ABSENCE OF CHYTRIDIOMYCOSIS IN THE SKIN OF AMPHIBIANS COLLECTED FROM VADODARA DISTRICT OF GUJARAT

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Received: August 7, 2008; Revised: August 26, 2008; Accepted: August 28, 2008

Abstracts: The reports of life threatening fungal infection chytridiomycosis on amphibian populations have emerged from various parts of the world and the fungus has been considered as major contributor to the decline of amphibian population worldwide. However, a meticulous scan through the literature revealed that this disease is yet to be identified in Asia. Therefore, it was thought pertinent to investigate the prevalence of the cutaneous disease the chytridiomycosis in the amphibian community in Vadodara, Gujarat by analyzing the histologic profile of the amphibian skins. A careful analysis of the histoarchitecture of epidermis revealed no evidence of fungal infection in the samples of the skin examined. The thickness of stratum corneum in all the cases remained within the normal range of 2-5µm. Thus, it could be concluded that the anurans collected from Vadodara might be free from Batrachochytrium dendrobatidis zoosporangia.

Key words: Amphibian, Batrachochytrium dendrobatidis, Chytridiomycosis

INTRODUCTION

Amphibian declines were first presented as a conservation issue in 1989 at the First World Congress of Herpetology held at Kent University in Canterbury, UK. However, with an initial skepticism (that this change in population is natural as it is known to vary through time), biologists have come to a consensus that declines in amphibian populations are a real and severe threat to biodiversity [1-3]. However, there has been a widespread controversy about their significance [4-6].

Dramatic mass mortalities observed in some of the amphibian populations suggest that among the various factors, diseases may also play a significant role in the decline of some of the species [7,8]. Undoubtedly the most worrying pathogen so far discovered is a chytrid fungus Batrachochytrium dendrobatidis. This has been implicated in mass mortalities and population decline of amphibians in America, Europe, Australia and New Zealand [7]. The chytrid reported is the first member of the phylum Chytridiomycota to be recognized as a parasite to the phylum Vertebrata [9]. It attacks the skin and causes death by impairing cutaneous respiration and osmoregulation. Disease outbreak may follow either 1) a weakened immune response in the amphibians caused, perhaps, by another stressor; or 2) an increased virulence of the pathogen [10]. However, it still remains uncertain as to whether they are primary or secondary causes. Through histological analyses of amphibian skin, Berger and coworkers [11] reported the presence of the disease causing fungus in a large number of species from diverse geographical regions viz. Australia, USA, Panama and Ecuador. Through surveys of extant and archived specimens, Batrachochytrium has been found in every continent that has amphibians, except Asia [12-14].

The disease chytridiomycosis, or frog chytrid fungus, which claims the lives of frogs worldwide, is so far not reported from Asia. One of the major reasons
Fig. 1: Skin section from *Mixophyes fasciolatus*. Note empty collapsing sporangium (arrow) & one containing bacteria (B). One spongium is divided by an internal septum (S). (Berger et al., 1999)

Fig. 2: Skin section of *Bufo stomaticus*. X 1200

Fig. 3: Skin section of *Hoplobatrachus tigerinus*. X 1200

Fig. 4: Skin section of *Euphyctis cyanophlyctis*. X 1200
Fig. 5: Skin section of *Fejervarya limnocharis.* X 1200

Fig. 6: Skin section of *Polypedates maculatus.* X 1200

Fig. 7: Skin section of *Uperodon systoma.* X 1200

Fig. 8: Skin section of *Duttaphrynus melanostictus.* X 1200
for this could be that no study related to this disease has yet been undertaken in this part of the world. In India also no such work has been carried out. Therefore, the focus of the present study was to understand the prevalence of the cutaneous disease chytridiomycosis in the amphibian community at the selected study sites in Vadodara by analyzing the histologic profile of the amphibian skins and since the fungus invades only the \textit{stratum corneum} and \textit{stratum granulosum}, in particular the subsurface layer [11], skin is the only organ used for diagnosis.

**MATERIALS AND METHODS**

**Collection of Specimen:** Different species of \textit{Anurans} \textit{viz.} \textit{Duttaphrynus melanostictus, Bufo stomaticus, Hoplobatrachus tigerinus, Euphlyctis cyanophlyctis, Fejervarya limnocharis, Polypedates maculatus} and \textit{Uperodon systoma}, were collected during a monitoring programme of amphibian population in Vadodara, Gujarat.

**Histology:** Strips of skin from the pelvic region of the animals were taken for the study of chytridiomycosis. Since the chytrid fungus invades only the \textit{stratum corneum} and \textit{stratum granulosum} [11], only these two regions were looked into for the diagnosis. Tissues were preserved in 10\% formalin. These were then embedded in paraffin, sectioned at 6 µm, stained with haematoxylin and eosin and observed under Leica DMRB photo microscope.

**RESULTS**

Stratum granulosum [SG] and stratum corneum [SC] (epidermis/keratin layers) in amphibians are much thinner than that found in the human skin. The stratum corneum in the amphibians studied was rarely more than a couple of cells thick, and these keratinised cells usually appeared flattened. In the present study, diagnosis of the skin of amphibians for chytridiomycosis showed a negative result in all the samples of the skin examined. Further, confirmations of the above result were done through peer reviewing of the images by two independent experts from the field-Rick Speare and Diana Mendez (James Cook University, Queensland). Usually the SC in the infected animals is loosely adhered to the SG (Fig. 1) and full as well as empty zoosporangia are embedded in this layer. There are no such features observed in the SC of the epidermal layer in the anurans studied (Figs. 2 to 8). The normal thickness of SC is 2-5 µm while the infected SC measure about 60 µm [7]. In the current study, SC and SG of the epidermal layer of the anurans collected from various sites were measured and recorded (Table 1). None of the skin samples studied showed the thickness of SC layer more than 8 µm. The thickness of SC ranged from 4-8 µm, while that of SG ranged from 20 µm to 41.25 µm (Table 1). There is no evidence of epidermal fungal infection in adult anurans based on the analysis of histological profile. Thus, it would be logical to surmise that the anurans from the present study site might be free of \textit{Batrachochytrium dendrobatidis} zoosporangia.

**DISCUSSION**

Typical clinical signs in Australian frogs with chytridiomycosis were lethargy, inappetence, skin discolouration, presence of excessive sloughed skin and sitting unprotected during the day with hind legs held loosely to the body. Frogs became moribund in terminal stages with loss of righting reflex, and death usually occurred a few days after the onset of clinical signs [15]. Diagnosis of skin of the amphibians, using histological techniques is an effective method for identifying the chytrid fungus [16,17]. During the current study, skin samples of amphibians collected from different study sites were diagnosed for the chytrid infection. These skin specimens of the amphibians studied showed non appearance of any chytrid fungus \textit{viz. Batrachochytrium dendrobatidis}. Stratum corneum may attain a

<table>
<thead>
<tr>
<th>Amphibian species</th>
<th>Thickness of stratum corneum (µm)</th>
<th>Thickness of stratum granulos (µm)</th>
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</thead>
<tbody>
<tr>
<td>\textit{Euphlyctis cyanophlyctis}</td>
<td>5.00 ± 1.02</td>
<td>28.13 ± 6.25</td>
</tr>
<tr>
<td>\textit{Hoplobatrachus tigerinus}</td>
<td>5.63 ± 0.72</td>
<td>20.94 ± 1.20</td>
</tr>
<tr>
<td>\textit{Bufo stomaticus}</td>
<td>7.19 ± 1.20</td>
<td>30.00 ± 2.70</td>
</tr>
<tr>
<td>\textit{Duttaphrynus melanostictus}</td>
<td>7.81 ± 0.63</td>
<td>25.63 ± 2.98</td>
</tr>
<tr>
<td>\textit{Fejervarya limnocharis}</td>
<td>6.25 ± 1.02</td>
<td>2.37 ± 3.51</td>
</tr>
<tr>
<td>\textit{Polypedates maculatus}</td>
<td>4.50 ± 0.58</td>
<td>40.31 ± 2.13</td>
</tr>
<tr>
<td>\textit{Uperodon systoma}</td>
<td>5.00 ± 1.02</td>
<td>24.06 ± 2.37</td>
</tr>
</tbody>
</table>
thickness of 60 μm during infection [11]. The zoosporangia may have a diameter ranging usually between 6 and 15 μm, this size helps in distinguishing zoosporangia from other bodies of stratum corneum [11]. In the present study the size of stratum corneum layer ranged from 4.50 -7.81 μm which is comparatively less than those seen in the infected animals. Zoosporangia were not observed in the SC layer. Thus, screening of amphibians for chytridiomycosis, confirms a negative result (no chytrid fungus, *Batrachochytrium dendrobatidis* found). However, during the current study the histological sections of the skin taken from selected amphibian species revealed that this pathogen has not yet infected the amphibians at least in the selected study sites.

Therefore, it can be inferred that the much hyped chytridiomycosis is not contributing to the decline of amphibian population, if any, in Gujarat. However, the study has to be extended to other regions of Gujarat as well as in other states of India.

Acknowledgements

The work was supported by grant no. SP/SO/C-37/2000 awarded by the Department of Science and Technology (DST), New Delhi, India.

References