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Septicaemia caused by *Edwardsiella tarda* and *Plesiomonas shigelloides* in captive penguin chicks

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Abstract

Three cases of fatal septicaemia due to *Plesiomonas shigelloides* and one due to *Edwardsiella tarda* were diagnosed in newborn penguins from the Basle Zoo, Switzerland from 2003–2007. The affected penguins were of two different species (king penguin, *Aptenodytes patagonicus*, and African penguin, *Spheniscus demersus*) and between 2 and 10 days old at the time of death. The causative agents, *E. tarda* and *P. shigelloides* are ubiquitous bacteria which are reported to be present in the normal intestinal flora of wild and captive aquatic animals, including penguins. Their occurrence and infectious potential is discussed.

Keywords: *Edwardsiella tarda*, *Plesiomonas shigelloides*, penguin chicks, septicaemia

Edwardsiella tarda und *Plesiomonas shigelloides* Septikämien bei Pinguinküken im Zoo

In den Jahren 2003–2007 wurden bei Pinguinküken im Zoo Basel fatale Septikämien diagnostiziert, in drei Fällen verursacht durch *Plesiomonas shigelloides* und einmal durch *Edwardsiella tarda*. Die betroffenen Pinguine gehörten zu zwei verschiedenen Arten (Königspinguin, *Aptenodytes patagonicus* und Brillenpinguin, *Spheniscus demersus*) und waren zum Zeitpunkt des Todes zwischen zwei und zehn Tagen alt. Die Erreger *E. tarda* and *P. shigelloides* sind ubiquitäre Bakterien, die in der intestinalen Normalflora von aquatischen Wild- und Zootieren, einschliesslich Pinguinen nachgewiesen wurden. Ihre Verbreitung und ihr Infektionspotential wird erörtert.

Schlüsselwörter: *Edwardsiella tarda*, *Plesiomonas shigelloides*, Pinguinküken, Septikämie

Introduction

Edwardsiella tarda (E. tarda) and *Plesiomonas shigelloides (P. shigelloides)* are opportunistic aquatic bacteria belonging to the family of the *Enterobacteriaceae*. These potential zoonotic organisms can be isolated from various fish, shellfish, reptile, bird and mammal species (White et al., 1973; Jagger, 2000), and the water sources they live in (White et al., 1973; Bauwens et al., 1983). These bacteria may be found in healthy animals but are occasionally reported to cause septicaemia, ulcerative skin lesions or intestinal infections in animals and humans (White et al., 1973; Coles et al., 1978; Janda and Abbott, 1993; Jagger, 2000; Abbot, 2003). In humans, diarrhoeic gastroenteritis is mostly food-borne (Van Damme and Vandepitte, 1980; Janda and Abbott, 1993; Jagger, 2000). In wild and captive penguins, lactose nonfermenting (like *E. tarda*) or lactose fermenting (like *P. shigelloides*) organisms are frequently cultured from faeces (Sieburth, 1959; Soucek and Mushin, 1970; Bauwens et al., 1983), and are therefore considered to be ubiquitous non-pathogenic bacteria of the intestinal tract. In young and/or immunosuppressed animals, however, they can be pathogenic (Jagger, 2000). In this report we describe three cases of fatal septicaemia due to *P. shigelloides* and one due to *E. tarda* in neonatal penguins.

History

At Basle Zoo 13 king penguins (*Aptenodytes patagonicus*) and 14 gentoo penguins (*Pygoscelis papua*) are housed

together in an indoor enclosure with a standing pool, at constant water and air temperature (8–10 °C). The African penguins (*Spheniscus demersus*), a group of 25 animals, live in an outdoor enclosure with a small pond in another area of the zoo. All penguins are fed with freshly thawed sea fish (herring, mackerel, sprat) which is supplemented daily with a commercial salt and mineral/vitamin tablet (Sea Tabs, Streuli Pharma, Uznach, Switzerland). None of the gentoo penguin chicks that hatched in the years 2003 to 2007 survived. In 2003 3 chicks died at 3, 4 and 7 days and remained unexamined. One chick that died at 12 days in 2005 was examined with the cause of death being septicaemia due to *Escherichia coli (E. coli)*. The survival rate of the king penguins was 62.5 %. In 2003 2 chicks survived and one died at 2 days of age (Tab.1), in 2004 1 chick survived and 1 perished at 3 days (Tab.1) and in 2007 2 survived and 1 died at 2 days (Tab.1). In the African penguins the survival rate was 33.3 %. In 2003 none of two chicks that hatched survived. One died at 11 days and was not sent for examination, the other died at 10 days and was examined (Tab.1). In 2004 a day-old chick died and was not examined. In 2005 both chicks born survived. In 2006 a 19 day-old chick died and was not examined. No previous clinical signs were noted in the penguin chicks.

Pathology

A complete necropsy was performed in 4 penguins and samples for histology were fixed in 4 % buffered formalin, embedded in paraffin, sectioned at 4µm, mounted on positively charged glass slides and stained with haematoxylin and eosin (HE). In gross pathology, all carcasses were slightly autolytic and in 3 penguins the body cavity was dilated and filled with grey-yellowish fluid, similar to that of the yolk sac. The stomachs and intestines only contained minimal amounts of mucoid fluid. The lungs of 2 penguins were diffusely hyperaemic and moist, whereas the lung of another chick presented multifocal hyperaemic areas. In 2 penguins the liver was slightly enlarged and disseminated pale areas were visible in the parenchyma. No other macroscopic lesions were observed. Histopathological findings are summarised in Table 1. The histology of the lungs revealed severe congestion and pulmonary oedema, as well as multifocal peribronchial macrophage infiltration in all four penguins (Fig. 1), associated with rod shaped bacteria in 3 of them. The liver in all animals was congested with multifocal hepatocellular necroses, which were associated with rod-shaped bacteria in 3 penguins. There were no signs of endoparasites or parasitic lesions in any of the penguin chicks.

Table 1: Pathological findings in 3 king penguin chicks (*Aptenodytes patagonicus*) and 1 African penguin (*Spheniscus demersus*) chick of the Basle Zoo 2003–2007.

organs and findings	penguin / bacteria isolated king penguin, 2 days, 2003	king penguin, 3 days, 2004	king penguin, 2 days, 2007	African penguin, 10 days, 2003
lung				
severe congestion and heterophilic leucostasis	+	+	+	+
oedema and macrophages in the parabronchi	+	+	+	+
associated with colonies of rod shaped bacteria	-	+	+	+
liver				
congestion	+	+	+	+
multifocal necrosis	+	+	+	+
multifocal lymphoplasmacytic and heterophilic perivascular infiltration	-	+	-	-
associated with intravascular colonies of rod shaped bacteria	+	-	+	+
kidney				
congestion	+	+	+	+
extramedullary haematopoiesis	+	+	+	+
bacteriology	*Plesiomonas shigelloides*	*Plesiomonas shigelloides*	*Edwardsiella tarda*	*Plesiomonas shigelloides*

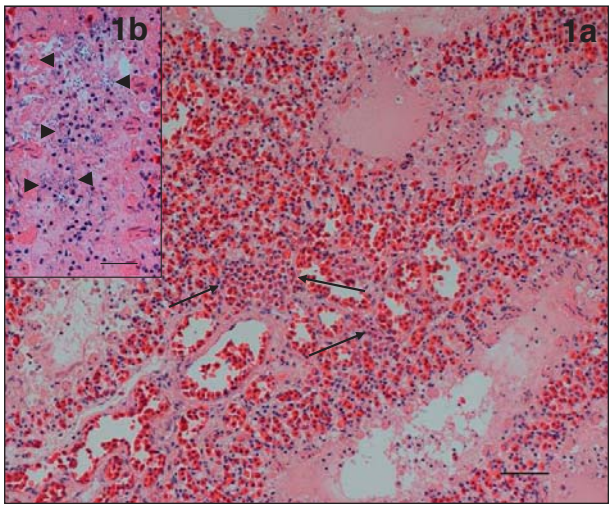

Figure 1: Histology of the lung, king penguin (*Aptenodytes patagonicus*), 2007. (a) Section with severe congestion, pulmonary oedema and clusters of heterophils (arrows). HE, 20x. Bar, 50 µm. (b) Close-up view of a parabronchus with basophilic, rod shaped bacteria (arrowheads). HE 40x. Bar, 25 µm.

Bacteriology

A selection of organs based on gross pathology and state of decay was submitted for bacteriology (Institute of Veterinary Bacteriology, University of Bern). *E. tarda* was strongly haemolytic on tryptone soy agar with 5 % sheep blood (TSA, Oxoid, Wesel, Germany), lactose negative on bromthymolblue lactose agar (Brolac; BioMérieux, Genève, Switzerland) and oxidase negative. Colonies of *P. shigelloides* were weakly incompletely haemolytic on TSA, late lactose fermenting and, as the only member of the *Enterobacteriaceae* family, oxidase positive. *P. shigelloides* was identified with the API ID 32 GN gallery (BioMérieux) and *E. tarda* with the VITEK 2 GN card (BioMérieux). A salmonella enrichment broth (Müller-Kauffmann tetrathionate-novobiocin broth MKTTn, Oxoid) was used for intestinal samples. Serotyping of the salmonella isolate was carried out at the National Centre for Enteropathogenic Bacteria (NENT), Switzerland.

In the king penguin that died in 2003, a pure culture of *P. shigelloides* was isolated from the liver and from the kidney. In the king penguin submitted in 2004, a pure culture of *P. shigelloides* was isolated from the liver and small intestine. In this animal, *Salmonella enterica* subsp. *diarizonae* serovar 47:k:z35 and *E. tarda* were additionally isolated only from the small intestine from the subculture of the salmonella enrichment broth. A pure culture of *E. tarda* was isolated in the king penguin from 2007 from the liver, lung, yolk sac and small intestine. In the African penguin, *P. shigelloides* together with *Staphylococcus* species was isolated from the lung. No additional parasitological or virological tests were carried out.

Discussion

In the 4 penguin chicks examined, septicaemia due to the cultured bacteria was diagnosed and considered to be the cause of death. Pathology results corresponded to the microbiological findings of pure cultures of either *E. tarda* or *P. shigelloides* isolated from different extraintestinal organs. By contrast, neither *P. shigelloides* nor *E. tarda* were isolated from extraintestinal organs in three adult penguins (one of each species), that had died of old age (22 to 25 years). In histopathology, observed inflammatory lesions associated with rod-shaped bacteria in all chicks were consistent with septicaemia (Tab.1). Thus, we exclude that *P. shigelloides* and *E. tarda* in the dead chicks simply derived from contamination from the intestines, where many *Enterobacteriaceae*, including *Salmonella* spp., are regularly detected in low numbers in the normal intestinal flora of healthy penguins (Sieburth, 1959; Soucek and Mushin, 1970; Bauwens et al., 1983). This is shown by the finding of *E. tarda* and *S. enterica* subsp. *diarizonae* serovar 47:k:z35 exclusively from the enrichment broth from faeces (and not from extraintestinal organs) from one penguin, that died of *P. shigelloides* septicaemia.

P. shigelloides and *E. tarda* as well as *Salmonella* spp. are not necessarily strictly pathogenic, indeed they are frequently isolated from intestines of free-ranging healthy birds (Sieburth, 1959; Soucek and Mushin, 1970; Bauwens et al., 1983; Rollin and Baylet, 1983, Hubálek et al., 1995). *P. shigelloides* was cultured from 17 % of 75 examined wild grey heron (*Ardea cinerea*) nestlings (Glünder, 1988) and *E. tarda* is known to be part of the normal intestinal flora of flamingoes (Rollin and Baylet, 1983). In Antwerp Zoo, 21 % of 254 examined animals of various species were positive for *P. shigelloides*, *Edwardsiella* spp. (including *E. tarda*) or both (Bauwens et al., 1983). The bacteria were also cultured from water sources, e.g. ponds and from faeces of free-ranging gulls living in the zoo. In comparison, the incidence of *E. tarda* in free-ranging animals from areas outside the zoo was much lower (Bauwens et al., 1983). The authors suggest that the high population density in a zoo may increase the infection rate. Interestingly, the agents could not be isolated from the frozen fish which was used for feeding. Therefore, a foodborne route of infection could not be confirmed in this study and other sources like contaminated water have to be considered. Nevertheless, fish-eating birds and mammals have a higher risk of ingesting the bacteria (Bauwens et al., 1983). Accordingly, in birds from Swiss zoos, apart from the two species of penguins, septicaemia due to either *P. shigelloides* or *E. tarda* was additionally diagnosed in two captive pink flamingos (*Phoenicopterus ru-*

ber roseus), a captive black stork (*Ciconia nigra*) and in a free-ranging grey heron (Institute of Veterinary Bacteriology, Bern, Switzerland), where in two of the mentioned species isolation of the organism had already been described (Rollin and Baylet, 1983; Glünder, 1988).
Extraintestinal infections in birds are often associated with concurrent infections with parasites, *Aspergillus* sp. or bacteria (Glünder, 1988; Jagger, 2000). However, the penguins described in this study were too young for a concurrent parasitic infection. Adult parasites and eggs are generally not expected in chicks up to 10 days old. Pathological findings of extraintestinal infections in birds include hyperaemia of parenchymatous organs and hepatic degeneration (Glünder, 1988), corresponding to the findings described in this study (Tab.1). The reservoir of *P. shigelloides* and *E. tarda* infection in birds is not clear. The sporadic presence of *P. shigelloides* and *E. tarda* in bird faeces may be due to transient colonisation following ingestion of fish or water, or water-borne contamination of food (Van Damme and Vandepitte, 1984).
In freshly hatched chicks, the most likely entry for environmental bacteria is the umbilicus, emerging to a yolk sack infection and, subsequently, to septicaemia. In the Basle Zoo no organic nesting material is offered and the enclosure is regularly cleaned. However, breeding pairs are very sensitive to disturbance and therefore a stringent hygiene is not feasible for the nesting site during breeding time. The chicks may also be infected at hatching through the faecal contamination of the egg shells (Barnes et al., 2008). In analogy to *E.coli* in chickens, other routes of infections have also to be considered in the older penguin chicks, e.g. via the conjunctiva or nasal cavity (Barnes et al., 2008).

Whenever an unusual bacteria is isolated the questions arises about its zoonotic potential. *P. shigelloides* and *E. tarda* mostly cause skin wound infections after contact with contaminated water (Janda and Abbott, 1993; Abbot, 2003), while human gastrointestinal infections are almost exclusively described in association with consumption of contaminated sea food in tropical countries (acute «traveller's diarrhoea») (Van Damme and Vandepitte, 1980; Rautelin et al., 1995), and in fact, intestinal infections of keepers have not been reported.

Conclusion

Septicaemia due to *E. tarda* and *P. shigelloides* in captive parent-reared penguin chicks occur, because the bacteria naturally prevail in the intestines of the adult birds and may be found in ponds and other water sources. It is not possible to follow a stringent hygiene during breeding, hatching and raising of chicks in the enclosure, because penguins do not appreciate disturbance. *E. tarda* and *P. shigelloides* are unusual *Enterobacteriaceae* which only rarely infect humans.

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Septicémies à *Edwardsiella tarda* et *Plesiomonas shigelloides* chez des jeunes manchots dans un zoo

Entre 2003 et 2007 on a diagnostiqué chez les poussins manchots du Zoo de Bâle des septicémies fatales, causées dans 3 cas par *Plesiomonas shigelloides* et dans 1 par *Edwardsiella tarda*. Les manchots concernés appartenaient à 2 espèces (Manchot royal, *Aptenodytes patagonicus et* Manchot du Cap, *Spheniscus demersus*) et étaient âgés au moment de leur mort de 2 à 10 jours. *E. tarda* et *P. shigelloides* sont des bactéries ubiquitaires présentes dans la flore intestinale normale des animaux aquatiques sauvages ou en captivité, y compris les manchots. On décrit leur répartition et leur potentiel infectieux.

Edwardsiella tarda e *Plesiomonas shigelloides*: setticemia nei pulcini di pinguino allo zoo

Tra il 2003 e il 2007 allo zoo di Basilea si è diagnosticata nei pulcini di pinguino una setticemia fatale, provocata in tre casi da *Plesiomonas shigelloides* e una volta da *Edwardsiella tarda*. I pinguini colpiti appartenevano a due specie diverse (Pinguino reale, *Aptenodytes patagonicus* e Pinguino africano, *Spheniscus demersus*) e al momento della morte avevano tra i due e dieci giorni di vita. L'agente patogeno *E. tarda e P. shigelloides* sono batteri ubiquitari che si trovano nella flora intestinale normale di animali acquatica selvatici e di animali dello zoo, inclusi i pinguini. La loro distribuzione e il loro potenziale infettivo è in via di discussione.

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