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# Diphtheric aspergillosis tracheitis with gastrointestinal dissemination secondary to viral infections in a dairy calf

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ABSTRACT

Diphtheric aspergillosis tracheitis is an uncommon syndrome described in human pathology, usually associated with immunosuppression in the affected individuals. Interestingly, no comparative/equivalent cases were found in domestic animals. This report describes the pathological and mycological findings associated with diphtheric aspergillosis tracheitis in an immunocompromised calf. The main pathological findings were diphtheric tracheitis and rhinitis, and necrotizing ruminitis associated with intralesional septate, acute branching fungal hyphae consistent with *Aspergillus* spp. Mycological culture and isolation confirmed the fungal hyphae as *A. fumigatus* due to characteristic features. Immunohistochemistry (IHC) assays identified intralesional antigens of bovine viral diarrhea virus (BVDV) and malignant catarrhal fever virus (MCFV) at the trachea and small intestine; IHC detected intralesional antigens of bovine alphaherpesvirus 1 (BoHV-1) only at the trachea. These findings confirmed the simultaneous occurrence of *A. fumigatus* with concomitant infections due to BVDV, MCFV, and BoHV-1 in this calf. Since ovine gammaherpesvirus-2 (OvHV-2) is the cause of MCF in Brail, it is likely that the intralesional MCFV antigens identified were those of OvHV-2. In this case, disseminated aspergillosis was probably associated with the undeveloped immunological status of the calf that was further impaired due to the combined immunodepressive effects of BVDV and BoHV-1 infections. Although BVDV and BoHV-1 are infectious disease pathogens frequently associated with the development of bovine respiratory disease (BRD) in feedlot and dairy cattle, the identification of intralesional OvHV-2-like antigens in several parts of the lungs suggest that this MCFV also played a role in the BRD-associated lesions identified in this calf.

## 1. Introduction

Aspergillosis is a mycotic disease of humans, mammalians, birds, and insects caused by some members of the genus *Aspergillus* [1,2]. Although most cases of aspergilloses described in domestic animals are associated with pulmonary disease due to *A. fumigatus* [1], gastrointestinal [3,4], placental [5–7], abortions [6,7] and mastitis [8] due to *A. fumigatus* have been described in cattle. Pulmonary infections associated with *A. fumigatus* occur due to the inhalation of airborne conidia present within internal and external environments [9], and are frequently fatal in ruminants [1]. In cattle, aspergillosis is more frequently observed in early suckling dairy animals and after antibiotic therapy [1]. However, descriptions of diphtheric aspergillosis tracheitis, characterized by the formation of an intact fungal layer at the tracheal mucosa without

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pulmonary involvement, in domestic animals were not found when major data bases were examined. Alternatively, in human medicine, there is a comparatively similar syndrome referred to as *Aspergillus* tracheobronchitis (AT) [10,11] tracheobronchial aspergillosis [11,12], or pseudomembranous tracheobronchitis [13]. In human medicine, AT is an infrequent clinical manifestation of pulmonary invasive aspergillosis (PIA) in which the mycotic infection is restricted to the tracheobronchial tree with sparing of the pulmonary parenchyma [10]. Moreover, aspergillosis is frequently seen in patients with predisposing conditions [12,14], such as organs transplants [10] or in immunocompromised individuals [11,12].

The pestivirus, bovine viral disease virus (BVDV), produces respiratory and gastrointestinal diseases in cattle worldwide [15,16], and is a well-known immunodepressive agent that frequently facilitates the occurrence of concomitant secondary infections in susceptible animals [16,17]. Ovine gammaherpesvirus-2 (OvHV-2) is the cause of sheep associated-malignant catarrhal fever (SA-MCF), a severe, lymphotropic, frequently fatal disease of cattle [18,19]. Bovine alphaherpesvirus 1 (BoHV-1) is an agent associated with several clinical syndromes in cattle, including respiratory, reproductive disease, abortions and neonatal multisystemic disease as well [20,21]. In Brazil, BVDV [22,23], OvHV-2 [22,23] and BoHV-1 [24] are endemic in virtually all regions holding commercial beef and dairy cattle herds. Moreover, BVDV and BoHV-1 are common viral disease agents associated with the development of bovine respiratory disease, BRD [15,16].

Although several diagnostic tests/assays are used to identify tissues and/or fluids of cattle infected with BVDV, BoHV-1 and/or OvHV-2 worldwide, immunohistochemistry (IHC) has the significant advantage over molecular and serological assays in that it identifies intralesional antigens *in situ* within tissues or tissue blocks derived from fluids. Consequently, IHC is one of the diagnostic methods that can efficiently confirm an active infectious process [25], due to the association of typical histopathologic alterations with tissue antigens in an individual with clinical manifestations. Accordingly, we have developed IHC assays to identify intralesional antigens associated with the development of respiratory diseases in cattle [26], and have shown that these assays are efficient to detect BVDV and BoHV-1 antigens within the lungs [22,26], and BVDV antigens within the intestine of cattle [22]. Furthermore, IHC is an efficient method for the diagnosis of diseases associated with BVDV [27,28] and BoHV-1 [15,26]. Moreover, we have successfully demonstrated that the monoclonal antibody 15A (MAb-15A) which detects a epitope present in all known MCF viruses, MCFV [29], can be used to identify intralesional antigens of SA-MCF from tissues of cattle infected with OvHV-2 [22].

This article presents the pathological, immunohistochemical, and mycological findings associated with triple viral infections with concomitant disseminated aspergillosis in a dairy calf, representing the first description of diphtheric tracheitis in domestic animals.

## 2. Materials and methods

### 2.1. Clinical history and post-mortem

A 40-day-old Girolando dairy calf with a clinical history of diarrhea for the past 15 days was admitted at the Veterinary Teaching Hospital, Universidade Estadual de Londrina (VTH-UEL). The owner indicated that the calf was maintained with the dam since calving, had diarrheic yellow feces and that has been medicated (florfenicol, sulfa, and trimethoprim) on-site at the farm. However, two days before arriving at the VTH-UEL, the calf was not sucking milk and remained in lateral recumbency; medication was not administered at that time.

The animal was evaluated clinically on arrival at the VTH-UEL. Blood and serum samples were colleceted for hematological analyses. Fecal samples were colleceted in duplicate: one part was used for parasitological analyses and the other submitted for the screening of bovine rotavirus (BoRV) by silver stained-Polyacrylamide Gel Electrophoresis (ss-PAGE) analysis and the identification of BVDV and bovine coronavirus (BoCV) RNA by reverse transcription-polymerase chain reaction (RT-PCR) assays. The animal received supportive and antibiotic therapy but was found dead three days after arriving at the VTH-UEL. The carcass was submitted for a routine post-mortem examination to determine the cause of death.

A routine post-mortem evaluation was done soon after death; tissue sections from the brain, trachea, nasal conchae, lung, liver, kidney, rumen, abomasum, cecum, and intestine were fixed by immersion in 10% buffered formalin solution, routinely processed for histopathologic evaluation and visualized with the hematoxylin and eosin (H&E) stain. Selected formalin fixed paraffin-embeded (FFPE) tissues sections (nasal conchae, rumen, colon, trachea, small intestine) were processed to identify intralesional fungal organisms by the Gomori methenamine-silver (GMS) histochemical stain. Additionally, duplicate sections of these tissues were used in IHC assays to identify intralesional antigens of specific viral organisms. Moreover, fresh fragments, collecetd aseptically, from the trachea were processed for mycological culture and identification.

### 2.2. Immunohistochemical identification of viral pathogens

The selected FFPE tissues mentioned above were used in IHC assays designed to identify antigens of BoHV-1, BVDV, and OvHV-2 (MCFV) as previously described [22,26]. Positive controls consisted of FFPE known to contain antigens of BVDV, BoHV-1 [26], and OvHV-2 [22]. Two negative controls were used: the first consisted of replacing the primary antibodies by its respective diluent, while in the second, the primary antibodies were administered on FFPE tissues known to demonstrate negative immunoreactive to the primary antibodies mentioned above. Negative and positive controls were included in all IHC assays.

### 2.3. Histological, histochemical, and mycological analyses of fungal organisms

Initially, the fungal organisms within the tissues were identified by routing histopathology (H&E) followed by the Gomori methenamine-silver (GMS) histochemical stain. The mycological identification and culture was done as previously described [30]; briefly the aseptically collected sample from the distal end of the trachea was plated in Sabouraud Dextrose Agar (SDA), and then incubated at 25 and 37 °C. Thereafter, the micromorphological characteristics of the fungal colonies were observed with the Lactophenol Cotton Blue stain.

## 3. Results

### 3.1. Clinical and laboratory findings

The calf arriving at the VTH-UEL was dyspenic, febrile (39.8 °C), dehydrated (10%), mildly infested by ticks, diarrheic with ulcerations at the tongue and oral cavity. The hematological results were within normal parameters and cryptosporidiosis was not identified by routine parasitological evaluations, but fungal hyphae were observed within the fecal sample, while blood gas analysis revealed acidosis. Fecal ss-PAGE analysis did not identify BoRV; RT-PCR assays did not amplify BVDV and BoCV RNA from the fecal samples. Accordingly, sodium bicarbonate and lactated Ringer's solutions were administered to control the acidosis and dehydration, respectively, and antibiotic therapy with ceftiofur (2.2 mg/kg/IM/SID) was initiated. At this time, suckling reflex was absent, and the heifer received milk via an esophageal tube. On the second day, suckling reflex remained absent, the animal was in esternal recumbency during most of the day, and thus continued to receive milk via esophageal tube. The calf was found dead the following day, i.e., three days after arrival at the VTH-UEL.

### 3.2. Pathological findings

The main gross findings were observed at the respiratory and gastrointestinal systems, with restricted involvement of the kidneys and liver. Grossly, there was a locally extensive accumulation of a soft, fluffy, white-colored material that was adhered to the mucosal surface of the distal trachea, which was diagnosed as mycotic tracheitis (Fig. 1A), while the nasal conchae were severely hyperemic (Fig. 1B). Gross gastrointestinal alterations included multifocal, ulcerative glossitis and stomatitis, with several light brown to clear, rounded to elongated, slightly elevated, foci at the ruminal mucosa (Fig. 1C). These foci varied from 0.2 cm to 0.5 cm diameter for rounded lesions; the elongated foci contained a centrally located, 1 × 2 cm, clear and slightly depressed area, that was surrounded by an irregularly shaped hyperemic zone. There was a focally extensive hyperemic region at the serosa of the colon; examination of the colonic mucosa revealed an extensive region of hyperemia that contained a centrally located, irregularly or T-shaped, slightly elevated, white-cream colored plaque that was adhered to the mucosal surface (Fig. 1D). The lesions observed at the mucosal surfaces of the rumen and colon were interpreted as mycotic related, with consequent gross diagnoses of fungal rumenitis and colitis, respectively.

Histopathologic alterations were severe at the respiratory and gastrointestinal system, with restricted involvement of the kidneys and liver. Respiratory alterations included severe, acute, locally extensive diphtheric tracheitis with multifocal to coalescing necrohemorrhagic rhinitis associated with intralesional accumulations of fungal hyphae. Diphtheric tracheitis was diagnosed due to the formation of an intact membrane at the destroyed tracheal mucosa that contained numerous, intralesional, acute branching, 3–6 μm wide, non-pigmented, closely septate, fungal hyphae consistent with members of the *Aspergillus* genus; these fungal organisms were more easily visualized with the GMS histochemical stain (Fig. 2A–D). Adjacent to this membrane of fungal hyphae, was a layer consisting of fungal hyphae embeded in an edematous fibrin-rich exudate, which was in contact with a layer of neutrophils. Additional alterations to the respiratory system included degeneration of tracheal, bronchial, and bronchiolar epithelial cells, degeneration of vascular endothelial cells, and proliferation of type II pneumocytes with moderate influx of lymphocytes. However, fungal hyphae were not identified within the pulmonary parenchyma. A similar diphtheric membrane, containing fungal hyphae, as observed at the distal trachea was identified at the nasal mucosa (Fig. 2E and F); in addition, there were accumulation of intralesional bacteria within the destroyed nasal mucosa.

Histopathologic findings within the gastrointestinal system were observed at the tongue, small intestine, colon, and rumen, with intralesional fungal hyphae being comparatively more predominant in the rumen. These lesions included transmural necrotizing fungal rumenitis associated with intralesional septate fungal hyphae (Fig. 3A and B), with intravascular accumulations (Fig. 3C) of fungal hyphae; multifocal ulcerative glossitis with severe ballooning degeneration of the squamous epithelium of the tongue; chronic lymphoplasmacytic enteritis of the small intestine; and transmural necrotizing colitis associated with intralesional bacterial colonies but reduced number of fungal organisms. The fungal hyphae identified within the gastrointestinal tract had similar histologic characteristics as those described in the trachea; fungal hyphae at the rumen were readily identified with the GMS histochemical stain (Fig. 3D and E), demonstrating the transmural dissemination of fungal hyphae. Additional significant histologic lesions observed at the rumen included severe ballooning degeneration of ruminal epithelial mucosa and subepithelial cleavage vesicles. Less severe histopathologic findings included lymphocytic portal hepatitis with portal biliary stasis and ballooning degeneration of bile duct epithelia of the liver, and lymphocytic interstitial nephritis with ballooning degeneration of the vascular and tubular endothelia of the kidneys.

### 3.3. Immunohistochemical identification of intralesional viral antigens

Positive intracytoplasmic immunolabelling of BVDV antigens was disseminated in multiple organs included degenerated respiratory epithelia and adjacent glandular epithelium (Fig. 4A), chondrocytes of the hyaline cartilage (Fig. 4B) and within cryptal epithelial cells of the small intestine (Fig. 4C). Immunoreactivity to OvHV-2 was more widespread within the respiratory system when compared to those of BVDV, with positive intracytoplasmic immunoreactivity occurring within leucocytes contained in the exudate at the destroyed tracheal mucosa (Fig. 4D), epithelial cells and epithelium of mixed glands of the trachea (Fig. 4E) and bronchi (Fig. 4F and G) within the pulmonary parenchyma. Additionally, MCFV antigens were identified within cryptal epithelial

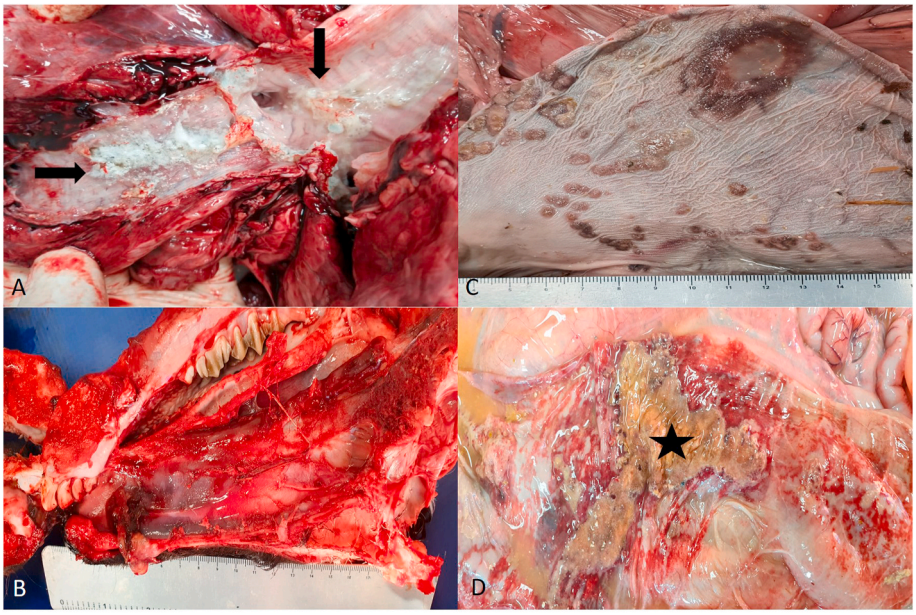


**Fig. 1.** Gross findings observed in disseminated aspergillosis in a calf. Observe the green, fluffy, material adhered to the mucosa of the distal extremity of the trachea (A), hyperemia of the nasal cavity (B), and multifocal, different-sized, slight elevated, lesions at the ruminal mucosa (C). The mucosa of the colon is severely hyperemic and contains a centrally located T-shaped (star), slightly raised layer (D).

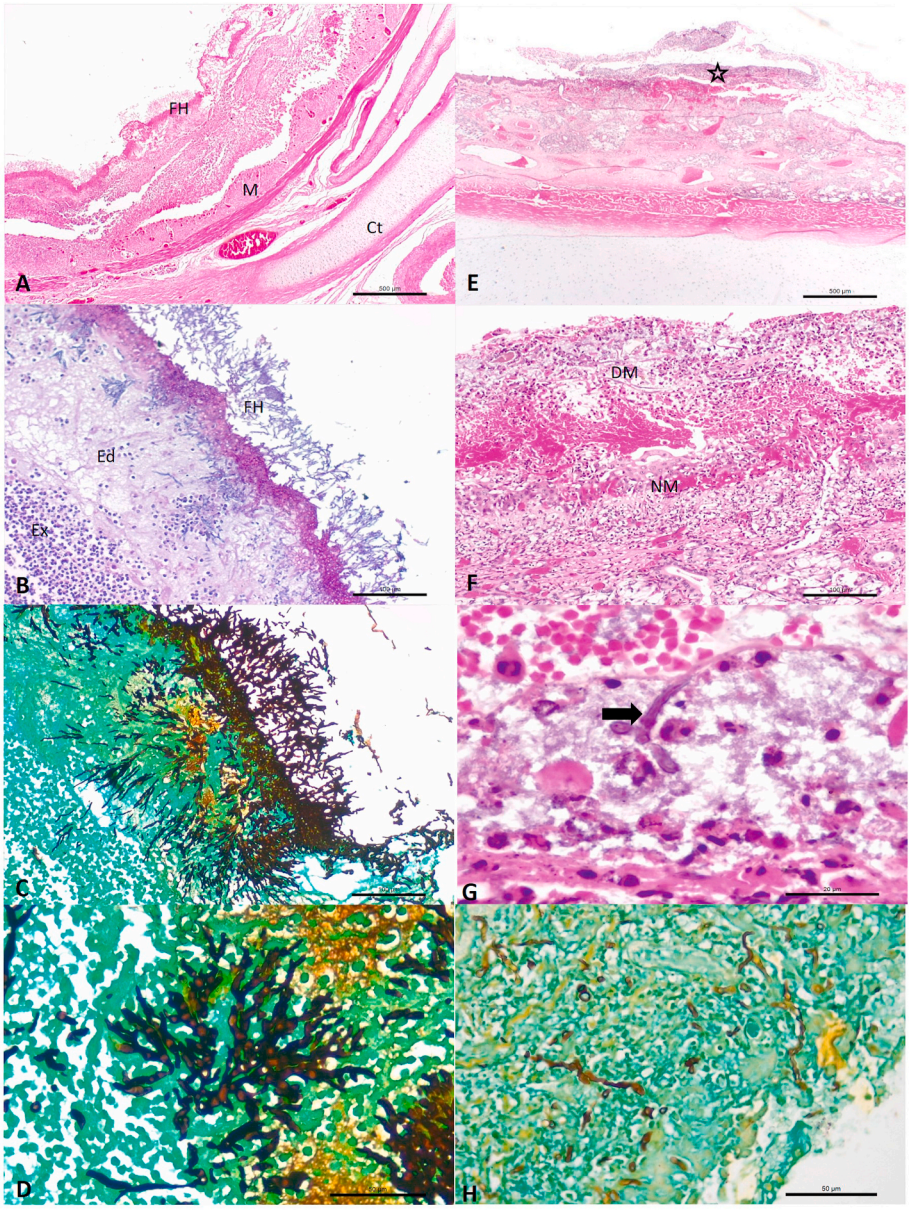


**Fig. 2.** Histopathological findings observed at the trachea and nasal cavity of a calf infected with *Aspergillus fumigatus*. Observe the diphtheric membrane containing numerous fungal hyphae (FH) attached to the mucosa (M) of the hyaline cartilage (Ct) of the trachea (A). Closer view of the diphtheric membrane (B) demonstrating the layer of fungal hyphae (FH), attached to an edematous layer (Ed) containing fungal hyphae (arrows) that is next to the predominantly neutrophilic exudate (Ex). Observe numerous septate hyphae at the diphtheric membrane of the trachea (C–D). There is a diphtheric membrane (star) attached to the mucosa of the nasal cavity (E); closer view showing the diphtheric membrane (DM) attached to the mucosa (NM) of the nasal cavity (F). There are intralesional fungal hyphae (arrow) within the nasal cavity (G–H). A-B, E–G: Hematoxylin and eosin stain; C-D, H: Gomori methenamine-silver histochemical stain. Bar: A and E, 500 μm; B and F, 200 μm; C, 100 μm; D, G-H, 50 μm.

cells of the small intestine (Fig. 4H) and within capillaries and degenerated tubular epithelial cells of the kidneys (Fig. 4. I-J). Positive immunoreactivity to antigens of BoHV-1 was restricted to the epithelial cells of mixed glands of the damaged trachea (Fig. 4K).

### 3.4. Mycological identification of Aspergillus fumigatus

There was prompt fungal growth at 37 °C. Grossly, the fungal colony on the SDA medium was blue green in color with an externally located white rim and of velvety texture (Fig. 5A and B); the reverse colony was cream colored (Fig. 5C). Microscopically, the stipes were long, smooth walled; vesicles were dome-shaped with uniserate conidial heads and dispersed conidia, measuring 2.1–2.5 μm in diameter (Fig. 5D); phialides were located principally at the upper surface of the vesicle and phialoconidia were arranged in rows (Fig. 5E).

## 4. Discussion

The intralesional fungal hyphae observed at the trachea, nasal cavity, rumen, and colon by routine histopathology and better visualized with the histochemical stain are consistent with previous descriptions of *Aspergillosis*-induced diseases in veterinary [31–35] and human [10–12, 36] medicine. Moreover, speciation to *A. fumigatus* was confirmed due to morphological characteristics observed from the specimens subjected to mycological evaluation; similar morphologic findings were described [37–39]. These histologic features are characteristic for *A. fumigatus* [2, 40]. Consequently, adequate mycological evaluation by experienced professionals can result in the diagnosis of *A. fumigatus* from tissues using classical mycology [41], without the need for molecular methods. Similar diagnostic strategies for aspergillosis were reported in horses [31,35]. Additionally, the intralesional identification of BVDV and MCFV antigens in multiple tissues and restricted identification of BoHV-1 antigens at the trachea demonstrated that this calf was infected by these viral pathogens, confirming four concomitant infections in this animal. Moreover, it must be highlighted that since BoCV and BoRV were not identified in fecal samples, these pathogens probably did not participate in the development of the intestinal lesions herein described. It must be highlighted that the MAb-15A detects epitopes of all known MCFV [29], while SA-MCF caused by OvHV-2 is the associated disease in Brazil [23]. Therefore, it is likely that the antigens identified were

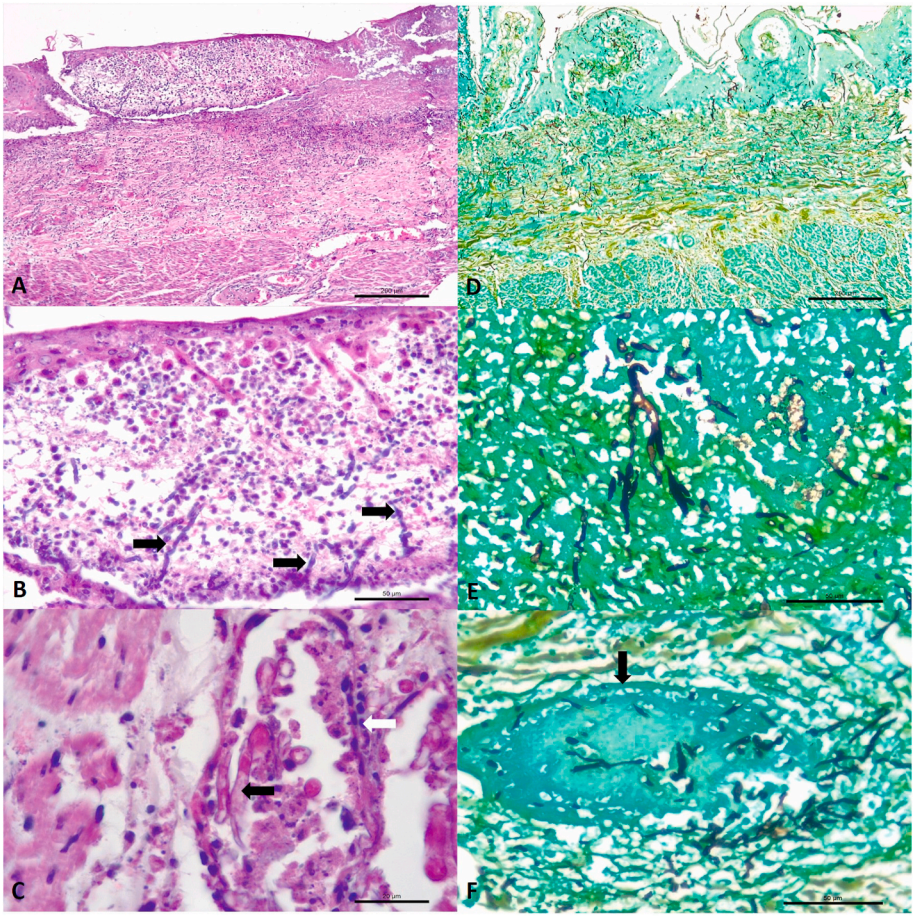


**Fig. 3.** Histopathologic findings associated with *Aspergillus fumigatus* in the rumen of a dairy calf. There is transmural mycotic rumenitis associated with intralesional fungal hyphae (A); closer view (B) showing intralesional septate hyphae (B). Observe invasive aspergillosis due to intravascular accumulations of fungal hyphae (black arrow) within a vessel (white arrow) of the rumen (C). The transmural dissemination of fungal hyphae across the ruminal wall is easily appreciated (D); a closer view (E) of the septate hyphae is provided. There is clear demonstration of invasive aspergillosis within the rumen with several fungal hyphae observed within the lumen and transfixed (F) across the wall of an artery (arrow). A-C; Hematoxylin and eosin stain; D–F: Gomori methenamine-silver histochemical stain. Bar: A and D, 500 μm; B, E, and F, 50 μm; C, 20 μm.

OvHV-2, but since molecular testing was not performed, we shall refer to this as MCFV-associated disease.

The gross and histopathologic findings observed at the tracheal confirmed a diagnosis of diphtheritic fungal tracheitis due to *A. fumigatus*; similar findings were not identified in ruminants nor in domestic animals. However, the tracheal findings herein identified are similar to syndromes described in human pathology known as *Aspergillus* tracheobronchitis [10,11], tracheobronchial aspergillosis [11,12], or pseudomembranous tracheobronchitis [13]. In human medicine, most cases of aspergillosis are associated with preexisting factors, including neutropenia due to chemotherapy or underlying hematologic disease, immunosuppression due to constant use of corticosteroids, solid-organ transplantation, and the acquired immunodeficiency syndrome [14]. A retrospective study that evaluated human patients with *Aspergillus* tracheobronchitis revealed that most (86.5%; 135/156) of these suffered from immunosuppression due either to neutropenia, chemotherapy, corticosteroid therapy, or infection by the human immunodeficiency virus [10]. In the case herein described, diphtheritic fungal tracheitis with gastrointestinal dissemination probably occurred due to primary infection by BVDV and/or BoHV-1, or due to the immature immunological status of the calf. Although BVDV is a well know immunosuppressive agent of ruminants [16,17,42], BoHV-1 also has immunosuppressive effects on the affected host [43,44]. Recent studies have shown that BVDV-induced immunodepression is probably associated with the interaction of the viral protein, $N^{pro}$, with subsequent inhibition of the cellular protein (S100A9) that is expressed in neutrophils, monocytes, dendrocytes and endothelial cells [17]. While immunosuppression due to BoHV-1 has been associated with the infection of lymphocytes and apoptosis of CD4 cells [44]. Moreover, the utilization of corticosteroids and/or antibiotics were predisposing factors relative to the occurrence of disseminated aspergillosis in a cow [45] and horses [31,46]. Consequently, the antibiotic therapy to which this calf had been subjected could have also contributed for the occurrence of aspergillosis; antibiotic therapy seems to predispose ruminants to infections by *A. fumigatus* [1,3].

Although the exact form of contamination in this case is uncertain, it is likely that the mycotic infection, herein described, probably occurred due to inhalation of *A. fumigatus* conidia, the most common form of environmental contamination [3,9,32]. Initial infection would have occurred at the nasal cavity resulting in mycotic rhinitis with consequent diphtheritic tracheitis. The most likely form of contamination in this case is deglutition, during which fungal particles adhered to the trachea suffered mucociliary clearance [40], and are dislocated towards the pharynx. On arrival at the pharynx, the fungal material probably suffered deglutition into the esophagus, finally arriving at the rumen resulting in necrotizing mycotic rumenitis. Hematological dissemination would then producing fungal colitis; hematological spread was also related to disseminated aspergillosis in a cow [45] and horses [31].

The IHC findings revealed that this calf was simultaneously infected with BVDV, MCFV, and BoHV-1 due to positive immunoreactivity to antigens of these viral disease pathogens in multiple tissue, with antigens of BVDV and MCFV being comparatively more widespread relative to those of BoHV-1. Moreover, the pattern of immunolabelling associated with each infectious disease pathogen herein identified was similar to that described in other studies for BVDV [28,39], OvHV2 [22,23], and BoHV-1 [26]. Consequently, the calf contained four identifiably pathogens (i.e., three viral agents *and A. fumigatus*), while the type of bacteria was not characterized. It must be highlighted that infections due to BVDV and BoHV-1 are frequently associated with BRD in dairy and feedlot cattle [15,16,25], and concomitant infections due to these agents

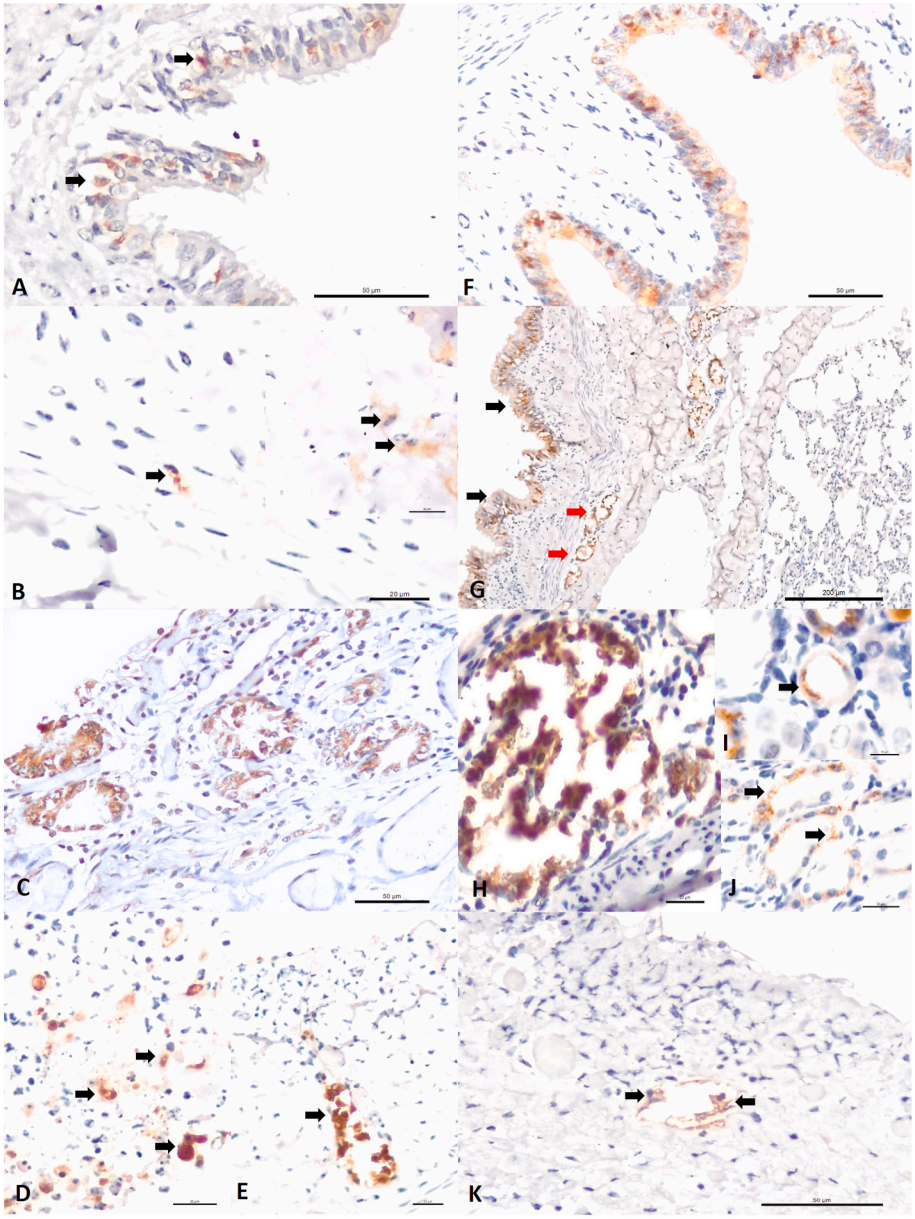


**Fig. 4.** Immunohistochemical identification of BVDV, MCFV, and BoHV-1 antigens in a calf with concomitant disseminated *Aspergillus fumigatus* infection. There is positive intracytoplasmic immunoreactivity to BVDV antigens within degenerated epithelium (arrows) of the trachea (A), chondrocytes (arrow) of the hyaline cartilage (B); closer view of immunoreactivity within chondrocytes (arrows; insert B), and cryptal epithelial cells of the small intestine (C). Observe positive intracytoplasmic immunolabeling for MCFV antigens within leucocytes (arrows) of the exudate (D) and glandular epithelial cells (arrow, E) of the trachea. There is positive reactivity to MCFV antigens within bronchial epithelium (black arrows) and adjacent glands (red arrows) of the lungs (F–G), cryptal epithelial cells of the small intestine (H), and within capillary endothelium (arrow, I) and epithelial cells of renal tubules (J) of the kidney. There is positive intracytoplasmic immunoreactivity to BoHV-1 antigens within epithelial cells of the mixed glands (arrows) of the necrotic trachea (K). A-K, Immunoperoxidase counterstained with hematoxylin. Bar; A, C, F, H, and K, 50 μm; B, D, E, I, and J 20 μm; G, 200 μm.

are common in BRD [25,26]. Although associations with MCFV/OvHV-2 in the development of BRD, as herein described, was not previously identified, we have diagnosed several cases in which this pathogen, acting isolated or in association with other pathogens, was associated with the development of BRD (manuscript in preparation) in dairy and beef cattle. Additionally, we have proposed that OvHV-2 should be considered as a possible pathogen for BRD [23].

The concomitant immunolabelling of MCFV and BVDV antigens within the small intestine is of significant interest, since there seems to be synergism between these two viral disease pathogens relative to the development of disease [22,23]. The histologic lesions observed in the kidneys of this calf are typical manifestations of infections due to OvHV-2 [18,19,22], with the kidney being the organ that is more frequently infected in outbreaks of OvHV-2 in cattle from Brazil [23]. Another intriguing result during this investigation was the negative amplification of BVDV nucleic acids from the fecal samples by RT-PCR while there was positive immunolabelling of BVDV antigens by IHC within the small intestine. This inconsistency may be associated with the specificity of each identification method relative to the type of sample used. Additionally, fecal samples are considered as poor [47] or reduced [48] sources for the detection of BVDV nucleic acids, since there is restricted shedding of BVDV viral particles via the feces [48].

In conclusion, diphtheric aspergillosis tracheitis with gastrointestinal dissemination was diagnosed in a calf that was concomitantly infected by three viral agents. The fungal disease probably initiated within the respiratory system and then gain entry to the digestive tract. Additionally, the mycotic infection occurred due to the immature immunological status of the calf coupled with simultaneous infections by BVDV and BoHV-1.

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## Ethics approval

All applicable international, national, and/or institutional guidelines

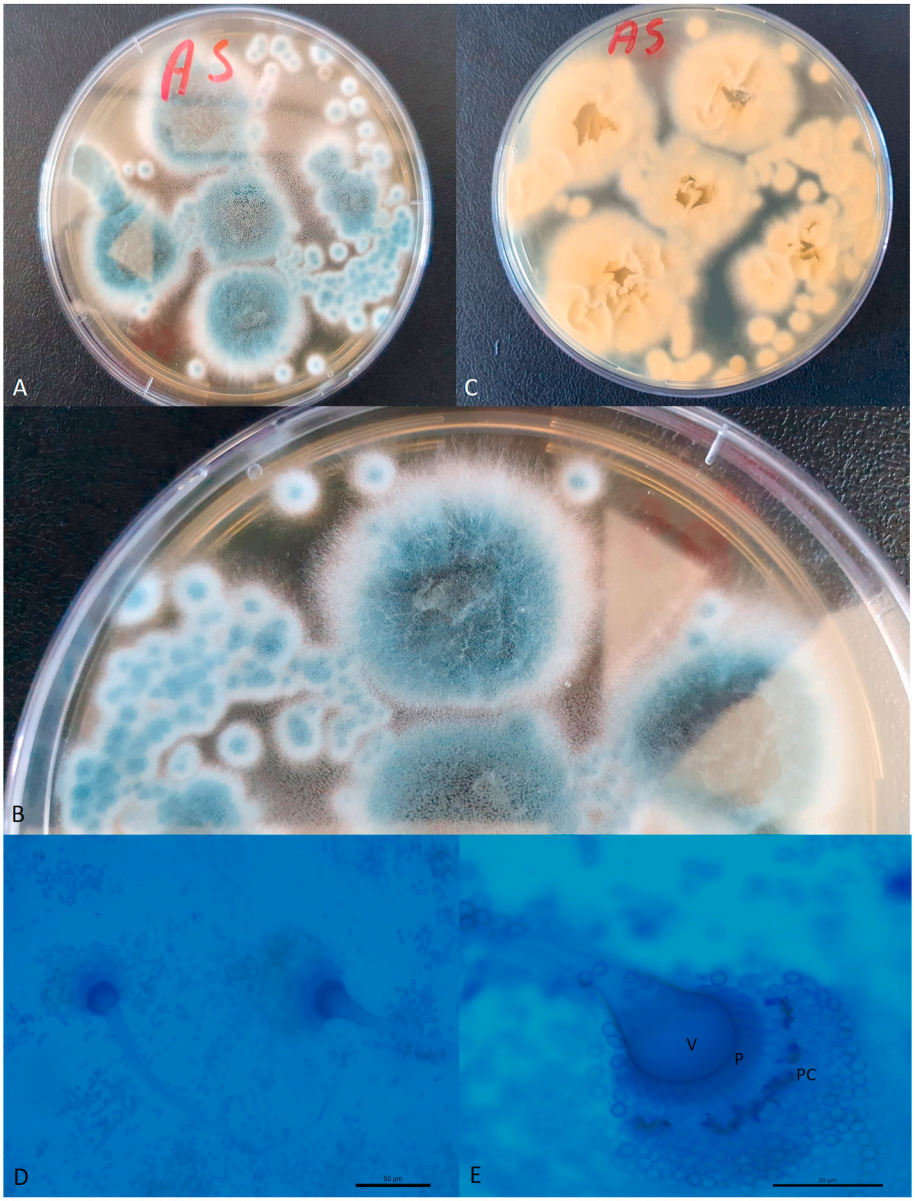


**Fig. 5.** Mycological identification of *Aspergillus fumigatus* in Sabouraud Dextrose Agar. The colony is blue green with an externally located white rim (A), closer view demonstrating the velvet texture of the fungal colony (B); the with reverse colony is cream (C). Microscopic identification of *A. fumigatus;* observe the dome-shaped vesicles of the fungus (D); closer view demonstrating dome-shaped vesicle (V), uniserate conidial head with phialides (P) located at the upper surface of the vesicle and phialoconidia (PC) arranged in rows (E). D-E, Lactophenol Cotton Blue stain; Bar, D, 50 μm, E 20 μm.

for the care and use of animals were followed. Additionally, permission was obtained from the owner of this animal relative to the utilization in scientific studies.

## Authors' contribution

Headley, S.A. contributed substantially to the conception and design of the study; drafted the manuscript, and contributed to the analysis, and interpretation of all pathological, immunohistochemical, and molecular data. Headley, S.A., Müller, M.C., and Oliveira, T.E.S. participated in the realization of the post-mortem autopsies and histopathological evaluations. Müller, M.C., and Oliveira, T.E.S. participated in realization of all histochemical and immunohistochemical stains and analyses. Duarte, C. A.B.G, Pereira, P.F.V., and Lisbôa, J.A.N. contributed towards the realization of all clinical analyses and interpretations. Vieira, M.V., and Pretto-Giordano, L.G. participated in the realization of all mycological analyses. Cunha, C.W. contributed to the interpretation of the immunohistochemical analyses. Flores, E.F., and Cunha, C.W., contributed to the realization of all molecular data. All authors have read, critically analysed, approved the final draft of this manuscript, and have agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Declaration of competing interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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