Bacteriological Features Of Rhinitis In Captive Greek Tortoises, *Testudo graeca*

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Introduction

Rhinitis does not appear to be a significant problem in free-ranging European tortoises. A prevalence of less than one percent in Greek tortoises, *Testudo graeca*, in Spain has been reported (Pons and Aguilar, 1992). In contrast, rhinitis is one of the most common problems in long-term captive *Testudo* tortoises. This condition is more frequent and is more severe in Greek tortoises compared to Herman's tortoises, *T. hermanni*. Similar observations have been seen in several reptile rehabilitation centers in Spain, France and Italy (personal communication, 1994).

The disease occurs at the beginning of the hibernation season (from September to February) and is characterized by bubbling from the external nares with nasal discharge ranging from serous to mucopurulent. There is a noticeable loss of weight resulting in a weakened physical condition which worsens the prognosis for recovery. Following emergence from hibernation in spring, some animals partially recover, gain weight and remain stable but frequently relapse with the onset of hibernation. Morbidity is high and mortality is low except in those cases with considerable weight loss or pneumonia.

Pharmacological treatment often is unsuccessful. Good husbandry procedures stabilize ill animals but will not improve the health of rhinitic tortoises.

It is unclear what role bacteria or viruses play in the development of this disease. Many microorganisms can be recovered from the oropharynx or nasal discharges of affected animals but some of these can be also recovered from apparently healthy tortoises (Snipes, *et al*, 1980, Lawrence and Needham, 1985). Recently, *Mycoplasma spp.* have been implicated as causative agents of rhinitis in desert tortoises, *Gopherus spp.* (Jacobson, 1993, Jacobson, *et al*, 1995). An avian serovar of *Chlamydia psittaci* has been associated with pneumonia in the Greek tortoise, *Testudo graeca*, (Vanrompay, *et al*, 1994). Some viruses, especially Sendai virus, have also been involved in this process. Moreover, stomatitis, glossitis and rhinitis has been reported in *Testudo* tortoises due to herpesvirus but the role of viruses as a respiratory pathogens in these species is not fully understood (Jackson and Needham, 1983). European wildlife rehabilitation centers euthanise affected tortoises and do not release doubtful animals for fear of spreading rhinitis (Pritchard, 1996).

This paper presents the results of a bacteriological and clinical survey of rhinitis in 17 ill Greek tortoises from a collection of 32 animals in a reptile rehabilitation center in Catalonia, Spain.

Material and Methods

Tortoises - The study was conducted with 17 Spanish Greek tortoises, *Testudo graeca graeca*, suffering from rhinitis in the Reptile Rehabilitation Center of Masquefa in Catalonia. Tortoises were maintained in several terraria in groups of two or three animals. Housing and management conditions were as recommended in the literature (Frye, 1991, Highfield, 1993). The diet consisted of a mixture of wild plants like dandelion, *Taraxacum officinale*, hawkbits, *Leontodon sp.*, trefoils, *Lotus sp.* and commercial vegetables and fruit such as lettuce, apple, pear and peach, supplemented with minerals, trace elements and vitamins. The animals were kept in an enclosure with native vegetation and trees. All became ill in the autumn of 1992. They were not treated with antimicrobial agents for at least one week before samples were taken. Control samples were taken from four unaffected wild Greek tortoises captured in Sierra de Almenara (Murcia, Spain).

Microbiological study – Swabs from nasal discharges were plated onto blood agar, Salmonella-Shigella and McConkey media. Swabs were placed into tripticase soy broth and incubated aerobically at 37°C (98.6°F). After 48 hours subcultures on blood agar were made from broths and incubated in the same conditions as described above. Bacterial isolates were presumptively identified by their cultural and staining characteristics, microscopic morphology, Gram stain, catalase and oxidase reactions. They were identified to species using the API-20E, API-Staph and API-Coryne systems (Bio-Merieux, Barcelona, Spain). In certain instances where API systems were not sufficiently accurate to provide a definitive identification conventional biochemical tests were used.

Antimicrobial susceptibility testing– All bacterial isolates were tested for sensitivity to antimicrobial agents by disc-diffusion method (ASM). Eleven antimicrobial agents were used in each case; penicillin G, amoxicillin, amoxicillin and clavulanic acid, cephalexin, ceftriaxone, erythromycin, chloramphenicol, gentamicin, enro-floxacin,

cotrimoxazole and tetracycline. In those cases where *Pseudomonas spp.* or *Acinetobacter spp.*, were isolated, ticarcillin, ceftazidime and tobramycin were also assayed. Cloxacillin was also evaluated when *Staphylococci* were isolated (all antimicrobial agents provided by Bio-Merieux, except enrofloxacin, provided by Bayer Quimico-Farmaceutica, Barcelona, Spain). *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC, *Ps. aeruginosa* ATCC, and *Streptococcus (Enterococcus) faecalis* ATCC, were used as control microorganisms.

Clinical trials – Tortoises were treated with the most reliable antimicrobial agent based on antimicrobial susceptibility testing. When more than one bacterium was isolated the antimicrobial agent chosen was active against all the strains. All antimicrobials were administered intramuscularly. Gentamicin was also instilled in the nares. Additionally, 5,000 I.U. of vitamin A in a single dose and intracoelomic electrolytes (25 ml/kg every 48 hours) were given. Environmental conditions were improved by increasing the temperature up to 30°C (86.0°F).

Results

Mixed cultures of *Bacillus sp, Pseudomonas fluorescens, Acinetobacter calcoaceticus, Corynebacterium sp.* and a mixture of saprophytic Gram positive flora were isolated from the healthy wild tortoises (Table 1).

From affected tortoises, a total of 27 bacterial strains were isolated (Table 2). In six cases only one strain was isolated, in seven cases two strains were isolated and in another two cases three and four strains were isolated. *Acinetobacter calcoaceticus, Achromobacter spp.* and *Chromobacterium spp.* were always isolated in pure culture. In contrast, *Staphylococci* were never isolated alone. The three strains of *Corynebacterium spp.* were isolated together with *P. fluorescens, Pasteurella multocida* and *Proteus rettgeri*, respectively. Tortoises kept together in the same terrarium had identical bacterial isolates.

Antimicrobial susceptibility tests showed that enrofloxacin inhibited the growth of all bacterial strains apart from *Corynebacterium spp.* which were also resistant to erythromycin and tetracycline but susceptible to gentamicin. All *Pseudomonas* and *A. calcoaceticus* isolates were susceptible to ticarcillin and ceftazidime but not to other β-lactams or tetracycline. They were also susceptible to gentamicin or tobramicin. *Staphylococci* were sensitive to all β-lactams. *P. rettgeri* showed more resistance to antimicrobials than other bacteria being susceptible only to enrofloxacin and aminoglycosides. *Achromobacter spp. and Chromobacterium spp.* were susceptible to all the antimicrobial agents tested.

Based on these findings, tortoises were treated with enrofloxacin (Baytril 2.5%, Bayer, Barcelona, Spain) 5 mg/kg IM q 12 hours for seven days. In those cases where *Corynebacterium* were isolated, gentamicin (Colircusi Gentamicina, 0.6 mg/ml, Laboratorios Cusi S.A., El Masnou, Barcelona, Spain) was also instilled in the nares (two drops of this solution in each nostril q eight hours) These latter cases were the only ones in which a marked clinical improvement was observed during the study. This treatment was given two months before hibernation (from September to November) and the tortoises were maintained at 30°C (86.0°F). The total hibernation time was three months. In spite of nursing and antimicrobial treatment, two animals died; nevertheless, at the beginning of the spring the surviving tortoises improved slowly and were in good physical condition by early summer.

Histopathological findings in dead tortoises showed a mixed mononuclear and granulocytic infiltrate in the nasal respiratory epithelium but no lesions in the tongue, trachea, lungs or liver.

Discussion

Although several bacteria and Sendai virus have been involved in the etiology of rhinitis in chelonians (Jackson and Needham, 1983, Lawrence and Needham, 1985, Jacobson, 1993), the causes remain unclear. The condition appears to be infectious since it spreads quickly in a collection of tortoises following the pattern of a contagious disease.

The role of bacteria in rhinitis of Greek tortoises has been discussed by other authors who found little difference between the nasal flora of ill and healthy *Testudo* tortoises (Lawrence and Needham, 1985). Moreover, they observed that *Staphylococcus aureus* and *Citrobacter freundii* were the predominant-species present in naso-oropharyngeal samples of rhinitic tortoises and that *Pseudomonas spp.* were only isolated sporadically. In contrast, in our study the predominant nasal flora of rhinitic *Testudo* tortoises were Gram-negative microorganisms, accounting for 15 of 27 strains isolated, with a predominance of Enterobacteriaceae, mainly *P. rettgeri* and *Pseudomonas spp.* These last bacteria have been implicated as important pathogens of chelonians (Frye, 1991).

Pasteurella spp. have been reported to be usually present in nasal flora of Mediterranean tortoises and they have been involved in stomatitis and respiratory disease (Snipes, *et al*, 1980, Holt and Cooper, 1976). Nevertheless, only one strain of *Pasteurella* was isolated in our study and seemed to be of little significance in our rhinitic tortoises.

Therapeutic management of rhinitis in tortoises remains difficult since the cause of the disease is unknown. Nursing care can be sufficient in mild cases but is not adequate alone when animals have shown a significant loss of body condition. Antimicrobial treatment is useful in some cases but, as found in the present study and in those reported previously (Vanrompay, *et al*, 1994, Frye, 1991), it does not effect a complete cure of rhinitic tortoises. Antimicrobial susceptibility tests showed that enrofloxacin was the one week of antimicrobial agent with activity against most of the bacteria. These results indicated that where bacteria, with the exception of *Corynebacterium spp.*, are involved, the use of fluoroquinolones is appropriate. However, one week of antimicrobial treatment was unsuccessful and in our opinion, bacteria (except mycoplasmas) seem to play a minor role in the disease. In short, the antimicrobial therapy would be directed to prevent growth of potentially pathogenic flora in an immunosuppressed tortoise.

Recently, *Mycoplasma spp.*, have been reported involved in the upper respiratory tract disease of tortoises (Jacobson, *et al*, 1991, Jacobson, 1993, Jacobson, 1994, Jacobson, *et al*, 1995). We did not attempt to isolate mycoplasmas. Although enrofloxacin has demonstrated to be very active against mycoplasmas in several avian and reptilian species prolonged therapy is required (Jordan, 1991, Schildger and Gobel, 1989).

Table 1. Bacterial strains isolated from nasal swabs of four healthy wild Greek Tortoises.

Microorganism isolated	Number of Strains
Bacillus spp	3 (42.8%)
Pseudomonas fluorescens	2 (28.5%)
Acinetobacter calcoaceticus	1 (14.2%)
Corynebacterium spp	1 (14.2%)

Table 2. Bacterial strains isolated from nasal exudates of 17 rhinitic captive Greek Tortoises.

Microorganism isolated	Number of Strains
Proteus rettgeri	7 (25.9%)
Corynebacterium spp.	3 (11.1%)
Acinetobacter calcoaceticus	2 (7.4%)
Pseudomonas aeruginosa	2 (7.4%)
Pseudomonas fluorescens	2 (7.4%)
Achromobacter spp.	2 (7.4%)
Staphylococcus aureus	2 (7.4%)
Staphylococcus intermedius	2 (7.4%)
Staphylococcus lentus	1 (3.7%)
Staphylococcus xylosus	1 (3.7%)
Pasteurella multocida	1 (3.7%)
Chromobacterium spp.	1 (3.7%)
Citrobacter freundii	1 (3.7%)

The following evidence suggests that rhinitis in European tortoises is a disease of infectious nature and that it could be caused by a virus or mycoplasma:

1. After the introduction of healthy tortoises in a colony of ill animals, the former became ill.
2. The disease spreads rapidly after its appearance in a colony of tortoises.
3. Intranuclear inclusion bodies resembling those of a herpesvirus have been observed in the oropharyngeal and respiratory tracts of dead *Testudo sp.* that suffered from rhinitis, stomatitis and glossitis (Blahak, 1995).
4. Herpesvirus - like particles have been isolated in rhinitic Greek tortoises in Spain, but their presence has not been determined in every ill animal (personal communication, 1996).
5. Histopathologic features in our Greek Tortoises are more similar with mycoplasma infections in desert tortoises than herpesvirus infections in other tortoises (Holt and Cooper, 1976, Blahak, 1995, Jacobson, *et al*, 1991, Mohnanty and Dutta, 1983, Jacobson, 1994). The absence of oropharyngeal lesions would not be typical for herpesvirus infection (Blahak, 1995, Cooper, *et al*, 1988).

Although not conclusive, the hypothesis of a virus or mycoplasma as the causative agent of the rhinitis would explain most of the features observed:

1. Both are known to affect tortoises in oropharyngeal location (Blahak, 1995, Jacobson, *et al*, 1991).
2. They have the ability to remain in a latent state with reactivation of pathogencity when immunosupressive conditions occur. Tortoises with complete remission of clinical signs may still develop the disease at a future date (Mohanty and Dutta, 1983, Jacobson, 1994).

3. The disease does not respond properly to short-term antibiotics based on bacterial isolates.
4. Both agents have the ability to predispose their hosts to secondary agents (Jacobson, *et al*, 1991, Mohanty and Dutta, 1983). Bacteria could also contribute to the disease as secondary pathogens and thus increase the severity of the illness. Some evidence to suggest this was that those tortoises treated with intra-nasal Gentamicin showed marked clinical improvement.

Aerobic bacterial isolates (not including mycoplasmas) from nasal discharges in Greek Tortoises with rhinitis seems to be secondary to the initial agent. Further investigations are needed to clarify the etiology of rhinitis in tortoises, especially the role of viruses or mycoplasmas since this disease can be a serious problem in reptile centers and zoological gardens.

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