A report of hepatic granuloma in free-living Brown anoles (*Anolis sagrei*) in Taiwan: two cases

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ABSTRACT – Brown anoles (*Anolis sagrei*) were collected for stomach-content analysis from an area surrounding a plant nursery (23º25'51"N, 120º28'30"E) in Santzepu, Sheishan District, Chia-Yi County. Tissue sampling revealed liver lesions, which were later identified as hepatic granulomas, in two individuals. This appears to be the first record of hepatic granuloma in *A. sagrei* in Taiwan.

A granuloma is a mass or nodule of chronically inflamed tissue, with granulations, that is usually associated with an infection (*Merriam Webster's Medical Dictionary*, 1995), and often resembles true neoplasms (Jacobson, 1981). These lesions are usually due to infections by bacteria (e.g., Sohn *et al*., 2003), fungi (e.g., Juopperi *et al*., 2002; Speare *et al*., 1997; Silva, 1985) or parasites (e.g., Junker *et al*., 1999; Weiss *et al*., 1987; Silva *et al*., 2000; Fogaca *et al*., 2000; Goldberg & Bursey, 1988), and may occur in almost any organ system (Jacobson, 1981). Viral, fungal, bacterial, protozoan and metazoan parasites can induce hepatitis in reptiles, a fairly common occurrence in captive individuals, and the formation of focal or multifocal granulomata is a typical chronic reaction to such infections (Frye, 1991).

The Brown anole (*Anolis sagrei*), also known as *Norops sagrei* (Köhler, 2000; Lee, 2000), is native to the Bahamas and Cuba (Campbell, 1996) and certian islands such as Swan Island (Rodriguez Schettino, 1999), Cayman Brac, and Little Cayman (Losos *et al*., 1993). An introduced population has also been reported from Taiwan (Norval *et al*., 2002) as well as some other localities, which have been reported elsewhere (Calderon *et al.,* 2003; Campbell, 2003; Conant & Collins, 1991; Goldberg & Bursey, 2000; Greene *et al*. 2002; McAllister *et al*., 2003; Landwer *et al*., 1995; Rodriguez Schettino, 1999; Roughgarden, 1995; Schwartz & Henderson, 1991; Steven & Lance, 1994), and these papers should be consulted for details. At present the population in Taiwan is being studied and here what appears to be the first report of hepatic granuloma in *A. sagrei* from this area will be discussed.

MATERIALS AND METHODS

Between the 10th of January 2002 and 28th of March 2003 a sample of about 30 Brown anoles (*Anolis sagrei*), consisting of males and females, was collected monthly for a stomach content study on these exotic lizards. The lizards were captured by hand or with a fine-meshed fishing scoop-net. At the point of capture, the GPS location was recorded with a GARMINI 2 PLUS handheld reader, the specimen was allocated a field number, and the date and time was recorded. Upon returning from the study area, each lizard was killed with ether; the snout-vent length (SVL) and tail length (TL) were measured to the nearest mm; the tail was scored as complete or broken; the specimen was weighed to the nearest 0.1g with a YC e68 digital scale and dissected by making a

mid-ventral incision. The stomach was removed for stomach content analysis, and from March 2002, to the end of the study period, the abdominal fat and the liver, as well as the right testis of the males, were also removed as part of a reproductive cycle study.

On the 18th of January 2002 (GN0020 - case 1) and on the 12th of December 2002 (GN0397 - case 2) lizards were collected that had liver lesions. The livers of these two individuals were submitted to the pathology laboratory of Chia-Yi Christian Hospital for examination. In addition to the livers, the brain, heart, tail, stomach, left testis, abdominal fat, pancreas and lungs of GN0020, as well as the pancreas and left lung of GN0397, were removed and submitted for examination. All the organs and tissue samples were fixed in 10% formalin, embedded in wax, sectioned at 5µ and stained with Ehrlich's haematoxylin and eosin (HE), Periodic-Acid-Schiff (PAS) and Ziehl-Nielsen (ZN) acid-fast stains. In addition to that, samples of the liver and pancreas of GN0397 were stained with Mayer's Mucicarmine and Grocott's Methenamine Silver Nitrate (GMS) stains.

RESULTS

Case 1: This male was collected at 23º25'44"N, 120º28'54"E, and had a SVL of 59 mm, TL of 45 mm, and a mass of 5g. The average SVL and mass of the other males collected in January 2001 were 51.87 mm and 3.71g respectively. As GN0020 experienced tail-loss its TL could not be compared with that of the other males that had complete tails, and because this individual was not part of the reproductive cycle study that was initiated in March 2001, its abdominal fat and liver were not weighed before tissue sampling and can thus not be reported on. The seminiferous tubules of the left testis contained spermatogonia, primary spermatocytes, secondary spermatocytes, and transforming spermatids with few spermatozoa.

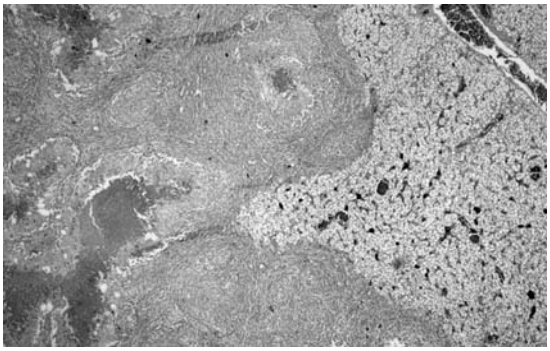
The stomach of the lizard contained the following: Hymenoptera (Apidae) x1, Coleoptera x1, Hemiptera (Lygaeidae) x2, Diptera (Dixidae) x1 and Mollusca x1.

The whitish nodule was focal on the ventral side of the liver, and slightly raised (Fig. 1). Histological examination of the liver nodule revealed a granulomatous reaction (Fig. 2), composed of pleomorphic histiocytes, with occasional central necrosis and haemorrhages (Fig. 3). In the PAS stained sections, fungal spores and hyphae were observed in the granuloma, morphologically similar to those of an *Aspergillus* species (Fig. 4). No acid-fast bacillus was observed in the ZN stained sections.

Case 2: This female was collected at 23º25'47"N, 120º28'53"E, and had a SVL, TL, mass, abdominal fat weight (AFW) and liver weight (LW) of 39 mm, 74 mm, 1.7g, 0.09g and 0.16g respectively. The average SVL, TL (n=5, excluding the individuals that suffered tail-loss), mass, AFW and LW of the other females collected in December 2001 were 39.17 mm, 73.4 mm, 1.64g, 0.09g and 0.11g respectively. In order to minimize variances due to different SVLs, the LW

Figure 1. The nodule on the surface of the liver of the *A. sagrei* male, as observed during dissection of this specimen.

Figure 2. A section of the liver, with the nodule on the left of the image and normal tissue on the right (40 x magnification, HE stain).



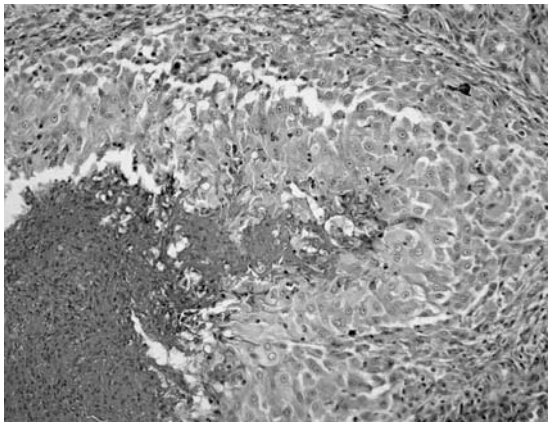


Figure 3. An enlarged section of the liver nodule (200 x magnification, HE stain).

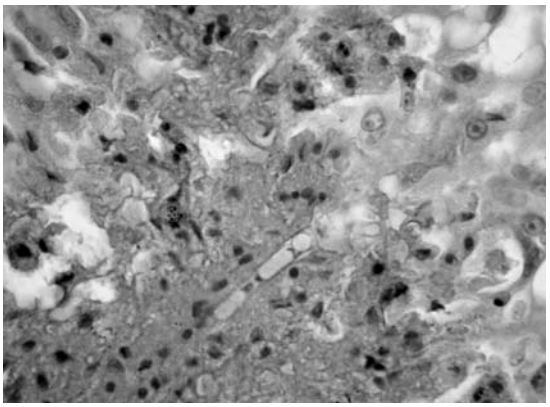


Figure 4. Fungal hyphae are visible in the centre lower portion of the image (400 x magnification, HE stain).

Figure 5. The dorsal (top) and ventral (bottom) views of the liver of the *A. sagrei* female, as observed during dissection of this specimen.

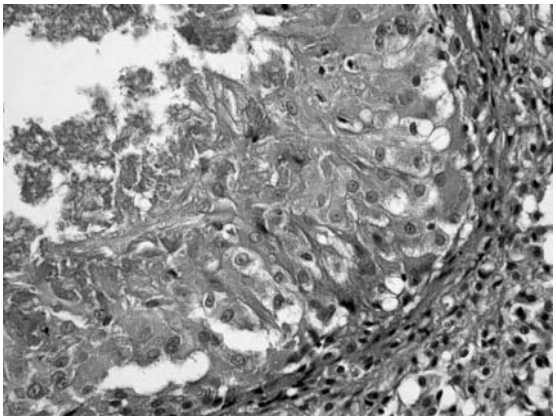


Figure 6. A section of the nodule of the *A. sagrei* female's liver, in which necrosis is visible in the top left hand corner of the image (400 x magnification, HE stain).

was also expressed as a proportionate liver weight (PLW = LW/SVL X 100); this female had a PLW of 0.41% while the monthly mean was 0.27%.

The stomach of the lizard contained the following: Coleoptera x1 and Lepidoptera (Noctuidae) x1. This female had one enlarged follicle and a contralaterally located oviducal egg.

The nodule was diffused, creamish in colour and not raised as in case 1 (Fig. 5). Histological examination of the liver nodule revealed the same chronic inflammatory pattern as in the granuloma described in case 1, but with central necrosis (Fig. 6). However, neither fungal hyphae or spores, nor acid-fast bacilli could be identified in the PAS and ZN stains.

DISCUSSION

Except for the presence of melanomacrophages, which are absent in avian and mammalian livers, the reptilian liver is similar in function and structure to that of other vertebrates, and the liver and biliary tree are sites of diseases similar to those found in mammals (Schaffner, 1998). The *A. sagrei* described herein were part of other studies, necessitating that the organs be treated as required for those investigations (i.e. fixation), and therefore culture of fungi and bacteria was not possible.

Reports of granulomata and abscesses associated with micro-organisms in reptiles are not uncommon (Huchzermeyer & Cooper, 2000). An

early account was by Reichenbach-Klinke & Elkan (1965) who reported a fungal infection of the liver of a Two-banded chameleon (*Chamaeleo bitaeniatus*). They stated that the animal was euthanised as it was not able to maintain a hold on branches. Dissection of this animal's liver revealed that it contained foci of necrotic material, surrounded by granulation tissue, which in turn was surrounded by fibrous tissue. A PAS stain showed the granulation zone to be heavily permeated by a yeast-like fungus – most likely *Candida albicans* (Reichenbach-Klinke & Elkan, 1965). In both the cases described in the present study, the *A. sagrei* appeared to be in good health when they were collected and did not seem particularly easier to capture. That both *A. sagrei* were probably, as active as the other brown anoles in the area is supported by the types of prey items in their stomachs; prey such as Hymenoptera and Diptera are highly mobile and generally not easy to capture.

In addition to the various functions of the liver, such as the part it plays in carbohydrate, protein, and hormone metabolism, the reptilian liver plays a vital part in fat metabolism, during which stored abdominal fat is used to provide material for vitellogenesis during the reproductive cycle of the females (Schaffner, 1998; Zug *et al.,* 2001). The fact that both the *A. sagrei* described here were in a reproducing state, with stored abdominal fat, is another indication that these lizards were in a relatively good condition.

Although detailed laboratory investigations of the lesions were not feasible, it is worth noting that this appears to be the first report of hepatic granulomata in free-living *A. sagrei* from Taiwan, and indicates that hepatic granulomata occur naturally in this population.

A plea to readers: The occurrence of pathological conditions in free-living reptiles and amphibians is poorly understood, and the majority of published cases of these conditions are from captive animals. We would thus like to encourage researchers – even if it is not part of the in study – to investigate lesions, or at least to submit them for examination, and to report the findings.

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