NOTE

A clinical case of ophidiomycosis in Esculapian snake (*Zamenis longissimus*) in France

Cas Clinique d'ophidiomycose chez une couleuvre d'Esculape (*Zamenis longissimus*) en France

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Résumé - *Ophidiomyces ophidiicola*, agent mycosique responsable d'ophidiomycose chez les reptiles, est une menace croissante pour les populations de serpents en Europe, en particulier les colubridés. Décrit en Europe pour la première fois en 2017, l'agent pathogène est détecté annuellement en France, dans le Doubs, depuis 2021. Nous décrivons ici le premier cas clinique avec évidence de l'implication du pathogène dans la mort d'un juvénile non captif de Couleuvre d'Esculape (*Zamenis longissimus*), investigué dans le cadre de la surveillance sanitaire événementielle du réseau SAGIR. Bien que la couleuvre d'Esculape ne soit pas, en France, une espèce fortement menacée, la circulation active d'*O. ophidiicola* associée à de la mortalité dans les populations non-captives est une menace pour l'ensemble des espèces de serpents en France, qui doit être prise en compte dans les actions de conservation des espèces.

Mots-clés - Epidémiologie ; *Ophidiomyces ophidiicola* ; Ophidiomycose ; SAGIR ; *Zamenis longissimus*

Ophidiomycosis is a fungal disease caused by *Ophidiomyces ophidiicola* (Lorch *et al.* 2015) affecting wild and captive snakes worldwide. It is a growing concern in free-ranging snakes, first described in the USA in 2008 and later in Europe in 2017 (Franklinos *et al.* 2017). In France, the first description occurred in 2021 from a *Natrix maura*. Afterwards, cases were detected annually in three species: *Zamenis longissimus* (N=19), *Natrix maura* (N=57), and *Hierophis viridiflavus* (N=14), all detected through systematic sampling protocols in a study area of the Doubs department with an *ex situ* conservation program and CMR protocols (Bourgogne-Franche-Comté, France) (Blanvillain *et al.* 2024, Joudrier 2024, com. pers. LPO Bourgogne-Franche-Comté).

Two clades of *O. ophidiicola* are known to circulate in Europe (Blanvillain *et al.* 2024). Although host susceptibility varies with the pathogen clade, both are known to affect (semi-aquatic species with higher prevalence (Joudrier *et al.* 2024). Nonetheless, in May 2024, a clinical case was identified in a juvenile *Zamenis longissimus* in the Doubs (France), the first one described through scanning surveillance (*i.e.*, opportunistic detection of wildlife mortality events that are collected for investigations on the cause of death). Although *O. ophidiicola* infection has already been described for *Z. longissimus* in Europe and in France (Blanvillain *et al.* 2024), only one previous description has been published of a clinical case with histological evidence of a contribution of *O. ophidiicola* to the death of a free-ranging individual of the species, in Spain (Martinez-Silvestre *et al.* 2024).

The individual was first sighted during a field session, apathic and with severe skin erosions (Fig. 1). Thirty minutes later, upon second observation, the individual was found dead in the same spot without any sign of predation or traumatic lesion. The carcass was collected and transferred to be frozen by the LPO Bourgogne-Franche-Comté Team for further analysis by the French network for wildlife health surveillance (SAGIR).

A local veterinary laboratory performed samplings. After slow defrosting at +4°C, external skin swabs were collected, using dry swabs without conservation media (Copan®). Three skin samplings (less than 0.25cm^2) centered on the lesions were frozen (-80°C). The whole carcass was submerged in nine volumes of 10% formalin after ventral incision of the coelomic cavity.

Histological analysis was performed after exhaustive sampling of major organs from the fixed carcass (skin, muscle, heart, lungs, liver, trachea, thyroid, kidneys, tongue, pancreas, stomach, intestine, bone, bone marrow, brain, and spinal cord). Samples were routinely processed as formalin-fixed paraffin-embedded (FFPE) blocks, sectioned at 5 µm thickness, and stained with hematoxylin and eosin for examination by light microscopy. An additional Periodic Acid Schiff (PAS) staining was performed on the cutaneous lesions.

Subsequently to histopathology results, swabs and one skin sample were used for culture on Sabouraud's Dextrose Agar (SDA) plates at 30° C, and fungal identification was then confirmed using the MALDI-TOF MS technique and the MSI-2 database (Normand *et al.* 2021). DNA was extracted from the second frozen skin sample using QIAamp® DNA Mini Kit (QIAGEN). A TaqMan qPCR *targeting a 68-bp sequence of the ITS1 region* was then performed using OphioITS primers (Allender *et al.* 2015) and *O. ophidiicola* probe coupled to a FAM fluorophore (probe sequence: 5'-CGATCGGCGCCCGTCGTCAAC-3', modified from Allender *et al.* 2015).

Histological examination of the whole body revealed a severe deep necrotizing dermatitis and myositis extending to the periphery of the underlying vertebrae. A thick fibrinous and proteinaceous crust filled the defect formed (Fig. 2). Similar lesions were observed in the oral cavity and on the rostral dermis. No lesions suggesting an extended inflammatory response were noted. No significant lesions were reported in other organs examined (heart, lungs, liver, trachea, thyroid, kidneys, tongue, pancreas, stomach, intestine, bone, bone marrow, and spinal cord).

The PAS staining revealed numerous hyphae in the crust and underlying tissues. The hyphae

Figure 1 – Partial views of the juvenile of *Zamenis longissimus* found dead in Doubs (France), May 25th, 2024. The individual exhibited apathy and severe skin erosions in the body and head (orange arrows).

Figure 1 – Vues partielles du cadavre de juvénile de *Zamenis longissimus* retrouvé dans le Doubs (France) le 25 mai 2024. L'animal présente un comportement apathique et des érosions cutanées sévères sur le corps et la tête (flèches orange).

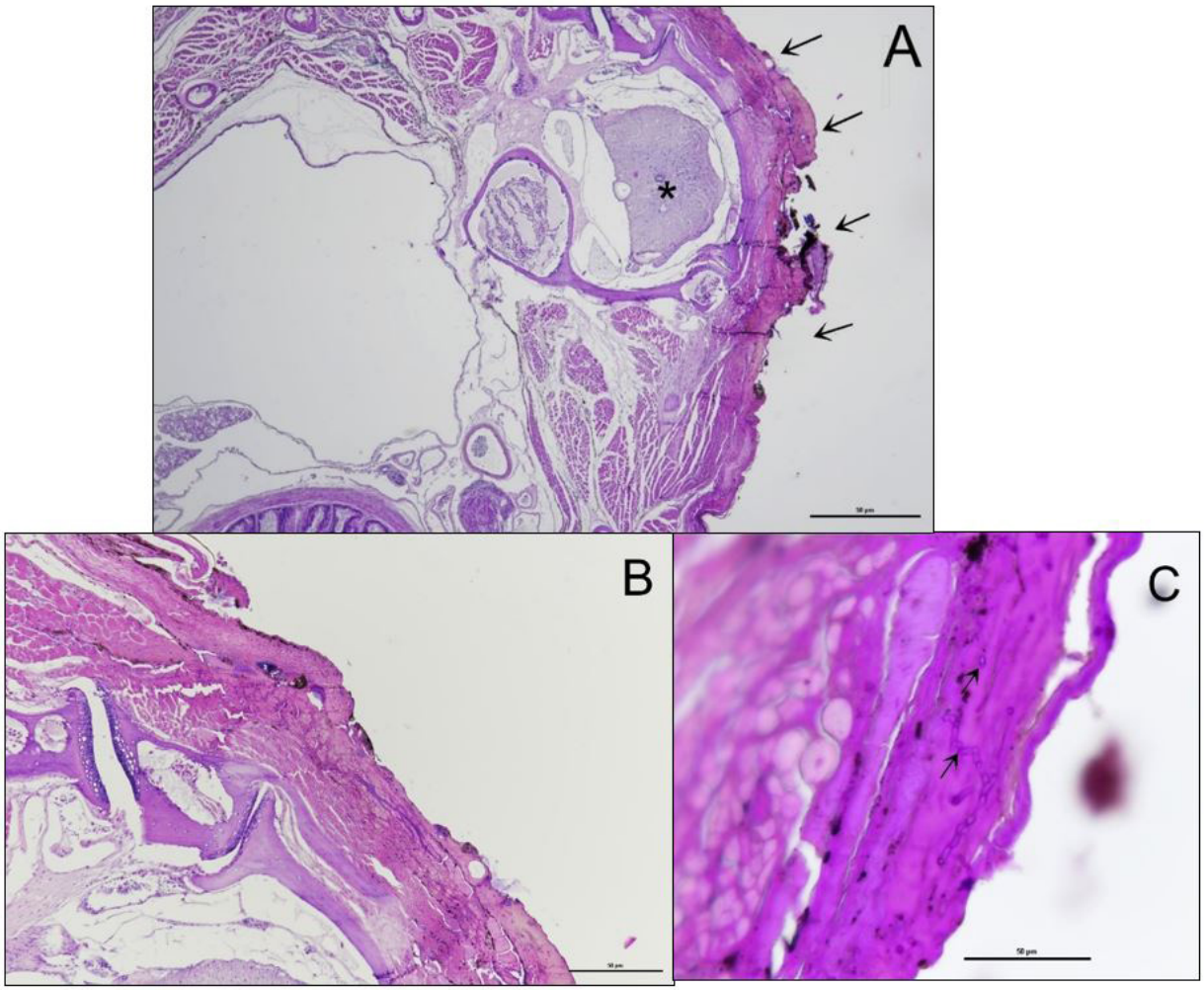

Figure 2 – Photomicrographs from histopathology on a juvenile of *Zamenis longissimus*, France. The scale represents 50µm on each photo. (A) cross section of the whole body showing a focally extensive area of deep necrotizing dermatitis and myositis (arrow heads →) extending to the periphery of the underlying vertebral body and spinal cord (*). HE stains, 2X magnification. (B) Close-up of the previous lesion showing the thick fibrinous and proteinaceous crust overlying the defect. HE stains, 4X magnification. (C) Periodic Acid Schiff stain revealing approximately 6um diameter segmented fungal hyphae with thick, almost parallel-walled with occasional apical bulges and irregular branching (arrow heads →). 10X magnification.

Figure 2 – Microphotographies à partir de lames histologiques d'un juvénile de *Zamenis longissimus*, France. L'échelle de chaque photo correspond à 50µm. (A) Section transversal du corps mettant en évidence une zone focalement extensive de nécrose profonde du derme, ainsi qu'une (flèches →) s'étendant jusqu'à la périphérie du corps vertébral sous-jacent et de la moelle épinière (*). Coloration HE, zoom X2. (B) Vue rapprochée de la lésion précédente montant la croute fibrino-protéique épaisse recouvrant la lésion. Coloration HE, zoom X4. (C) Coloration PAS (Periodic Acid Schiff) mettant en évidence des hyphes fongiques segmentés, d'environ 6µm de diamètre, avec des parois épaisses, quasi-parallèle avec ponctuellement des renflements apicaux et des branches irrégulières (flèche →). Zoom X10.

measured approximately 6 µm in diameter, were segmented, thick, almost parallel-walled, with occasional apical bulges and irregular branching (Fig. 2). These observations were consistent with cutaneous and muscular fungal infection, without further identification of the causative fungi. The responsibility of the lesions in the death of the individual is possible but not certain.

Fungal SDA culture from swabs and further identification by MALDI-TOF MS revealed no fungal element, and from the skin sample, only showed the presence of Mucorales (compatible with *Rhizopus* sp.). However, PCR analysis from the skin samples was positive for *O. ophidiicola* with a Ct value of 28.97 (mean value of triplicates). Although morphological features identified by histology were not sufficient to strictly identify *O. ophidiicola*, it was compatible with a hyaline septomycete (among which Oo) and ruled out the hypothesis of mucorales due to the presence of walls on the hyphae.

We report a clinical case of ophiodomycosis in a juvenile *Z. longissimus* in France. Previous identifications of *O. ophidiicola* in the Doubs department had been made in the context of a mark-recapture targeted protocol coordinated by the LPO Bourgogne-Franche-Comté, with the contribution of several teams for diagnosis (G. Blanvillain, Laboklin, Gent University, Karch info fauna), with samplings on live animals with suspect lesions and investigations focused on typing the pathogen rather than epidemiology and lesions on the individuals. Then, this case (although in the same area as previous detections) is the first one in the context of scanning surveillance based on the opportunistic detection of mortality events, coordinated by the SAGIR Network, and the first case in snakes for the network, since reptiles were officially added to the list of targeted groups of SAGIR's surveillance in January 2025.

The investigations did not identify a formal cause of death; nonetheless, the clinical presentation and the absence of lesions in internal organs suggest a death caused by severe apathy and final wasting, probably consequent to skin and muscle necrosis as well as a likely generalized inflammatory response caused by the fungus. Since, upon first encounter, the individual was manipulated, measured, and swabbed (as part of the mark-recapture protocol), we cannot exclude that the consecutive stress played a part in the final decompensation of the individual.

Fungal isolation by SDA culture was not successful when using skin swabs and frozen (-80°c) samples, probably because *O. ophidiicola* fungal elements did not survive due to the freezing of the skin and are not on the skin surface (swabbed) but infiltrate the skin and muscles, as confirmed by histopathology. On the other hand, qPCR on skin samples with lesions succeeded in identifying the mold. These results are consistent with a lower sensitivity of the fungal culture when it is done from swabs or after freezing and thawing, and the higher risk of contamination from common fungi, which could therefore inhibit *O. ophidiicola* growth in culture. Consequently, we encourage the use of complementary tools for the etiological diagnosis, including for individuals with compatible lesions. Although not applicable in our case, we encourage the development of IHC to understand better and characterize the physiopathology and specific lesions associated with Oo. In this case, we were not able to identify the clade of *O. ophidiicola* involved. Since both clades are known to be circulating in the neighboring country of Switzerland (Joudrier *et al.* 2024) and have different pathological impacts, although the circulation of clade II is known in this French department (Blanvillain *et al.* 2024), efforts will be made in future cases to determine which clade is involved.

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AUTHOR'S CONTRIBUTION

LP coordinated the investigations and wrote the manuscript with contributions from all authors concerning their part in the investigation. AM and SC collected the specimen. LP, AM, CS, and TC contributed to the conception of the article and to the epidemiological investigation. MH performed necropsy and coordinated lab analysis, KL performed histopathology, PLC, GJ, and RC conducted mycoculture and PCR analysis and interpretation.

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