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PATHOLOGY

Fatal systemic *Mortierella wolfii* infection in a neonatal calf in southern Brazil

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ABSTRACT: *This study described the pathological and microbiological aspects of a fatal systemic Mortierella wolfii infection in a neonatal calf in southern Brazil. The calf was born clinically normal, but on the third day of life it presented apathy, unilateral hypopyon, and neurological signs, and in the next day it was euthanized. At necropsy, multiple soft, and white-yellow nodules were observed in the liver, spleen, kidneys, mesenteric lymph nodes, heart, and lungs. In the brain, reddish, and friable areas were found. In the eye, there were anterior and posterior synechiae, diffuse thickening of choroid, and anterior chamber filled by whitish and friable material. Microscopically, areas of necrosis, pyogranulomatous inflammation, vasculitis, thrombosis, and intralesional fungal hyphae were observed, and the latter were better evidenced through Grocott Methenamine Silver technique. The fungus was identified as M. wolfii through mycological culture and molecular methods. To our knowledge, this is the first description of systemic disease caused by M. wolfii in a neonatal calf outside of Oceania.*
Key words: *cattle diseases, fungal diseases, pathology, molecular diagnosis.*

Infecção sistêmica fatal por *Mortierella wolfii* em um bezerro neonato na região Sul do Brasil

RESUMO: *Descrevem-se os aspectos patológicos e microbiológicos de uma infecção sistêmica fatal por Mortierella wolfii em um bezerro neonato na região Sul do Brasil. O bezerro nasceu clinicamente normal, porém no terceiro dia de vida apresentou apatia, hipópion unilateral e sinais neurológicos, e no dia seguinte foi submetido à eutanásia. Na necropsia, foram observados múltiplos nódulos macios e branco-amarelados no fígado, baço, rins, linfonodos mesentéricos, coração e pulmões. No encéfalo, havia áreas avermelhadas e friáveis. No olho, notou-se sinéquia anterior e posterior, espessamento difuso da coroide, e câmara anterior preenchida por material brancacento e friável. Histologicamente, foram observadas áreas de necrose, inflamação piogranulomatosa, vasculite, trombose e hifas fúngicas intralesionais, que foram melhor visualizadas por meio da técnica de Prata Metenamina de Grocott. O fungo foi identificado como M. wolfii por meio da cultura micológica e técnicas moleculares. Com base no conhecimento dos autores, este é o primeiro relato de doença sistêmica causada por M. wolfii em um bezerro neonato fora da Oceania.*
Palavras-chave: *doenças de bovinos, doenças fúngicas, patologia, diagnóstico molecular.*

Mortierella wolfii is a saprophytic fungus, belonging to the zygomycetes class (MUNDAY et al., 2006; CURTIS et al., 2017). It is an environmental fungus mainly present in foods, such as moldy silage and hay (KNUDTSON & KIRKBRIDE, 1992; JENSEN et al., 1994; GABOR, 2003; CURTIS et al., 2017), however, it can also be found in soil and pastures (KNUDTSON & KIRKBRIDE, 1992). Zygomycetes are often implicated as a cause of abortion in cattle (KNUDTSON & KIRKBRIDE, 1992), and *M. wolfii* is the most common agent in New Zealand, where it is considered the causative agent of a "distinctive mycotic abortion-pneumonia syndrome" (CARTER et al., 1973), though it rarely induces systemic disease in adult cattle (NEILAN et al., 1982; MUNDAY et al., 2006; DAVIES et al., 2010; CURTIS et al., 2017) or in neonatal calves (CORDES et al., 1967; MUNDAY et al., 2010). Therefore, the aim of this report was to describe the pathological, and microbiological aspects of a systemic *M. wolfii* infection in a neonatal calf in southern Brazil.

A four-day-old male Angus calf was born clinically normal, ingested colostrum and milk normally, but on the third day of life it presented anorexia, apathy, unilateral hypopyon, and neurological signs characterized by nystagmus, opisthotonus, bruxism,

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lateral decubitus, and pedaling movements. In the next day, due to the poor prognosis, it was euthanized.

At necropsy, multiple soft, and white-yellow nodules (0.2-1.0 cm in diameter), were observed in the liver (Figure 1A), spleen, kidneys, mesenteric lymph nodes, heart, and lungs. On the cutting surface of the cerebellum, a locally extensive reddish, soft, and friable area was observed (Figure 1B). Reddish circular lesions of 0.5 to 1.0 cm in diameter, similar to those described in the cerebellum, were also presented in the basal nuclei, thalamus (Figure 1B), mesencephalon, and telencephalon. In the left eye, there were anterior peripheral synechia and posterior synechia, choroid with diffuse thickening, white

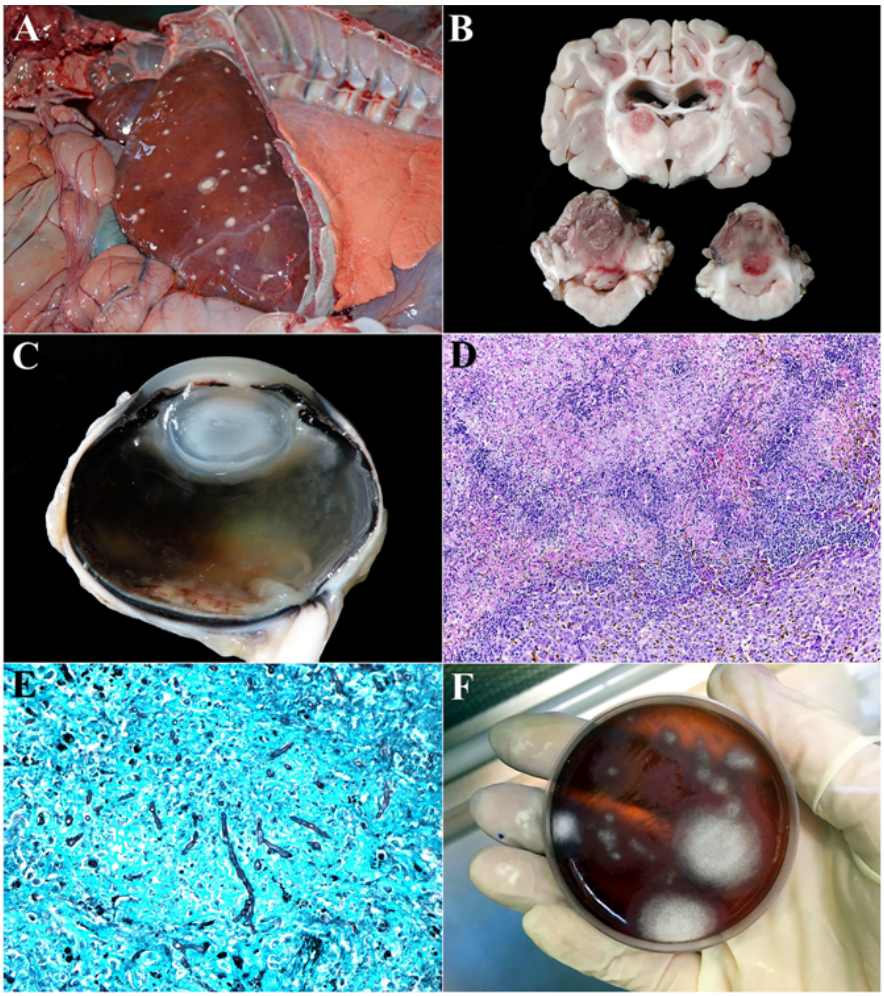

Figure 1 – Fatal systemic *Mortierella wolfii* infection in a neonatal calf. (A) Multiple soft and white-yellow nodules, ranging from 0.2 to 1.0 cm in diameter, are observed on the liver capsular surface. (B) The cut surface of the brain shows locally extensive reddish, soft, and friable area in the cerebellum. Similar reddish circular lesions of 0.5 to 1.0 cm in diameter, are also observed in the wall of the right lateral ventricle and thalamus, respectively. (C) The cut surface of the eye shows anterior peripheral synechia and posterior synechia; choroid with diffuse thickening, white and granular aspect, and anterior chamber filled with whitish and friable material. (D) Locally extensive area of coagulation necrosis in the liver associated with intense fibrin deposition, and marked inflammatory infiltrate. Cholestasis is also observed. H&E, magnification 10×. (E) Large fungal hyphae, with scarce septations, non-parallel walls, non-dichotomous branching, and occasional bulbous dilatations are observed in the brain. Grocott methenamine silver, magnification 40x. (F) White, downy, and fluffy fungal colonies are observed on blood agars.

and granular aspect, and the anterior chamber was filled with whitish and friable material (Figure 1C). Fragments of different organs were collected, fixed in 10% neutral buffered formalin, processed routinely, and stained with hematoxylin and eosin (HE).

Histologically, multifocal areas of coagulative necrosis associated with intense fibrin deposition, hemorrhage, hyaline hyphae, and marked inflammatory infiltrate composed of intact and degenerate neutrophils, macrophages, lymphocytes, plasma cells, and occasional multinucleated giant cells were observed in the liver (Figure 1D), brain, left eye, spleen, kidneys, heart, thyroids, adrenal glands, and mesenteric lymph nodes. The inflammatory infiltrate often invaded the blood vessels wall (vasculitis), which also presented wall fibrinoid necrosis and thrombosis.

The ciliary body, iris, sclera, choroid, and retina of the left eye were affected. There was adherence of part of the iris to the cornea (anterior synechia), as well as amorphous eosinophilic material interspersed with intact and degenerate neutrophils inside the anterior, posterior and vitreous chambers.

Sections of different organs were also subjected to the Grocott methenamine silver technique. Through this, many large (3-8 µm wide) fungal hyphae, with scarce septations, non-parallel walls, non-dichotomous branching, occasionally forming bulbous dilatations (Figure 1E), were evidenced amidst the areas of necrosis and inflammation, as well as surrounding and infiltrating the blood vessels wall.

Fresh liver and brain fragments were collected and inoculated in Sabouraud dextrose agar (Kasvi®), MacConkey agar (Kasvi®) and Blood agar base (Kasvi®) with 5% sheep blood. The plates were incubated aerobically at 37 °C. The MacConkey and Sabouraud plates did not have any growth after 72 hours. In both the blood agars (brain and liver), within 24 hours of incubation, the growth of a white to grayish-white, downy, and fluffy fungal colonies was observed (Figure 1F). Microscopically, the growth showed hyaline, non-septate hyphae with swellings and containing vacuoles.

To identify the fungus, total DNA was extracted from these colonies by taking a mycelium portion with a piece of agar under the mycelium center, to which it was added 50 µL of Tris-EDTA (TE) buffer and heated at 100 °C for 12 minutes. After centrifugation at 14,000 rpm for 5 minutes, the genetic material was recovered with supernatant and 80 µL of ultrapure water was added. The partial 18S rDNA was amplified by PCR reaction as previously described (MEDLIN et al., 1988), using the primers EukA-F: 5′-AACCTGGTTGATCCTGCCAGT-3′ EukA-R: 5′-GATCCWTCTGCAGGTTCACCTAC-3′. PCR amplicon was precipitate using 1 µg of tRNA (Invitrogen™) and 2.5V of absolute ethanol, following analysis by Sanger sequencing. The generated sequences were compared using BLAST (NCBI database), and matched to *M. wolfii*, showing 99% of identity in the 18S rDNA partial sequence. The generated sequence was deposited in GenBank under the accession number MK256350. For confirmation of the identity of the fungus, a phylogenetic analysis was performed within the phylum Zygomycota. For this, the sequence generated from the isolate of *M. wolfii* (LBV147-18) and a 18S rDNA partial sequence of subphylum were used as Kickxellomycotina, Zoopagomycotina, Mucoromycotina, and Mortierellomycotina. The tree was inferred with Tamura-Nei distance model and UPGMA method with 1,000 replicates in Geneious software (version 9.1). The phylogenetic tree illustrated the previously described heteregenicity in the Zygomycota evolutionary history. However, *M. wolfii* isolate LBV147-18 was positioned in the Mortierellomycotina subphylum clade together with other *M. wolfii* sequences (Figure 2).

Samples of blood serum, spleen, and lymph nodes were also submitted to the reverse transcription-PCR assay to identify the bovine viral diarrhea virus (BVDV) (BIANCHI et al., 2016); however, the results were negative for all tested samples.

Lesions observed in several organs, associated with vascular lesions, indicated a probable hematogenous dissemination of the fungus. Zygomycetes present tropism by blood vessels through a process called angioinvasion. Hyphae have the ability to invade endothelial cells, gain access to the circulatory system, and spread to other parts of the body. The presence of infarcts, necrosis, and vasculitis are considered characteristic lesions that occur secondary to the fungal invasion of the blood vessels (MUNDAY et al., 2006; ZACHARY, 2017), as observed in this case.

It is suggested that the pathogenesis for *Mortierella* infection in neonatal calves, as well as for other zygomycetes, is intra-uterine (VASCONCELOS & GRAHN, 1995; MUNDAY et al., 2010). The fungus establishes a primary infection in the dam's lungs, enters the bloodstream and reaches the uterus, where it can induce acute metritis and placentitis, that results in abortion due to fetal hypoxia (VASCONCELOS & GRAHN, 1995; MUNDAY et al., 2006). However, subacute or chronic fungal metritis and placentitis allow

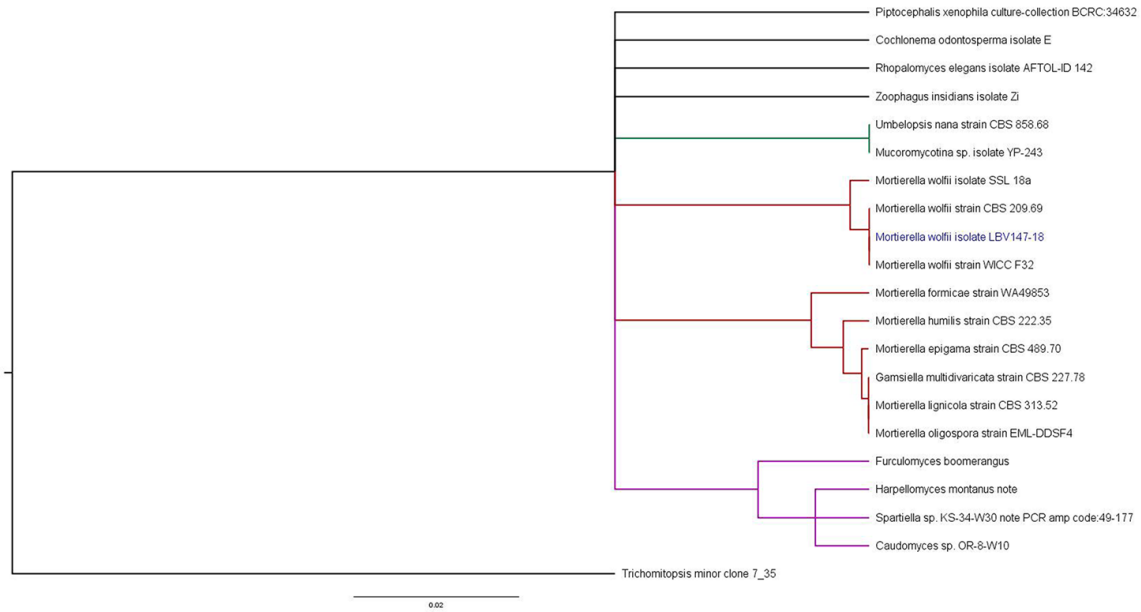

Figure 2 - Fatal systemic *Mortierella wolfii* infection in a neonatal calf. UPGMA tree calculated by genetic distance model of Tamura-Nei from 18S rDNA partial gene of the subphyluns from Zygomycota phylum. The taxa are color-coded in accordance to the four-subphylum of Zygomycota. Red bars: Mortierellomycotina clade. Pulple bars: Kickxellomycotina clade. Green bars: Mucoromycotina clade. Black bars: Zoopagomycotina clade. *Mortierella wolfii* isolate LBV147-18 is detached in blue. Trichomitopsis minor was used as outgroup.

fetal infection, but calves are usually born alive and clinically normal, and may develop lesions and clinical disease after some time of life (CORDES et al., 1967; VASCONCELOS & GRAHN, 1995), similar to what is described in this case. In older calves and adult cattle, disseminated mycoses may occur due to a primary gastrointestinal or respiratory infection (CHIHAYA et al., 1992). Previous reports have predisposing factors such as ruminal acidosis (CHIHAYA et al., 1992), prolonged antibiotic therapy (JENSEN et al., 1994), traumatic reticulopericarditis (CHIHAYA et al., 1986), parasitic anemia (NAKAGAWA et al., 1986), ketosis (MUNDAY et al., 2006), and cutaneous surgical wound (CURTIS et al., 2017).

We highlight that the mycological culture was pure, and negative in the Sabouraud agar, which is related to the hematogenous chemotaxis of *M. wolfii*. Moreover, the 18S rDNA partial sequencing and analysis was able to confirm the identity of the isolated fungus as *M. wolfii*, with 99% of identity in the matching BLAST search.

Because of the presence of neurological clinical signs and hypopyon; bacterial septicemia is the main differential diagnosis of this case. This has mostly been observed in neonatal calves that did not receive adequate colostrum, and this condition is predominantly associated with *Escherichia coli* infection (KONRADT et al., 2017). Infectious thrombotic meningoencephalitis caused by *Histophilus somni* is characterized by similar gross lesions in the brain, but the diagnosis can be made by distinct histological lesions and bacterial culture (MOMOTANI et al., 1985; CURTIS et al., 2017). Other fungi, especially *Aspergillus* spp. and zygomycetes, may cause similar lesions as those observed in this case, and the differential diagnosis should be done mainly through culture and molecular tests (VASCONCELOS & GRAHN, 1995; MUNDAY et al., 2006; CURTIS et al., 2017).

The diagnosis of a fatal systemic *M. wolfii* infection in a neonatal calf was based on pathological, and microbiological findings. To our knowledge, this is the first description of this condition in this age group outside of Oceania.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

BIANCHI, M. V. et al. Natural outbreak of BVDV-1d–induced mucosal disease lacking intestinal lesions. **Veterinary Pathology**, v.54, p.242-248, 2016. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27586238>. Accessed: Jun. 10, 2019. doi: 10.1177/0300985816666610.

CARTER, M. E. et al. Fungi isolated from bovine mycotic abortion and pneumonia with special reference to *Mortierella wolfii*. **Research in Veterinary Science**, v.14, p.201-206, 1973. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/4616297>. Accessed: Jun. 10, 2019.

CHIHAYA, Y. et al. A case of bovine cerebral mucormycosis. **Japanese Journal of Veterinary Science**, v.48, p.1271-1274, 1986. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/3820928>. Accessed: Jun. 10, 2019.

CHIHAYA, Y. et al. A pathological study of bovine alimentary mycosis. **Journal of Comparative Pathology**, v.107, p.195-206, 1992. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/1452813>. Accessed: Jun. 10, 2019.

CORDES, D. O. et al. Systemic mycosis in neonatal calves. **The New Zealand Veterinary Journal**, v.15, p.143-149, 1967. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/5235333>. Accessed: Jun. 10, 2019. doi: 10.1080/00480169.1967.33714.

CURTIS, B. et al. Embolic mycotic encephalitis in a cow following *Mortierella wolfii* infection of a surgery site. **Journal of Veterinary Diagnostic Investigation**, v.29, p.725-728, 2017. Available from: <https://journals.sagepub.com/doi/pdf/10.1177/1040638717710684>. Accessed: Jun. 10, 2019. doi: 10.1177/1040638717710684.

DAVIES, J. L. et al. Systemic infection with *Mortierella wolfii* following abortion in a cow. **The Canadian Veterinary Journal**, v.51, p.1391-1393, 2010. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2978994/>. Accessed: Jun. 10, 2019.

GABOR, L. J. Mycotic pneumonia in a dairy cow caused by *Mortierella wolfii*. **Australian Veterinary Journal**, v.81, p.409-410, 2003. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/15084053>. Accessed: Jun. 10, 2019. doi: 10.1111/j.1751-0813.2003.tb11548.x.

JENSEN, H. E. et al. Gastrointestinal aspergillosis and zygomycosis of cattle. **Veterinary Pathology**, v.31, p.28-36, 1994. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/8140723>. Accessed: Jun. 10, 2019. doi: 10.1177/030098589403100104.

KNUDTSON, W. U.; KIRKBRIDE, C. A. Fungi associated with bovine abortion in the Northern plains states (USA). **Journal of Veterinary Diagnostic Investigation**, v.4, p.181-185, 1992. Available from: <https://journals.sagepub.com/doi/pdf/10.1177/104063879200400211>. Accessed: Jun. 10, 2019. doi: 10.1177/104063879200400211.

KONRADT, G. et al. Suppurative infectious diseases of the central nervous system in domestic ruminants. **Pesquisa Veterinária Brasileira**, v.37, p.820-828, 2017. Available from: <http://www.scielo.br/pdf/pvb/v37n8/1678-5150-pvb-37-08-00820.pdf>. Accessed: Jun. 10, 2019. doi: 10.1590/S0100-736X2017000800007.

MEDLIN, L. et al. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. **Gene**, v.71, p.491-499, 1988. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/3224833>. Accessed: Jun. 15, 2019. doi: 10.1016/0378-1119(88)90066-2.

MOMOTANI, E. et al. Histopathological evaluation of disseminated intravascular coagulation in *Haemophilus somnus* infection in cattle. **Journal of Comparative Pathology**, v.95, p.15-23, 1985. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/3973108>. Accessed: Jun. 10, 2019.

MUNDAY, J. S. et al. Meningoencephalitis in an adult cow due to *Mortierella wolfii*. **Journal of Veterinary Diagnostic Investigation**, v.18, p.619-622, 2006. Available from: <https://journals.sagepub.com/doi/pdf/10.1177/104063870601800620>. Accessed: Jun. 10, 2019. doi: 10.1177/104063870601800620.

MUNDAY, J. S. et al. Disseminated *Mortierella wolfii* infection in a neonatal calf. **The New Zealand Veterinary Journal**, v.58, p.62-63, 2010. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20200577>. Accessed: Jun. 10, 2019. doi: 10.1080/00480169.2010.65062.

NAKAGAWA, M. et al. Bovine cerebellar zygomycosis: a case report. **Japanese Journal of Veterinary Science**, v.48, p.809-812, 1986. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/3761786>. Accessed: Jun. 10, 2019.

NEILAN, M. C. et al. Mycotic pneumonia, placentitis and neonatal encephalitis in dairy cattle caused by *Mortierella wolfii*. **Australian Veterinary Journal**, v.59, p.48-49, 1982. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/7150132>. Accessed: Jun. 10, 2019. doi: 10.1111/j.1751-0813.1982.tb02715.x.

VASCONCELOS, D. Y.; GRAHN, B. H. Disseminated *Rhizopus* infection with ocular involvement in a calf. **Veterinary Pathology**, v.32, p.78-81, 1995. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/7725604>. Accessed: Jun. 10, 2019. doi: 10.1177/030098589503200115.

ZACHARY, J. F. Mechanisms of microbial infections. In: Zachary, J.F. **Pathologic basis of veterinary disease**. 6th. St. Louis, Missouri: Elsevier, 2017. Cap.4, p.132-241.