Ciência Rural, Santa Maria, v.53:2, e20210807, 2023
http://doi.org/10.1590/0103-8478cr20210807
ISSNe 1678-4596
PATHOLOGY

Rhinitis in goat by *Exserohilum rostratum* (*Setosphaeria rostrata*)

Millena de Oliveira Firmino[1*] Rodrigo Cruz Alves[1] Karoline Lacerda Soares[2] Tatiane Rodrigues da Silva[3] Fernanda Harumi Maruyama[4] Valéria Dutra[4] Glauco José Nogueira de Galiza[1] Antônio Flávio Medeiros Dantas[1]

[1]Programa de Pós-graduação em Ciência e Saúde Animal, Laboratório de Patologia Animal, Hospital Veterinário Universitário, Universidade Federal de Campina Grande (UFCG), 58708-110, Patos, PB, Brasil. E-mail: millena_deoliveira@yahoo.com.br. *Corresponding author.
[2]Programa de Residência em Medicina Veterinária, Laboratório de Patologia Veterinária, Campus Darcy Ribeiro, Universidade de Brasília (UnB), Brasília, DF, Brasil.
[3]Clínica Médica e Cirúrgica de ruminantes, Hospital Veterinário Universitários, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande (UFCG), Patos, PB, Brasil.
[4]Laboratório de Microbiologia e Biologia Molecular Veterinária, Universidade Federal de Mato Grosso (UFMT), Cuiabá, MT, Brasil.

ABSTRACT: *We described a case of rhinitis case caused by Exserohilum rostratum in a 3-year-old, mixed breed, afemale goat, presenting with decreased appetite, dyspnea, and face deformity, with evolution of approximately 2 months. Necropsy revealed an extensive brownish yellow, irregular, ulcerated, and friable focal lesion in the nasal cavity. Purulent secretion drained from the lesion, affecting the nasal vestibule and extending the caudal portion of the dorsal concha, associated with nasal meatus obstruction, destruction of the nasal septum, and nasal bone palatine and vomer. Histologically, the lesion was characterized as rhinitis and pyogranulomatous and necrotizing osteomyelitis (multifocal to coalescent, sharp, and chronic) and associated with a myriad of fungal structures. Morphologically, the hyphae were characterized by thin, slightly tortuous, and rarely septate walls (2–6 µm diameter), multiple chains of individual conidia or in groups (8–10 µm diameter), and pigmented chlamydoconidia (7.5–15 µm diameter). They showed positive results for the special histochemical techniques such as Fontana Masson, methenamine silver nitrate by Grocott, and periodic acid by Schiff. E. rostratum was identified as the causative agent using microbiological isolation associated with molecular identification. Pyrogranulomatous rhinitis caused by E. rostratum in goats is unprecedented, being the first isolation of the agent in animals in Brazil and the first case of nasal phaeohyphomycosis in the goats.*
Key words: *nasal cavity, dyspnea, dematiaceous fungi, phaeohyphomycosis.*

Rinite em caprino por *Exserohilum rostratum* (*Setosphaeria rostrata*)

RESUMO: *Descreve-se um caso de rinite por Exserohilum rostratum em um caprino, sem raça definida, fêmea, três anos, com diminuição do apetite, dispneia e deformidade facial, com evolução de aproximadamente dois meses. Durante a necropsia, observou-se que na cavidade nasal havia área focal extensa, amarelo acastanhada, irregular, ulcerada e friável, que drenava secreção purulenta acometendo o vestíbulo nasal e estendendo-se da porção caudal da concha dorsal, associada a obstrução do meato nasal, destruição do septo nasal e osso nasal palatino e vômer. Histologicamente, a lesão foi caracterizada como rinite e osteomielite piogranulomatosa e necrosante, multifocal a coalescente, aguda, crônica, associada a uma miríade de estruturas fúngicas. Morfologicamente, as hifas foram caracterizadas por paredes finas, discretamente tortuosas e septadas medindo 2 a 6 µm de diâmetro. Observamos algumas cadeias de conídios ou grupos com 8 a 10 µm de diâmetro e clamidoconídios pigmentados medindo de 7,5 a 15 µm de diâmetro. Foram positivos na histoquímica especial de Fontana Masson, Nitrato de Metenamina de Prata de Grocott e Ácido Periódico de Schiff. O agente foi determinado por meio de isolamento microbiológico associado à identificação molecular. A rinite piogranulomatosa causada por E. rostratum em caprinos é inédita, sendo o primeiro isolamento do agente em animais no Brasil e o primeiro caso de feo-hifomicose nasal na espécie caprina.*
Palavras-chave: *cavidade nasal, dispneia, fungos dematiáceos, feo-hifomicose.*

The saprophytic and dematiaceous fungi of *Exserohilum* spp. (*Setosphaeria rostrata*) are important plant pathogens (POLTRONIERI et al., 2008; GAUTHIER et al., 2013; KATRAGKOU et al., 2014) that rarely infect humans (GAUTHIER et al., 2013; KATRAGKOU et al., 2014) or animals (WHITFORD et al., 1989; MORE et al., 2019). In humans, cases occur in immunosuppressed individuals in different clinical forms, including systemic, cutaneous, subcutaneous, and ocular (ADLER et al., 2006; GAUTHIER et al., 2013; KATRAGKOU et al., 2014) forms.

Received 11.11.21 Approved 03.07.22 Returned by the author 05.05.22
CR-2021-0807.R1
Editor: Rudi Weiblen

Rare cases of *E. rostratum* rhinitis have been described in horses (MORE et al., 2019) and humans (ADLER et al., 2006; GAUTHIER et al., 2013; KATRAGKOU et al., 2014). However, data on *E. rostratum* infection in domestic animals remain scarce, especially the involvement of the nasal cavity in goats. This study described a case of rhinitis caused by *E. rostratum* in a goat, highlighting the clinical and anatomopathological aspects of the infection.

A three-year-old mixed-breed goat, from São José do Bonfim-PB, presented with decreased appetite, dyspnea, and increased volume in the face, with an evolution of approximately 2 months. The animal was raised alongside 32 sheep and 10 goats in a semi-extensive system, with commercial feed, native pasture, and free access to a pond.

On clinical examination, the animal was cachectic in lateral decubitus, comatose, dehydrated, and had pale mucous membranes and mucopurulent bilateral nasal discharge. Moreover, in addition to dyspnea, a facial deformity characterized by an irregular, bilateral volume increase located in the nasal sinuses and nasal cavity was present. Radiographic examination of the face revealed a loss of continuity in the nasal bone and increased radiopacity in the nasal cavity. This seriously ill goat died and was sent for necropsy.

Macroscopically, the head showed facial deformity, characterized by an increase in bilateral, firm volume, including the nasal cavities. The head sagittal section revealed an extensive yellowish-brown, irregular, ulcerated, and friable focal lesion in the nasal cavity, which drained purulent secretion affecting the nasal vestibule. It extended into the caudal portion of the dorsal concha and was responsible for nasal meatus obstruction and destruction of the nasal septum and nasal bone, palate, and vomer. (Figure 1A).

The abomasum mucosa showed marked infestation by nematode parasites identical to *Haemonchus* sp. In addition, subcutaneous edema, pulmonary edema, hydropericardium, and mild ascites were observed in the submandibular region.

Tissue samples of all organs were collected and fixed in 10% buffered formaldehyde, processed routinely, and stained with hematoxylin and eosin (HE). To visualize the morphological characteristics of the fungal infective agent, tissue sections of the nasal cavity lesions were treated with Grocott's methenamine silver (GMS), Schiff's periodic acid (PAS), and Fontana Masson (FM) stains.

Histologically, the lesions were characterized by rhinitis, pyogranulomatous, and necrotizing osteomyelitis (multifocal to coalescent, severe, and chronic) and associated with myriad fungal structures. In the nasal cavity, multifocal and coalescent areas of marked thickening of the lamina propria are caused by inflammatory infiltrates (consisting of macrophages, epithelioid macrophages, multinucleated giant cells, and neutrophils) were observed. Occasionally, central and extensive areas of necrosis are associated with myriads of intralesional fungal structures, delimited by a moderate proliferation of fibrous connective tissue and lymphocytic infiltrate.

Destruction of the nasal septum and bone tissue adjacent to the inflammation site was observed. Fungi were also frequently observed in the cytoplasm of the multinucleated giant cells. Slight congestion and occasional blood vessels with fibrin thrombus formation, partially occluding the vascular lumen, were also observed.

In the longitudinal and transverse HE-stained nasal sections, the fungi presented themselves as tubuliform structures, with basophilic to brownish walls (Figure 1C). Numerous fungal structures were observed in previously deparaffinized and unstained slides, showing slightly brownish, septate, and thin walls (Figure 1C, inset). GMS and FM staining revealed heavily black metallic silver–deposited hyphae (Figure D and E). PAS staining revealed intense rosy hyphae (Figure 1F). Hyphae exhibited thin, slightly tortuous morphology with rarely septate walls (2–6 µm diameter), multiple individual conidial chains or groups (8–10 µm diameter), and pigmented chlamydoconidia (7.5–15 µm diameter).

Microbiological isolation of the infective fungal agent was done by incubating samples of the nasal cavity lesions on Sabouraud agar culture medium at 25 °C for 14 days . The colonies had a black cottony texture. On direct examination, cotton blue, septate, and branched hyphae with dilations, devoid of sporulation and conidiophores formation, were observed.

To determine the fungal infective species, the colony stored in mineral oil was sent for DNA extraction according to DEL POETA et al. (1999). The extracted DNA was subjected to PCR with oligonucleotides from the internal transcribed spacers (ITS) 4 (5-TCCTCCGCTTATTGATATGC) and ITS5 (5-GGAAGTAAAAGTCGTAACAAGG-3') regions, as described by WHITE et al. (1990). DNA analysis was performed using CLC Genomics Workbench 4.9 software (CLC bio, Denmark), and the species was subsequently identified by comparing the sequence in the GenBank database using the BLAST software (NCBI server http://www.ncbi.

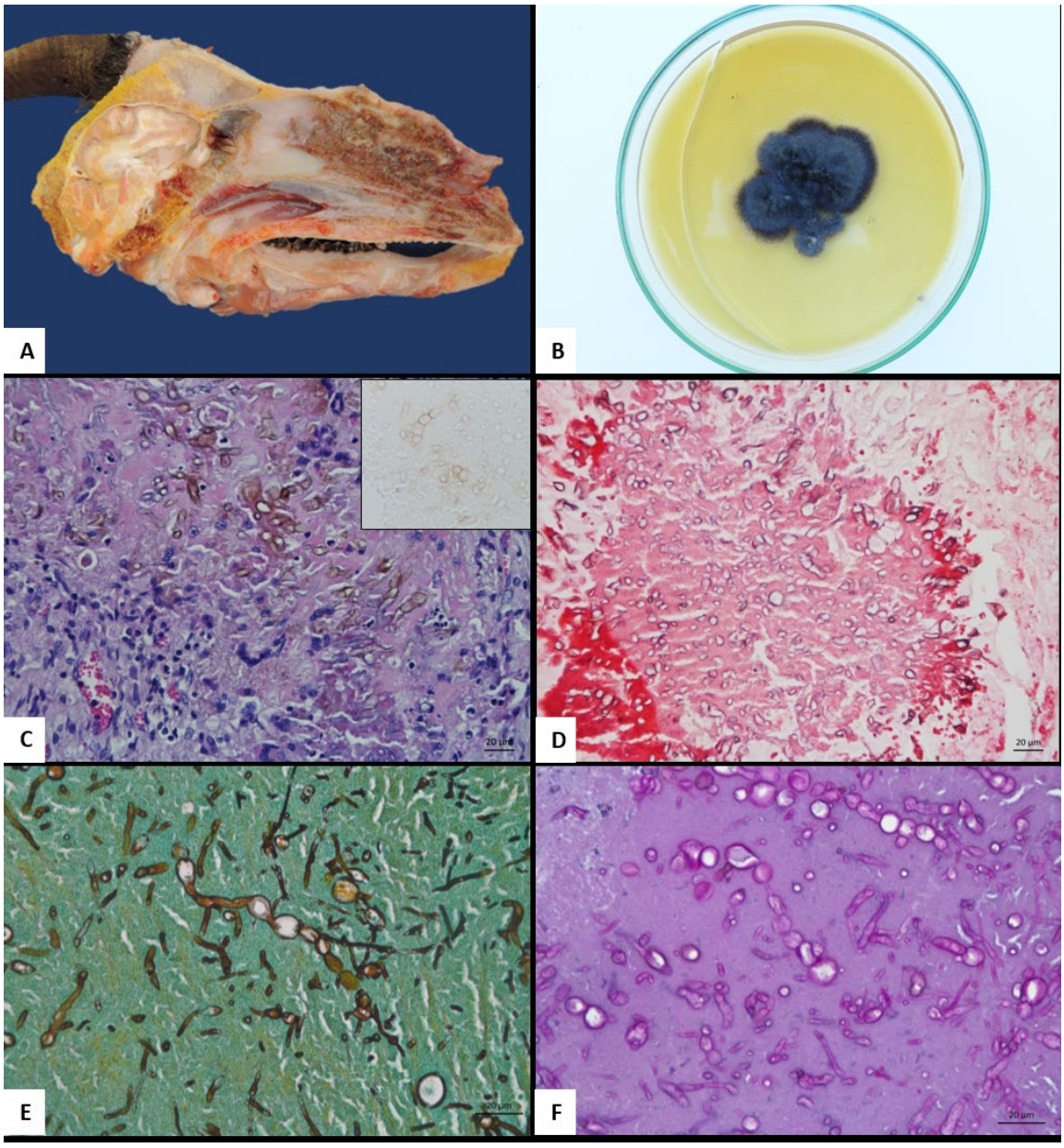

Figure 1 – A) Sagittal section of the nasal cavity with enlarged volume characterized by a focal area, which is yellowish brown, irregular, friable, and ulcerated, affecting the nasal vestibule and extending the caudal portion of the dorsal concha associated with destruction of the nasal septum, nasal bone, palate and vomer. B) Microbiological isolation of the fungal infective agent—*Exserohilum rostratum*—a blackish fungal colony with a cottony aspect is seen. C) HE-stained sections of the nasal cavity. Numerous pigmented hyphae are observed in transverse and longitudinal sections with rarely septate thin walls amid necrosis and in the cytoplasm of multinucleated giant cells [Bar = 20 µm]. Inset: Hyphae with a pigmented wall, thin and rarely septate, evident in deparaffinized and unstained lamina [Bar = 20 µm]. D) and E) GMS- and FMS-stained sections of nasal cavity—numerous hyphae, chains of conidia, and chlamydoconidia strongly impregnated in black are seen [Bar = 20 µm]. F) PAS-stained sections of the nasal cavity—multiple fungal structures, stained intensely in pink, similar to (D) and (E) are observed [Bar = 20 µm].

nlm.nih.gov/BLAST). This molecular PCR testing resulted in amplification of an approximately 623-bp fragment with 99.84% identity to *Exserohilum rostratum* (*Setosphaeria rostrata*).

The goat was diagnosed as having *E. rostratum* rhinitis based on the microscopic morphological characteristics of the infective agent found in the focal lesions. The diagnosis was further confirmed through isolation and molecular testing. In histopathological examinations, the morphology of the fungal infective agent can be indistinguishable from other pathogenic fungal agents (RAI et al., 2021). Thus, fungal infections must be carefully diagnosed after microbiological isolation and molecular identification of the agent involved, individually. Here, the goat was infected by *E. rostratum* probably by inhaling spores present in the soil around the weir, which had a marked amount of organic matter. Similar epidemiological conditions have been reported for nasal cavity diseases (including pythiosis and conidiobolomycosis) in sheep in this region (PORTELA et al., 2010).

Data related to the pathogenesis and behavior of *E. rostratum* in veterinary medicine are rare, with cases of skin infections in cattle (WHITFORD et al., 1989) and a recent case of rhinitis in horses (MORE et al., 2019), being the only ones reported. Thus, reporting—the first isolation of *E. rostratum* in Brazilian animals and the first case of nasal phaeohyphomycosis in goat.

Several clinical presentations of *E. rostratum* human infections have been described, including systemic, cutaneous, subcutaneous, and ocular (ADLER et al., 2006; KATRAGKOU et al., 2014). An isolated case of involvement of the central nervous system during severe *E. rostratum* outbreak (KATRAGKOU et al., 2014) is also known. The fungus is opportunistic and predominantly infects immunosuppressed individuals (ADLER et al., 2006; GAUTHIER et al., 2013; KATRAGKOU et al., 2014). Here, severe hemonchosis in the goat may have triggered immunosuppression via general body weakness.

Lesions characterized by pyogranulomas with extensive areas of necrosis are typical of *E. rostratum* infection (WHITFORD et al., 1989; MORE et al., 2019), as observed here, which may be related to the chronicity of the lesion. Few histopathological studies of *E. rostratum* morphology exist; however, it presents differently, including hyphae, chlamydoconidia, and conidial chain (MORE et al., 2019) structures with fully pigmented or basophilic walls. Similarly, *E. rostratum* presented here with various presentations, located mainly in necrotic areas. The degree of basophilic coloration of the hyphae has been described (LYONS et al., 2012) in a woman with severe vasculitis and cerebral infarction associated with angioinvasive *exserohilum* infection. Here; although, *E. rostratum* was not observed inside the vessels, vascular changes, such as congestion and venous thrombi, were present.

In the present study, the changes observed during necropsy, including subcutaneous edema, pulmonary edema, hydropericardium, and ascites, were attributed to hypoproteinemia secondary to the high parasitic burden of *Haemonchus* sp., which possibly culminated in animal death. However, the lesions observed in the nasal cavity were severe and extensive, associated with the destruction of the nasal septum and adjacent bone structures and affected approximately 80% of the nasal cavity bilaterally, thus justifying the clinical signs of facial deformity and severe dyspnea. Therapeutic and surgical treatments become unfeasible and often inefficient in cases where lesions are extensive in the nasal cavity, as observed in sheep with conidiobolomycosis and pythiosis (PORTELA et al., 2010).

Given the anatomopathological findings, a differential diagnosis considering other infectious rhinitis (such as aspergillosis (PORTELA et al., 2010) and protothecosis (CAMBOIM et al., 2011) already described in goats is necessary. However, it is possible to morphologically identify and differentiate these agents during histopathological examination. Because other fungal species that trigger phaeohyphomycosis stain similar to that observed here, they must be considered in such studies (RAI et al., 2021).

Pyogranulomatous rhinitis caused by *E. rostratum* in goats is unprecedented, this being the first isolation of the agent in animals in Brazil and the first case of nasal phaeohyphomycosis in a goat breed. Thus, differential diagnosis of chronic rhinitis must consider all infectious agents and neoplasms. Diagnosis must be done carefully—after histopathological examination, microbiological isolation, and genetic sequencing of the infective agent—to exclude infections by algae, oomycetes, and other fungi of the phaeohyphomycosis group.

ACKNOWLEDGMENTS

The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) designed the doctoral scholarship of the first author.

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of this manuscript. All authors critically reviewed and approved the final version of the manuscript.

REFERENCES

ADLER, A. et al. *Exserohilum*: an emerging human pathogen. **European Journal of Clinical Microbiology and Infectious Diseases**, v. 25, n. 4, p. 247–253, 2006. Available from: <https://www.researchgate.net/publication/7265549_Exserohilum_An_emerging_human_pathogen>. Accessed: Mar. 23, 2020. Doi: 10.1007/s10096-006-0093-3.

CAMBOIM, E. K. et al. Protothecosis by *Prototheca wickerhamii* in goats. **Mycoses**, v. 54, n. 4, p. 196–200, 2011. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/j.1439-0507.2010.01864.x>. Accessed: Mar. 17, 2020. Doi: 10.1111/j.1439-0507.2010.01864.x.

DEL POETA, M. et al. Topoisomerase I is essential in *Cryptococcus neoformans*: role in pathobiology and as an antifungal target. **Genetics**, v. 152, p. 167–178, 1999. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1460578/pdf/10224251.pdf>. Accessed: Mar. 23, 2020.

GAUTHIER, G. M.; KELLER, N. P. Crossover fungal pathogens: the biology and pathogenesis of fungi capable of crossing kingdoms to infect plants and humans. **Fungal Genetics and Biology**, v. 61, p. 146–157, 2013. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1087184513001564?via%3Dihub>. Accessed: Mar. 23, 2020. Doi: 10.1016/j.fgb.2013.08.016.

KATRAGKOU, A. et al. *Exserohilum* infections: review of 48 cases before the 2012 United States outbreak. **Sabouraudia**, v. 52, n. 4, p. 376–386, 2014. Available from: <https://academic.oup.com/mmy/article/52/4/376/1046249>. Accessed: Apr. 12, 2020. Doi: 10.1093/mmy/myt030.

LYONS, J. L. et al. Fatal *Exserohilum* meningitis and central nervous system vasculitis after cervical epidural methylprednisolone injection. **Annals of Internal Medicine**, v. 157, p. 835–836, 2012. Available from: <https://www.acpjournals.org/doi/10.7326/0003-4819-158-1-201212040-00557>. Accessed: Feb. 17, 2021. Doi: 10.7326/0003-4819-158-1-201212040-00557.

MORE, S. N.; HERNANDEZ, O.; CASTLEMAN, W. L. Mycotic rhinitis and sinusitis in Florida horses. **Veterinary Pathology**, v. 56, n. 4, p. 586–598. 2019. Available from: <https://journals.sagepub.com/doi/10.1177/0300985818817046>. Accessed: Feb. 17, 2021. Doi: 10.1177/0300985818817046.

POLTRONIERI, L.S.; et al., Primeiro registro de *Exserohilum rostratum* (anamorfo de *Setosphaeria rostrata*) causando manchas foliares em açaizeiro no Brasil. **Summa Phytopathologica**, v. 34, n. 2, p. 195, 2008. Available from: <https://www.scielo.br/j/sp/a/vm5JRjfp7DRTt3RNG4fMFfc/?lang=pt>. Accessed: Nov. 04, 2020. Doi: 10.1590/S0100-54052008000200022.

PORTELA, R. A. et al. Doenças da cavidade nasal em ruminantes no Brasil. **Pesquisa Veterinária Brasileira**, v. 30, n. 10, p. 844–854, 2010. Available from: <https://www.scielo.br/j/pvb/a/Ktg3XH96nqGxFgTcZ4RCjLg/?lang=pt>. Accessed: Mar. 23, 2020. Doi: 10.1590/S0100-736X2010001000007.

RAI, M. et al. Recent advances on mycotic keratitis caused by dematiaceous hyphomycetes. **Journal of Applied Microbiology**, v. 131, n. 4, p. 1652–1667, 2021. Available from: <https://sfamjournals.onlinelibrary.wiley.com/doi/10.1111/jam.15008>. Accessed: Aug. 12, 2021. Doi: 10.1111/jam.15008.

WHITE, T. J.; BRUNS, T.; LEE, S. J. W. T.; TAYLOR, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS, M. A.; GELFAND, D. H.; SNINSKY, J. J.; WHITE, T. J. **PCR protocols**: **a guide to methods and applications**, v. 18, n. 1, p. 315–322, 1990.

WHITFORD, H. W.; SCHWARTZ, W. L.; RICHARDS, H. *Exserohilum* dermal granulomas in a bovine. **Journal of Veterinary Diagnostic Investigation**, v. 1, p. 78–81, 1989. Available from: <https://journals.sagepub.com/toc/vdia/1/1>. Accessed: Mar. 23, 2020. Doi: 10.1177/104063878900100123.