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VIA E-MAIL ONLY

Dear Dr. Egrie,

The AVMA appreciates the opportunity to provide comments on the new draft chapter 2.1.1., titled “Infection with *Batrachochytrium dendrobatidis*,” which has been proposed for inclusion in the OIE *Manual of Diagnostic Tests for Aquatic Animals*. As requested, when we have comments, our responses are formulated as follows:

- a. identification of the text on which we are commenting;
- b. description of the changes we believe should be made, to include suggested language, if any, to implement the changes we believe should be made; and
- c. scientific justification or rationale for such changes; or comments if no specific changes are suggested.

When language revisions are suggested, recommended deletions are ~~struck through~~, and recommended additions are underlined.

The AVMA reviewers noted several typographical and grammatical errors throughout the document. For example, missing closed parentheses, missing commas, lack of italics. However, in the interest of space, not all such errors are documented below. We suggest that the document be carefully edited to correct such errors during the next review. Specific comments follow—

- a. TEXT: **Section 1. Scope, first sentence:** For the purposes of this chapter, chytridiomycosis as a disease resulting from infection with the zoosporic fungus *Batrachochytrium dendrobatidis* (Fungi, Chytridiomycota, Rhizophydiales).
 - b. CHANGES & REVISIONS SUGGESTED: For the purposes of this chapter, chytridiomycosis ~~as~~ is a disease resulting from infection with the zoosporic fungus *Batrachochytrium dendrobatidis* (Fungi, Chytridiomycota, Rhizophydiales).
 - c. RATIONALE: Correction of a typographical error. Also, italicized the genus and species name of the disease agent. This needs to be done throughout the document.
- a. TEXT: **Section 1. Scope, final sentence**
 - b. CHANGES & REVISIONS SUGGESTED: None
 - c. COMMENT: The AVMA recommends re-examining the claim that “All sampling,

histology, histochemistry and TaqMan techniques have been validated” (Hyatt et al., 2007). In examining the actual information provided in Hyatt et al (2007) it appears that the diagnostic validation has only been done for stage 2 of the OIE standard approach, with validation for stages 3 and 4 not completed.

- a. TEXT: **Section 2.1.1. Aetiological agent, agent strains**
 - b. CHANGES & REVISIONS SUGGESTED: None
 - c. COMMENT: The AVMA recommends that this section address possible different host preferences or pathogenicity of the different strains of *Batrachochytrium dendrobatidis*. Strain differences make movement of susceptible animals from one area to another, particularly movement between two areas known to have infected individuals, very problematic. See, for example, Berger, L., Marantelli, G., Skerratt, L.F., & Speare, R. (2005). Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. *Dis. Aquat. Org.*, 68: 47–50.
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- a. TEXT: **Section 2.1.1.; second-to-last sentence of the second paragraph:** The second suggests that damage to skin function results in disturbance of water and electrolyte balance (osmoregulation) of water or electrolyte balance resulting in death (Berger et al., 1998).
 - b. CHANGES & REVISIONS SUGGESTED: The second suggests that damage to skin function results in disturbance of water and electrolyte balance (osmoregulation) ~~of water or electrolyte balance~~ resulting in death (Berger et al., 1998).
 - c. RATIONALE: Deletion of a repeated phrase.
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- a. TEXT: **Section 2.1.3.; first sentence of the first paragraph:** Bd is susceptible to a broad range of chemical and physical treatments (Phillott et al., 2010).
 - b. CHANGES & REVISIONS SUGGESTED: Bd is susceptible to a broad range of chemical and physical treatments *in vitro* (Phillott et al., 2010).
 - c. RATIONALE: We believe this sentence needs to be clarified to ensure agents toxic to amphibians are not used to treat infected populations
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- a. TEXT: **Section 2.1.3.; second-to-last sentence of the second paragraph:** Heating to above 37°C for 4 hours results in death of sporangia.
 - b. CHANGES & REVISIONS SUGGESTED: Heating to ~~above~~ greater than 37°C for 4 hours results in death of sporangia
 - c. RATIONALE: Grammatical.
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- a. TEXT: **Section 2.4.2.; sentence that begins on line 9:** However, itraconazole baths have been widely used in amphibian rescue and conservation programmes and anecdotal evidence suggests that it is effective for adults and subadults.
 - b. CHANGES & REVISIONS SUGGESTED: However, itraconazole baths have been widely used in amphibian rescue and conservation programmes and anecdotal evidence suggests that ~~it~~ this method is effective for adults and subadults.
 - c. RATIONALE: Clarification.
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- a. TEXT: **Section 2.4.8. General husbandry practices, paragraph ii:** Livestock including food animals must be enclosed in such a way as to prevent escape to the external environment.
 - b. CHANGES & REVISIONS SUGGESTED: ~~Livestock including food animals~~ Captive amphibians must be enclosed in such a way as to prevent escape to the external environment.
 - c. RATIONALE: Although enclosing livestock, including food animals, in such a way as to prevent their

escape into the external environment is an important animal husbandry and environmental safeguard practice, the relevancy of including this for all livestock as a control point in preventing the spread of *B. dendrobatidis* is unclear. Preventing escape of captive amphibians, regardless of the reason they are captive, provides a more direct and clear control point.

- a. TEXT: **Section 3.1.1, heading:** Toe clips (adults), oral discs (tadpoles/larvae, and swabs (adults and tadpoles).
 - b. CHANGES & REVISIONS SUGGESTED: Toe clips (adults), oral discs (tadpoles/larvae), and swabs (adults and tadpoles).
 - c. RATIONALE: Adding the final parenthetical mark in the above two places provides closure to the examples and completes the heading.
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- a. TEXT: **Section 3.1.2, final sentence of the first paragraph:** Alternatively, bath water can be filtered and filters (e.g. 0.45 µm filter stored dried (room temperature or 4°C) until analysis.
 - b. CHANGES & REVISIONS SUGGESTED: Alternatively, bath water can be filtered and filters (e.g. 0.45 µm filter) stored dried (room temperature or 4°C) until analysis.
 - c. RATIONALE: Adding the end parenthetical mark following “filter” in the last sentence provides closure to the example and completes the sentence.
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- a. TEXT: **Sections 7.1. Definition of suspect case and 7.2. Definition of confirmed case**
 - b. CHANGES & REVISIONS SUGGESTED: None.
 - c. COMMENT: Clarification is needed. In 7.1, a “suspect” case is one in which the presence of sporangia has been confirmed through use of immunohistochemistry. In 7.2 the definition of a “confirmed” case includes an apparently healthy amphibian deemed positive based on the results of a TaqMan assay alone. We question whether a suspect case identified by immunohistochemistry must then be confirmed through use of PCR, or is testing by PCR alone sufficient to confirm an apparently healthy animal as infected? It is also worth noting that in Table 5.1 (Methods for Targeted Surveillance and Diagnosis), under targeted surveillance, immunohistochemistry (ie, immunoperoxidase stain) is designated as “c”, which is defined as meaning “the method has application in some situations, but cost, accuracy, or other factors severely limits its application.” It, therefore, appears that information in Table 5.1 and the case definitions in Sections 7.1. and 7.2.1 create a situation in which a single positive TaqMan result obtained during targeted surveillance of a population of apparently healthy amphibians is enough to label that population as officially positive for infection with Bd. If so, we find that unacceptable.

As always, we appreciate the opportunity to provide input. Should you have questions or require additional information about our comments, please do not hesitate to contact Dr. Beth Sabin, International Coordinator and Assistant Director in our Education and Research Division (esabin@avma.org; 800-248-2862, ext 6675).

Sincerely,

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Elizabeth A. Curry-Galvin, DVM
Assistant Executive Vice President