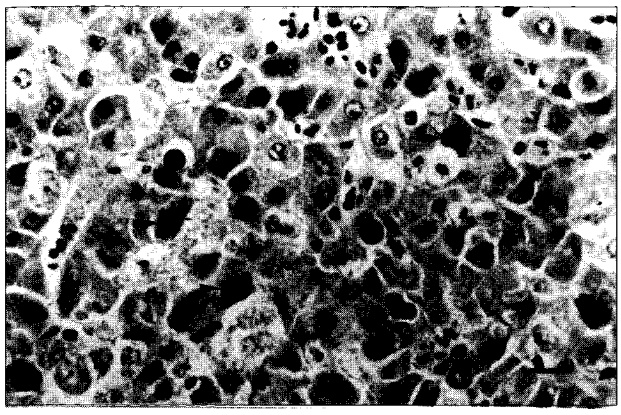

Figure 2. Histological section of the liver of a shama. The swollen nuclei (arrow heads) contain intranuclear inclusions and condensed chromatin clumps at the periphery.
Bar = 10 µm.

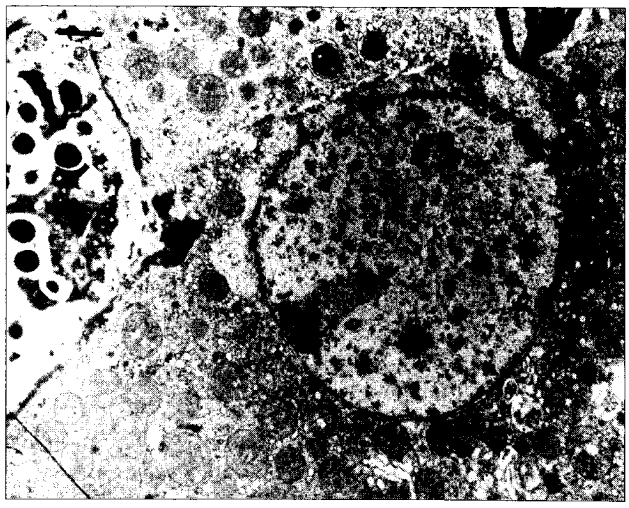

Figure 3. Electron micrograph of an ultra thin section of liver from a shama, showing numerous icosahedral polyomavirus particles in the cytoplasm of a hepatocyte.
Bar = 0.25 µm.

merous bacteria were present, ranging in shape from coccoid to coccobacillary. The blood vessels were congested. The kidneys histologically displayed severe congestion and the presence of similar large pale intranuclear inclusions in a limited number of tubular epithelial cells. No inflammation was observed in the kidney.

Enterococcus faecalis was isolated from the liver, spleen and kidneys of the first shama. Organs of the second bird contained coagulase-negative staphylococci. No bacteria were isolated from the organs of the third shama. Electron microscopic examination of the liver of one shama revealed intranuclear and intracytoplasmic electron-dense non-enveloped icosahedral viral particles with a diameter of 45-50 nm (Figure 3). The size and morphology of these particles fit polyomavirus characteristics.

PCR analyses of liver and kidney samples for the detection of DNA from avian polyomaviruses or avian adenoviruses all had a negative outcome.

DISCUSSION

The disease outbreak in the three shamas described in this paper was related to infection with a polyomavirus-like agent. Indeed, the morphological features of the viral particles observed by electron microscopy fit those of polyomaviruses rather than those of adenoviruses, having a diameter of 60-90 nm and thus being much larger than polyomaviruses (Ritchie, 1995). Although electron microscopic examination was performed in only one bird, it is very likely that the same viral agent caused infection in the two other diseased birds since in all three birds similar viral inclusion bodies were observed during histological and cytological examination of the liver and kidneys.

Clinical disease associated with polyomavirus infections in passerine birds has been described in black-bellied seed crackers (*Pyrenestes ostrinus*), bluebills (*Spermophaga haematina*), canaries (*Serinus canarius*), crimson crackers (*Pyrenestes sanguineus*), cordon blues (*Uraeginthus bengalus)*, goldfinches (*Carduelis carduelis*), gouldian finches (*Erythrura gouldiae*), green finches (*Carduelis chloris*), painted finches (*Emblema picta*) and royal starlings (*Cosmopsarus regius*). These birds all belong to the superfamily *Fringilloidae*. As far as we know, this is the first report of a polyomavirus infection in a *Muscicapoidae* species, namely the shama.

In passerine outbreaks of polyomavirosis described in the literature, poor general condition and the death of infected birds were often reported in the clinical history (Forshaw *et al.*, 1988; Garcia *et al.*, 1993; Johnston & Ridell, 1986; Sandmeier *et al.*, 1999, Sironi, 1991). Similar complaints occurred in the present case. It is not known whether polyomavirus can also be held responsible for feather and/or beak abnormalities in shamas, such as described in Gouldian finches infected at a very young age and in budgerigars (Bernier *et al.*, 1984; Bozeman *et al.*, 1981; Forshaw *et al.*, 1988; Hirai *et al.*, 1984; Marshall, 1989; Müller & Nitschke, 1986). A common feature in all outbreaks of polyomavirosis in birds is the infection of the liver and/or the kidneys. This was also observed in the present cases.

The source of infection with the viral agent in the three shamas is not clear. As the incubation period for polyomavirus infections in non-budgerigar psittacine birds has been estimated at between 2 and 14 days (Gaskin, 1989; Graham and Calnek, 1987), and as the first shama died at 14 days after arrival, it seems possible that the bird was already infected before its introduction in the aviary. If this is true, it may have subsequently spread the agent to the other birds. Another explanation may be that the shamas were infected with virus excreted by latently infected birds present in the aviary.

It was remarkable that only the newly purchased shamas developed clinical disease. This may indicate that other shamas in the aviary possessed protective immunity or that they did not experience factors predisposing to a clinical course of the polyomavirus infection. It seems rather unlikely that these birds were not infected during the outbreak since the occupation density in the aviary was high and the hygienic circumstances were poor. Polyomavirus is a highly contagious agent that spreads - at least in psittacine birds - through contaminated droppings, feather dust, respiratory secretions and crop secretions (Ritchie *et al.*, 1994).

Internal organs of shamas infected with *Enterococcus faecalis*, *Atoxoplasma* and coagulase-negative staphylococci aredescribed in this article. *Enterococcus faecalis* and *Atoxoplasma* have been recognised as passerine pathogens (Cooper *et al.*, 1989; Devriese *et al.*, 1990a; Devriese *et al.*, 1990b; Dorrestein, 1979), while coagulase-negative staphylococci are generally accepted as being non-pathogenic. Concurrent infections of polyomavirus and other pathogens such as poxvirus, *Mycobacterium*, *Atoxoplasma* and *Eimeria* have been described (Garcia *et al.*, 1994; Sironi, 1988). Although these pathogen interactions occur frequently, it is not clear to what extent they mutually influence the course of the disease. They may possibly contribute to the morbidity and mortality in polyomavirus infected aviaries.

PCR analyses failed to detect DNA from avian polyomavirus or avian adenovirus in paraffin-embedded liver and kidney samples. This may be due to the degradation of viral DNA resulting from the formalin fixation into fragments that are too small for primer recognition. Nevertheless, Garcia *et al.* (1994) demonstrated polyomavirus in the embedded tissues of seed crackers and bluebills using a VP1 probe in DNA in situ hybridization. This may indicate nucleotide differences between polyomaviruses from shamas, some other passerines and psittacine birds.

A polyomavirus vaccine for use in psittacine birds has been commercialised in recent years (Ritchie *et al.*, 1996). It is not known whether this vaccine can be used in passerine birds to protect expensive birds such as shamas.

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