SEVERE ORAL CANDIDIASIS IN COMMERCIAL TURKEYS

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ABSTRACT

Commercial 28-day-old male turkeys with severe oral lesions were submitted for examination with a presumptive diagnosis of mycotoxicosis. Oral lesions were characterized by erosions, ulcers and crusts extensively affecting the beak rim and the outer portions of the palate and tongue. Necropsy revealed no gross lesion other than mild aerosacculitis and entero-typhlitis. Histopathology of the oral tissues showed diffuse, severe ulcerative glossitis and stomatitis associated with myriads of 3-6 µm diameter, roundish, pale staining, thin-walled yeasts (Candida sp) often arranged in short chains of pseudohyphae. Retrospective molecular investigations confirmed the involvement of Candida albicans in those lesions. The possible role of recent manual debeaking in these unusual Candida outbreak and the differential diagnosis of trichothecene toxicity are discussed.

INTRODUCTION

Candida species have a worldwide distribution and are part of the microflora of the healthy digestive system of humans, animals, and birds (**3**). Perturbance of the mucosal microflora, young age, concurrent infections and debilitation of the host can lead to candidiasis. Birds are particularly susceptible to oral and crop candidiasis, which resembles thrush in humans. In poultry production the occurrence of candidiasis is sporadic, but outbreaks can be costly. Diagnosis of candidiasis requires a multidisciplinary approach based primarily on conventional methods (direct microscopic examination of fresh smears, isolation, histopathology, etc.) often associated with molecular genetic techniques necessary to obtain more accurate and detailed insight (**2**).

MATERIALS AND METHODS

Animals – Seven 28-day-old commercial male turkeys out of a flock of 10000 were submitted for necropsy because of increased mortality and severe oral lesions compatible with a presumptive diagnosis of mycotoxicosis (trichothecene mycotoxins). Recent manual debeaking (second week of age) was reported as anamnestic data.

Histopathology - Samples of oral tissues (roof and floor of the buccal cavity and tongue), thymus, bursa of Fabricius, spleen, liver, intestine and kidney were fixed in 10% buffered formalin and routinely included for histopathology. 4-micron sections were stained with haematoxylin and eosin (HE) and PAS.

PCR - DNA was retrospectively extracted from a selection of paraffin blocks from the archival tissue material shown to contain structures consistent with Candida on histopathology. Sterile razor blades were used to harvest 30-µg samples of tissue from the centres of the larger lesions. Samples were collected in sterile 1.5-ml tubes and dewaxed in 1 ml for 20 min, centrifuged at 1000 × *g* for 3 min, and washed twice with 100% ethanol. DNA extracted from the pellet by using a DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The identification of the yeasts was based on the amplification with universal primers (ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS2 5'-TCCTCCGCTTATTGATATGC-3') and sequence analysis of a region of the Internal Transcribed Spacer (ITS) of the rRNA gene as previously described (**4**). Negative and positive controls were included alongside the amplification reactions. Presence and size of the PCR products were assessed by agarose gel electrophoresis and ethidium bromide staining. PCR products of the expected length (218 bp) were purified from agarose gel slices using a QIAquick PCR purification kit (Qiagen) and sequenced using an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA). The sequence were submitted to the GenBank database (HQ845604). The percentage of similarity with reference sequences was evaluated by BLAST search in the NCBI website.

RESULTS

Necropsy – All the birds showed unsatisfactory growth associated with severe oral lesions characterized by erosions, ulcers and crusts extensively affecting the upper beak rim and the outer portions of the palate and tongue (Figures A-C). Signs of recent debeaking appeared evident in the seven birds. Mild entero-typhlitis with few oocysts in the cecal content was detected in two turkeys. Gizzards appeared normal. Mild fibrinous aerosacculitis was observed.

Histopathology- Diffuse, severe ulcerative glossitis and stomatitis with abundant necrotic debris associated with myriads of 3-6 µm diameter, roundish, pale staining, thin-walled yeasts (consistent with Candida yeast) often arranged in short chains of pseudohyphae (Figures E-F). Scattered aggregates of cocci were also found. Mild ballooning degeneration of the oral epithelium was focally observed in few sections. Mild to moderate lymphocytic depletion was found in all the spleen sections. As additional finding, mild, chronic typhlitis associated with coccidia at different developmental stages was observed in one section.

PCR and Sequencing – Molecular analysis identified *Candida albicans* (similarity of 95%) as the yeast massively present in oral lesions.

DISCUSSION

The presumptive diagnosis of trichothecene mycotoxicosis was discarded as the other gross lesions typically associated with the oral involvement (yellow-tan, friable livers, swollen kidneys, urate deposits in the ureters, focal ulceration and inflammation of crop mucosa, and a thickened, rough lining in the gizzard) were lacking (**1**). Histopathological evaluation of all the oral samples from each bird revealed erosions and ulcers were constantly and massively colonized by structures consistent with blastoconidia and pseudohyphae of Candida as retrospectively confirmed by molecular analysis. The differential diagnosis of fowlpox was ruled out for lack of typical microscopic lesions (epithelial hypertrophy and Bollinger bodies).

It was not possible to clearly assess which of the predisposing factors were primarily involved in this outbreak of oral candidiasis. Nevertheless, it is possible to hypothesize the recent manual debeaking played a major role. Matter-of-factly the oral lesions were significantly more severe in the upper beak which was completely affected from the debeaking scar to the beak commissure including the whole oral roof. On the contrary, in the oral floor the lesions were limited to the beak and tongue margins. The turkeys had mild aerosacculitis but it was not possible to ascertain whether they had received antibiotic treatment which could have enhanced Candida proliferation. Unfortunately, the isolation of this Candida strain was not performed. This could have been essential to characterize possible virulence factors related to the severity of this uncommon outbreak of candidiasis.

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Figures

A B C E F

Legends

A-B-C: erosions, ulcers and crusts extensively affecting the upper beak rim and the outer portions of the palate and tongue. Note in A and B the signs of recent debeaking. D: section of tongue with abundant necrotic debris associated with numerous yeast cells (HE stain, bar: 150 µm). E: section of tongue, aggregates of pseudohyphae and yeast cells within necrotic debris layer and infiltrating the tongue epithelium (PAS stain, bar: 50 µm).