JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

JZAR Evidence-based practice

OPEN ACCESS

Evidence-based practice

Intestinal adenocarcinoma in a Montserrat mountain chicken (*Leptodactylus fallax*)

J. E. Jaffe[1,2], E. J. Flach[1], Y. Feltrer[1], S. Rivers[3], F. J. Lopez[4] and A. A. Cunningham[1]

[1]Zoological Society of London, Regent's Park. London NW1 4RY, United Kingdom
[2]Present address: J. E. Jaffe, Tacugama Chimpanzee Sanctuary, Freetown, Sierra Leone
[3]Abbey Veterinary Services, 89 Queen Street, Newton Abbot, Devon TQ12 2BG, United Kingdom
[4]Chester Zoo, Cedar House, Caughall Road, Upton by Chester, Chester CH2 1LH, United Kingdom
Correspondence: E. Flach, Zoological Society of London, Regent's Park. London NW1 4RY, United Kingdom; e-mail: edmund.flach@zsl.org

Keywords:
captive breeding, conservation, histopathology, pathology

Article history:
Received: 20 November 2013
Accepted: 9 December 2014
Published online: 31 January 2015

Abstract
The mountain chicken (*Leptodactylus fallax*) is a critically endangered, exceptionally large frog, found only on the Caribbean islands of Montserrat and Dominica. A wild-caught adult female Montserrat mountain chicken housed at the Zoological Society of London was euthanased after a history of lethargy and loss of body weight. On gross pathology, a 1 cm diameter mass was seen in the wall of the large intestine and a 2 mm diameter nodule was present on the cranial pole of the left kidney. On histopathological examination, both masses were identified as adenocarcinomas. Comparison of the histological appearance of the two masses, and characteristics on special staining, suggest that it was an intestinal adenocarcinoma with metastatic spread to the kidney. In mountain chickens, the only neoplasms previously reported are two cases of ovarian dysgerminoma. No aetiology was detected in the case described here. However, we recommend vigilance for future cases in this species, to ensure the success of the breeding programme and the survival of the species.

Introduction

The mountain chicken (*Leptodactylus fallax*) is a critically endangered, exceptionally large frog, found only on the Caribbean islands of Montserrat and Dominica. The major threats to the species are hunting, volcanic activity and, most importantly, the infectious disease amphibian chytridiomycosis (Fa et al. 2010). As a result of this last threat, caused by infection with the fungal pathogen *Batrachochytrium dendrobatidis,* captive breeding and reintroduction programmes have been established to safeguard the species' survival. Here we report a case of intestinal adenocarcinoma in a captive mountain chicken frog.

Methods

An adult female Montserrat mountain chicken, wild-caught on Montserrat in 2009 and housed since capture at the Zoological Society of London, was examined post-mortem in December 2011. It had a history of lethargy and loss of body weight and condition, and was euthanased by immersion in a 3 g/L tricaine methane sulphonate (MS222; Pharmaq Ltd) aqueous solution followed by an intra-cardiac injection of pentobarbitone (Pentobarbital solution 20%; J.M. Loveridge Ltd).

Results

At post-mortem examination, the frog's skeletal musculature was found to be very pale and the animal was in poor body condition. There was a firm, 1 cm diameter mass in the wall of the distal large intestine (Figure 1). At this point the intestine was adhered to the wall of the urinary bladder, which was mildly thickened. The liver had a grossly normal appearance, colour and texture. The gall bladder was distended with turbid bile. Within the gall bladder, and adherent to the mucosa, was a disc-shaped calculus, 7 mm diameter x 4 mm thick. The cranial pole of the left kidney contained a small (approximately 2 mm diameter) cream-coloured, spherical nodule (Figure 1b).

Numerous white, elliptical, rice-grain-like structures (approximately 4 mm long x 1 mm diameter) were found attached to the coelomic wall – on the outer, sub-cutaneous, surface – and within the thigh muscles. These were consistent with those identified previously in wild-caught mountain chickens, which were determined to be larval acanthocephalans in the genus *Centrorhynchus* (E. Harris, pers. comm.). Bacteriological examination of coelomic fluid, liver and faeces, using routine aerobic culture on horse blood agar at 25˚ C, failed to grow any significant pathological bacteria. Specific tests for chytrid fungus and ranavirus were also negative.

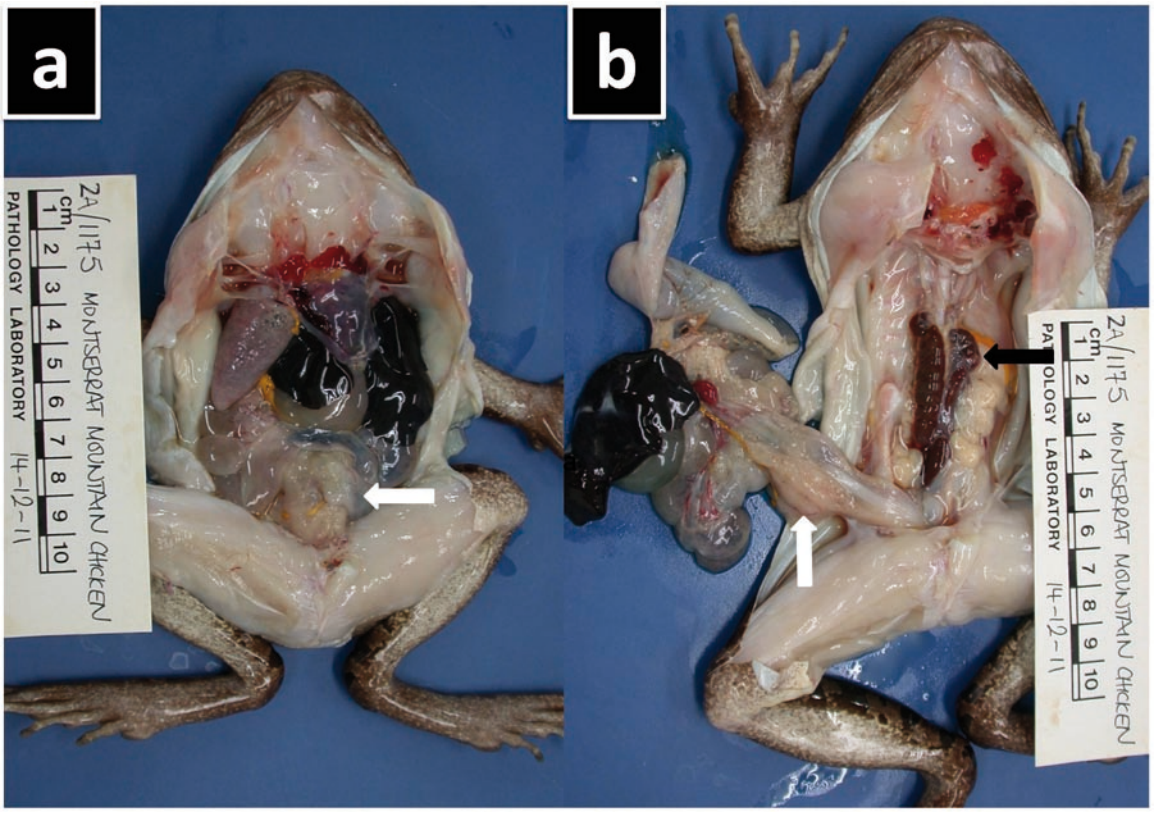

Figure 1. Post-mortem examination of the mountain chicken showing the viscera in-situ. (a) White arrow indicates intestinal mass adherent to the urinary bladder wall. (b) Some of the organs removed to reveal the kidneys. Note the pale nodule in the left kidney (black arrow) and the large-intestinal mass (white arrow).

A range of tissue samples was fixed in 10% buffered formalin, routinely processed, sectioned and stained with haematoxylin and eosin, Gram, PAS (periodic acid–Schiff), Giemsa and Ziehl–Neelsen stains for light microscopic examination.

On histopathological examination, the intestinal mass was identified as an adenocarcinoma (Figure 2). It was composed of polyhedral cells with marked anisokaryosis arranged in fragmented islands and trabeculae; in some areas these formed tubule-like structures (Figure 2b). Mitotic figures averaged 1–2 per 400X field. The pale nodule seen grossly in the left kidney also comprised tissue consistent with that of an adenocarcinoma (Figure 3). Tubule formation was much more pronounced in this mass (Figure 3b), however the cells closely resembled those comprising the intestinal mass. It was therefore suspected to be part of the same neoplastic process and, given its small size and sharply demarcated appearance, assumed to be a metastasis from the intestinal mass.

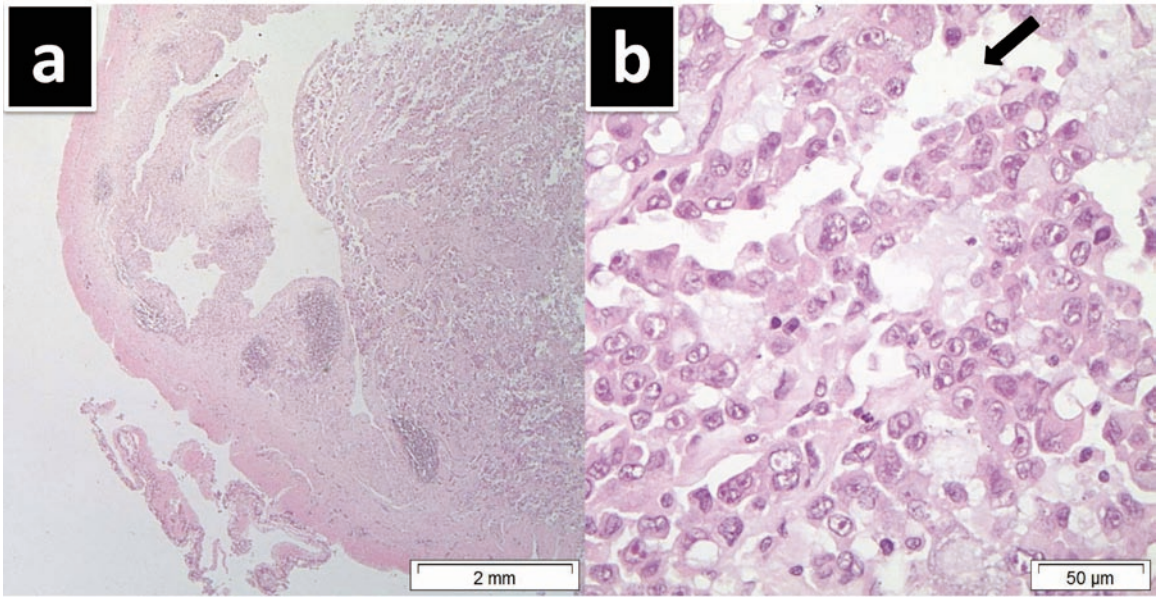

Figure 2. Microphotograph of the intestinal mass. (a) Adenocarcinoma originating from normal intestinal mucosa (on left) with neoplastic mass filling right side of image. Haematoxylin and eosin. (b) Higher magnification of the neoplastic cells, showing marked anysokaryosis and the suggestion of tubule formation (black arrow). Haematoxylin and eosin.

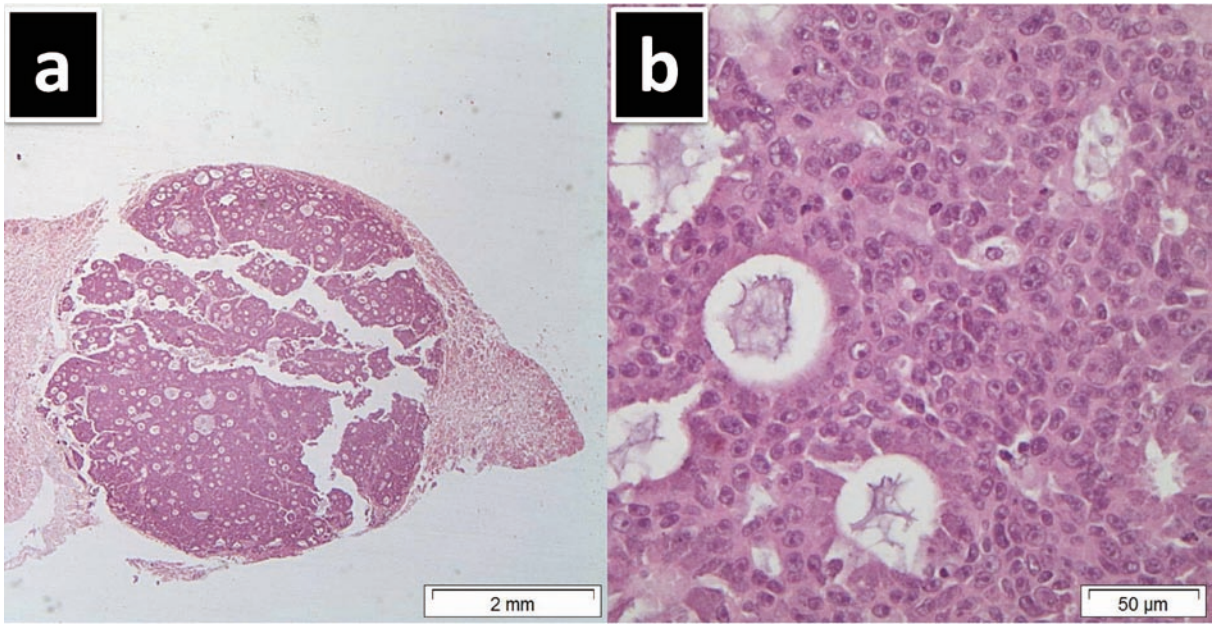

Figure 3. Microphotograph of the renal mass. (a) The smaller neoplastic mass in the kidney is well circumscribed. Haematoxylin and eosin. (b) Higher magnification of neoplastic cells, showing tubule formation is more pronounced but cells are similar to those in the intestinal mass. Haematoxylin and eosin.

Both tumour sections were stained with Alcian blue/Diastase PAS, which showed that both tumours expressed mucus. In human beings, renal adenocarcinomas rarely produce mucus whereas intestinal adenocarcinomas usually do (Woolf 1998). Therefore, it is most likely that the neoplasm was an intestinal adenocarcinoma with metastatic spread to the kidney.

In addition, histological examination detected occasional metazoan parasites, thought to be *Centrorhynchus*, within the gastric serosa and (in degenerated form) also in the kidney and liver. No abnormalities of the heart, lung, ovary, spleen, small intestine, pancreas or oviduct were detected.

Discussion

Published reports of neoplasia in amphibians are relatively uncommon, although the occurrence of renal adenocarcinoma and dermal gland tumours are well known in certain species (Green and Harschbarger 2001; Stacy and Parker 2004; Khudoley and Mizgireuv 1980). Only four cases of gastrointestinal neoplasia in amphibians have been documented previously: Elkan (1970) described a metastasising adenocarcinoma of gastric or pancreatic origin in an African clawed frog (*Xenopus laevis*); Green and Harschbarger (2001) described an intestinal adenocarcinoma in a marine toad (*Bufo marinus*) and a cloacal adenocarcinoma of possible intestinal origin in a Mexican axolotl (*Ambystoma mexicanum*); and Vaughan et al. (2006) described a metastasising adenocarcinoma in a splendid tree frog (*Pelodryas splendida*). In mountain chickens, the only neoplasms reported to date are two cases of ovarian dysgerminoma (Fitzgerald et al. 2007).

Specific aetiologies have been identified for some amphibian neoplasms. For example, ranid herpesvirus-1 is the cause of Lucké renal adenocarcinoma in the northern leopard frog (*Rana pipiens*) (McKinnell and Carlson 1997), and a genetic basis for a high rate of spontaneous renal cell carcinoma in hybrids of Japanese and Chinese toads (*Bufo japonicus and B. raddei*) is suspected (Masahito et al. 2003). Environmental stimuli, specifically contaminants (Stacy and Parker 2004), can predispose amphibians to neoplasia. The effect of temperature on the growth of some tumours has been studied. Colder temperatures inhibit the growth of Lucké renal adenocarcinoma (Marlow and Mizell 1972) and of melanomas in crested newts (Zavanella 1985). Temperatures both below and above 10–13˚ C inhibit the development of epidermal papillomas in Japanese newts (Asashima et al. 1985). In theory, a parasite burden could also cause carcinogenic mutations in host cells through chronic infection and inflammation, although this has only been shown in human beings (Kuper et al. 2000). No aetiology was detected in the case described here, but we recommend vigilance for future cases in this and other amphibian species.

Acknowledgements

We thank Belinda Clark, Matt Rendle and Matt Perkins, ZSL, and the technicians at Abbey Veterinary Services for technical assistance. Tony Sainsbury, ZSL, kindly reviewed the manuscript and Ken Smith, Royal Veterinary College took the photomicrographs used in Figures 2 & 3. We also thank Amar Paul Dhillon, Department of Cellular Pathology, UCL Medical School for assistance with the identification of the tumours. The Department of Environment, Ministry of Agriculture, Land, Housing and the Environment, Government of Montserrat kindly provided the frogs for the captive breeding programme. This case was originally submitted by J. Jaffe in part fulfilment of the requirements for the degree of Master of Science in Wild Animal Health, University of London, 2012.

References

Asashima M., Oinuma T., Matsuyama H., Nagano K. (1985) Effects of temperature on papilloma growth in the newt, *Cynops pyrrhogaster. Cancer Research* 45: 1198–1205.

Elkan E. (1970) A spontaneous anaplastic intestinal metastasizing carcinoma in a South African clawed toad (*Xenopus laevis* Daudin). *Journal of Pathology* 100: 205–207.

Fa J., Hedges B., Ibéné B., Breuil M., Powell R., Magin C. (2010) *Leptodactylus fallax*. In: *The IUCN Red List of Threatened Species. Version 2014.3.* <www.iucnredlist.org>. Downloaded on 24 November 2014

Fitzgerald S.D., Duncan A.E., Tabaka C., Garner M.M., Dieter A., Kiupel M. (2007) Ovarian dysgerminomas in two mountain chicken frogs (*Leptodactylus fallax*). *Journal of Zoo and Wildlife Medicine* 38: 150–153.

Green D.E., Harshbarger J.C. (2001) Spontaneous neoplasia in Amphibia. In: Wright K.M., Whitaker B.R. (eds). *Amphibian Medicine and Captive Husbandry*. Malaber, Florida: Krieger, 335–400.

Khudoley V.V., Mizgireuv I.V. (1980) On spontaneous skin tumors in Amphibia. *Neoplasma* 27: 289–293.

Kuper H., Adami H.O., Trichopoulos D. (2000) Infections as a major preventable cause of human cancer. *Journal of Internal Medicine* 248: 171–183.

Marlow P.B., Mizell S. (1972) Incidence of Lucké renal adenocarcinoma in *Rana pipiens* as determined by histological examination. *Journal of the National Cancer Institute* 48: 823–829.

Masahito P., Nishioka M., Kondo Y., Yamazaki I., Nomura K., Kato Y., Sugano H., Kitagawa T.L. (2003) Polycystic kidney and renal cell carcinoma in Japanese and Chinese toad hybrids. *International Journal of Cancer* 103: 1–4.

McKinnell R.G., Carlson D.L. (1997) Lucké renal adenocarcinoma, an anuran neoplasm: studies at the interface of pathology, virology, and differentiation competence. *Journal of Cellular Physiology* 173: 115–118.

Stacy B.A., Parker J.M. (2004) Amphibian oncology. *Veterinary Clinics of North America: Exotic Animal Practice* 7: 673–695.

Vaughan R.J., Vitali S.D., Payne K.L., Eden P.A. (2006) A splendid tree frog with edema syndrome and intestinal adenocarcinoma. *Veterinary Clinics of North America: Exotic Animal Practice* 9: 583–587.

Woolf N. (1998) *Pathology: Basic and Systemic.* London: Saunders.

Zavanella T. (1985) Environmental temperature and metastatic spread of melanoma in the crested newt. *Cancer Letters* 27: 171–179.