Pasteurella testudinis associated with respiratory disease and septicaemia in leopard (*Geochelone pardalis*) and other tortoises in South Africa

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ABSTRACT

The first recorded isolates of *Pasteurella testudinis* from South African tortoises kept in captivity is presented. *P. testudinis* was found in association with respiratory disease in affected animals.

Key words: chelonian, *Pasteurella testudinis*, respiratory disease, tortoise.

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Pasteurella species are commonly found on the mucous membranes of the respiratory tract of mammals and birds. The various species of *Pasteurella* are widely distributed, both geographically and with regard to host species. Some species of *Pasteurella*, such as *P. multocida*, are found in many different hosts[1] while others, such as *P. canis* in dogs[8] and *P. caballi* in horses[11], have a narrow host range. *Pasteurella* species generally cause respiratory and septicaemic disease.

Pasteurella testudinis was first described by Snipes *et al.*[12,13], who associated it with respiratory disease in the Californian desert tortoise, *Gopherus agassizii*. It has also been isolated from the mucous membranes of the respiratory tract as part of the normal flora[5,13], in both captive and free-ranging California desert tortoises[5,7,12,13]. Respiratory disease is often precipitated by stress related to captivity[12] as well as *Mycoplasma* or viral infection[2,7,10]. *P. testudinis* has not previously been reported from southern African chelonians.

The family *Testudinidae* is represented by 43 species of tortoises, 14 of which occur in southern Africa[3]. The leopard tortoise, *Geochelone pardalis,* is widespread in central and southern Africa, ranging from Ethiopia and the Sudan in the north-east of the continent, to Montagu in the Western Cape Province. Adult leopard tortoises have a variable colour pattern of either a light brown or yellow background with darker brown patches, or a darker brown background with lighter patches. This results in a leopard-like pattern.

Terrestrial tortoises are protected wild animals in the whole of South Africa and they may only be kept in captivity under permit from conservation authorities. Five outbreaks of disease, from 3 locations, are described here. The bacterial isolates reported below were all made from tortoises that had been kept in captivity and had shown respiratory tract disease. The specimens were submitted to the Onderstepoort Veterinary Institute between 1981 and 1993.

All 5 outbreaks or cases were in 3 populations of adult tortoises kept in the Pretoria area. The first 2 outbreaks were in a group of leopard tortoises kept in a suburban garden in Pretoria. The tortoises showed nasal discharges and some deaths occurred in January 1981, when they were first sampled. Thereafter they were kept together as an isolated group without showing any signs of illness for 8 years until December 1988. At that stage another group of leopard tortoises, which had been kept by a different owner, was introduced to the group. Tortoises from both groups subsequently showed respiratory distress and nasal discharges, and 2 from each group died.

The 3rd set of samples was from a group of tortoises of an unknown species kept at the Onderstepoort Veterinary Institute to test the efficacy of various anthelmintics. These tortoises showed pneumonia and nasal discharges in December 1991.

The last 2 cases were from a group of tortoises of an unknown species kept in a zoological garden. The 1st one was found dead in August 1992. At necropsy, ascites, lung oedema, perianal oedema and intestinal wall oedema was seen. The urinary bladder contained a large amount of beige, viscous exudate, which was partially solidified. The urinary bladder contents, lung and intestine from this case were sampled. The same group of tortoises at the zoo showed chronic nasal discharges 7 months later, from which a further isolation was made.

Only nasal discharges from affected tortoises in each of the 5 outbreaks were sampled, except the one from the zoo, where the internal organs were sampled. The samples from these cases were all cultured on blood tryptose agar containing bovine blood, as well as on MacConkey agar, and incubated at 37 °C overnight. *P. testudinis* was isolated from all these cases in pure, or nearly pure growth, except from the organs of the tortoise from the zoo that had died from septicaemia, where it was isolated together with *Edwardsiella tarda*. The isolates of *P. testudinis* grew on blood agar as greyish, slightly mucoid, beta-haemolytic colonies, which were tiny after overnight culture, and much larger (2–3 mm diameter) after 2 days of incubation. They were V and X factor independent. There was either no growth or very tiny colonies on MacConkey agar. The bacteria were Gram-negative pleomorphic rods, which were facultatively anaerobic. They were non-motile, catalase positive and weakly oxidase positive. Conventional biochemical tests were used for identification. The isolates were positive for nitrate reduction, methyl red, *o*-nitrophenyl-*beta*-D-galactopyranoside (ONPG), indole production and aesculin hydrolysis. Tests for urease, gelatine hydrolysis and arginine dehydrolase were negative. The isolates fermented glucose, without the production of gas, as well as adonitol, galactose, inositol, mannitol, maltose, sucrose and xylose. They did not ferment arabinose, dulcitol, rhamnose, sorbitol or trehalose. Lactose and salicin were either negative or showed weakly positive fermentation. Hydrogen sulphide was produced in small quantities, only detectable with lead acetate strips and not on triple sugar iron agar. These results correspond with the

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description of *P. testudinis*[12,13], except for fermentation of rhamnose[13].

It is unlikely that this bacterium could have originated from chelonians from other continents, as only the tortoises from the zoo may have had either direct or indirect contact *via* attendants with exotic species.

Only 4 other cases from tortoises were received for diagnosis of bacterial diseases at the Onderstepoort Veterinary Institute from 1970 to 2001. Two cases yielded no significant bacterial isolates and 2 others from unknown tortoise species were submitted for *Salmonella* typing. One was from a healthy imported tortoise, which yielded *Salmonella* Zaire[15], and the other was a tortoise from the Western Cape with hepatitis, which yielded *Salmonella* Windermere, in 1980.

Since no other investigations were carried out on the cases it is unknown whether viruses or *Mycoplasma* were also present, as was found in Californian desert tortoises[2,7,10]. The stress of being held kept in captivity, as well as the mixing of 2 groups in the 2nd case, probably contributed to clinical disease in those tortoises.

A primary viral aetiology was suspected as a cause of high mortality in a South African tortoise colony in which some of the tortoises showed acute dyspnoea[9]. Of the 31 tortoises that died in that outbreak, Herpesvirus-like inclusions were found in material from 1 tortoise and Paramyxovirus-like inclusions in another. Bacterial culture was only attempted in a few cases, and *Pseudomonas aeruginosa* was isolated from an abscess of 1 case, the liver of another, the mouth of a 3rd (*Homopus areolatus*) and the lungs of a 4th (*Chersina angulata*). It was not clear from the report which species the first 2 referred to, but of the 31 tortoises that died, only 2 were leopard tortoises. One of the leopard tortoises showed dyspnoea and stomatitis, and had a plastic bag in the colon, and the other dyspnoea, sudden death and pneumonia. No attempt was, however, made to isolate viruses from this group, and it appears that no bacterial culture was attempted on either leopard tortoise.

The family Pasteurellaceae is currently under intensive taxonomic investigation, and proposals for nomenclatural changes as well as reclassification of some species are regularly published, such as *Mannheimia* in ruminants, *Lonepinella* in koalas and *Phoecanobacter* in porpoises[1,6]. *P. testudinis* should not belong to the genus *Pasteurella*, as it displays less than 50 % DNA homology with the type species, *Pasteurella multocida*[8]. Its exact taxonomic position has not yet been determined, and a new genus will probably be defined to accommodate it[1,4,6]. Molecular methods, such as ribotyping, whole cell and outer membrane protein determinations have been described, which distinguish between strains isolated from tortoises with and without disease[14]. These isolates were, however, not subjected to further examination.

Further investigations should be carried out to see whether *P. testudinis* is more widespread in South Africa. Leopard tortoises were definitely identified only in the first 2 outbreaks, and investigations should include all 14 southern African tortoise species, both free-living and in captivity.

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