Austral Journal of Veterinary Sciences

Austral J Vet Sci 58, e5804 (2026)
https://doi.org/10.4206/ajvs.58.04

SHORT COMMUNICATION

Erysipelothrix rhusiopathiae, a re-emerging pathogen: a case report in laying hens from a cage-free farm

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Article History
Received: 04.11.2025
Accepted: 19.01.2026
Published: 30.03.2026

ABSTRACT. Erysipelas, caused by *Erysipelothrix rhusiopathiae*, has re-emerged as a significant threat in cage-free poultry systems. In May 2025, we investigated a sudden increase in mortality on a commercial layer farm in Chile. Eleven hens were necropsied, revealing systemic lesions consistent with septicemia. *Erysipelothrix rhusiopathiae* was isolated from multiple organs and confirmed through biochemical testing, histopathology, and PCR, identifying serotype 1b. Antimicrobial susceptibility testing showed marked sensitivity to β-lactams and resistance to fluoroquinolones, macrolides and tetracyclines. The outbreak was successfully controlled with amoxicillin therapy. This case highlights the increased risk of infectious diseases associated with cage-free production and underscores the need for continued diagnostic surveillance and antimicrobial resistance monitoring.

Keywords: free-range; poultry; cage-free; infections; zoonosis; erysipelas

INTRODUCTION

The global human population is projected to reach 9.8 billion by 2050, increasing the demand for animal protein and reinforcing the poultry industry's role as a primary food provider (United Nations, 2019; FAO, 2023). Concurrently, consumer-driven adoption of cage-free, free-range, and organic systems is improving animal welfare but also increasing the risk of emergence and re-emergence of infectious diseases due to outdoor access and greater environmental exposure (Eriksson *et al*., 2014).

Erysipelas, caused by *Erysipelothrix rhusiopathiae*, is an acute septicemic disease affecting turkeys, chickens, fish, mammals, and humans, and is strongly associated with cage-free poultry systems (Fossum *et al*., 2009; Butler-Lund *et al*., 2025). *Erysipelothrix rhusiopathiae* is a small, Gram-positive, nonmotile, non–spore-forming, facultative anaerobic bacillus that grows in slender filaments and produces small colonies with mild alpha-hemolysis. The genus *Erysipelothrix* currently includes at least 13 recognized species within the family *Erysipelotrichaceae* (Brooke & Riley, 1999; Dec *et al*., 2024; Eriksson *et al*., 2025). *Erysipelothrix rhusiopathiae* is classified into 17 serotypes based on differences in peptidoglycan antigens: 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, 23, and N (Eriksson & Swayne, 2020; Shimoji *et al*., 2020). Among poultry isolates, the most commonly detected serotypes are 1a, 1b, 2, and 5 (Takahashi *et al*., 1992; Eriksson *et al*., 2025). Transmission generally occurs through breaches in the skin or via mechanical arthropod vectors, such as Dermanyssus gallinae (Eriksson *et al*., 2010). Capsule formation, mediated by the cps gene cluster, is a well-established virulence factor that plays a central role in immune evasion and pathogenicity (Shimoji, 2000).

With the transition from conventional cages to cage-free systems in the 21st century, erysipelas has re-emerged as a significant health issue in laying hens, with an increasing incidence and multiple recent outbreaks reported in Europe and the United States (Eriksson *et al*., 2025). Birds with outdoor access are at a substantially higher risk than caged birds (Eriksson *et al*., 2003; Stokholm *et al*., 2010; Shamoun *et al*., 2023). Consequently, timely and accurate diagnosis is essential to limit the spread of emerging and re-emerging infectious agents, particularly in cage-free production systems, where increased bird contact and greater environmental exposure may facilitate pathogen transmission.

MATERIALS AND METHODS

In May 2025, the Avian Pathology Laboratory at the University of Chile received and necropsied 11 59-week-old laying hens from a cage-free commercial farm housing more than 200,000 birds in the Ñuble Region of Chile. The outbreak lasted an estimated 7–10 days and began with a sudden increase in mortality, accompanied by lethargy and a decline in egg production in the affected hens, resulting in a cumulative mortality rate of 2.1%. Standard

necropsy examinations were performed. A longitudinal incision was made in the oral cavity through the pharynx and trachea to expose the upper respiratory tract. The palatine sinus was subsequently opened with a longitudinal incision along the hard palate to allow full evaluation of the mucosa and internal contents. The skin and fascia between the leg and abdomen were incised, the legs were rotated laterally, and the femoral head was disarticulated to access the coelomic cavity through the ventral tip of the sternum and examine the viscera.

For bacteriological analysis, samples of the spleen (n = 2), liver (n = 2), kidney (n = 2), and bone marrow (n = 2) were collected aseptically and inoculated onto tryptone soy agar supplemented with 5% sheep blood and MacConkey agar. The plates were incubated at 37 °C and examined at 24 and 48 h. Pure subcultures were obtained from single colonies on blood agar and processed for Gram staining, catalase and oxidase testing, and biochemical identification using the VITEK® 2 Compact system (BioMérieux, Marcy-l'Étoile, France), following the manufacturer's instructions.

Antimicrobial susceptibility testing for ten antimicrobials was performed by determining the minimum inhibitory concentration (MIC) for *E. rhusiopathiae* isolates obtained from bone marrow using Sensititre™ AVIAN1F Vet AST plates (Thermo Fisher Scientific, Waltham, MA, USA), as previously described by Chang *et al*. (2024). Results were interpreted according to CLSI document VET06, 1st edition, *Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated from Animals* (CLSI, 2017), and relevant published literature (Dec *et al*., 2023; Dec *et al*., 2024).

To exclude Newcastle disease virus (NDV) and avian influenza virus (AIV) infections, spleen samples were aseptically collected and homogenized in 10% phosphate-buffered saline (PBS; pH 7.2) supplemented with 200 U/mL penicillin and 0.2 mg/mL streptomycin. Homogenates were vortexed for 10 s, subjected to three freeze–thaw cycles, and centrifuged at 3000 × g for 20 min at 4 °C. The supernatant was tested using a standard hemagglutination assay (Alexander, 2000). For histopathological analysis, spleen samples collected at necropsy were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 µm, mounted on glass slides, and stained with hematoxylin and eosin (H&E) for microscopic examination.

Genomic DNA was extracted from pure cultures using the PureLink™ Genomic DNA Mini Kit (Invitrogen, Waltham, MA, USA). PCR amplification of a gene fragment encoding a capsular biosynthesis–associated polypeptide was performed to confirm the identity of *E. rhusiopathiae*, following the protocol described by Shimoji *et al*. (1998). Serotyping was conducted using multiplex PCR targeting serovars 1a, 1b, 2, and 5, as previously described by Shiraiwa *et al*. (2018). PCR products were resolved on a 1% agarose gel stained with GelRed® (Millipore, Burlington, MA, USA) and visualized using a TransLum SOLO transilluminator (Biotop, Jing'an District, Shanghai, China). Primer sequences are listed in Table 1.

Table 1.
Primers used in this study.

Primer	Sequence (5' to 3')	PCR Product (bp)	Reference
ER1	CGA TTATATTCTTAGCACGCAACG	937	Shimoji *et al*., 1998
ER2	TGCTTGTGTTGTGATT TCTTGACG		
1a-F	CTCCTAACGCTTTAGCACGC	356	Shiraiwa *et al*., 2018
1a-R	TGATCCTTTGCCACTAATGC		
1b-F	CGAAAGCATCCCTGTAATCAGTTGC	1.357	
1b-R	TGCGTGTAAAACCTGATCGTGTAAATC		
2-F	CCACGTCTTCCCACACTACAAAAAAGTAAATTC	541	
2-R	TCATCCTAATGCATATCATTATGTGGATATGAA		
5-F	GCACGTTTCCAAATATTGTATCGAGTCT	194	
5-R	GAAATAATGCCGATAGATGGAGCAC		

RESULTS AND DISCUSSION

At necropsy, the lesions were consistent with a systemic infection and included generalized congestion of skeletal muscles (8/11) and internal organs (9/11), regression of ovarian follicles (11/11), hepatomegaly with multifocal hemorrhages (9/11), friable hepatic texture (9/11), pale foci of variable size (7/11), and marked splenomegaly characterized by a dark, enlarged spleen (11/11) (Figure 1). No valvular endocarditis or respiratory lesions were observed. These findings are consistent with previously reported descriptions of systemic erysipelas in poultry, including generalized congestion, hepatomegaly, splenomegaly, and acute regression of preovulatory ovarian follicles (Eriksson *et al*., 2025). Nevertheless, a recent case in broiler chickens demonstrated an atypical presentation characterized by swollen head syndrome associated with *E. rhusiopathiae* infection (Shamoun *et al*., 2023), supporting the notion that this pathogen is re-emerging in poultry and may produce diverse clinical manifestations.

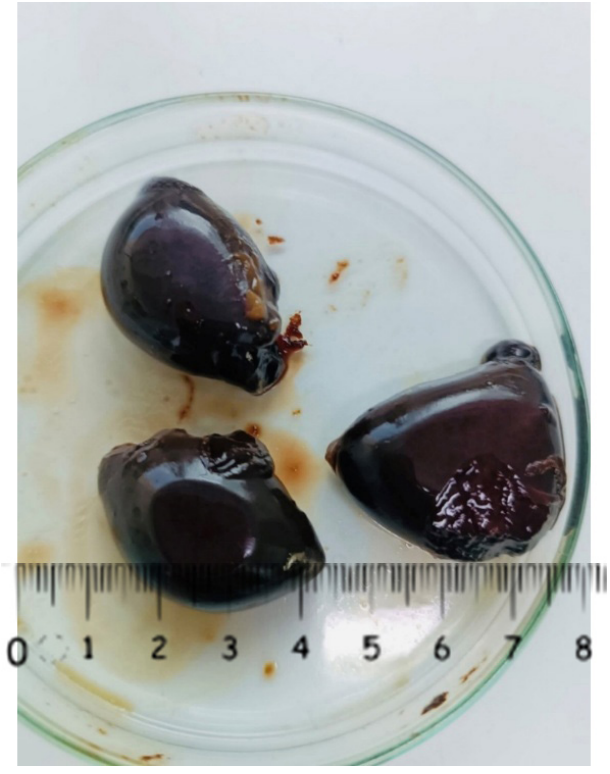
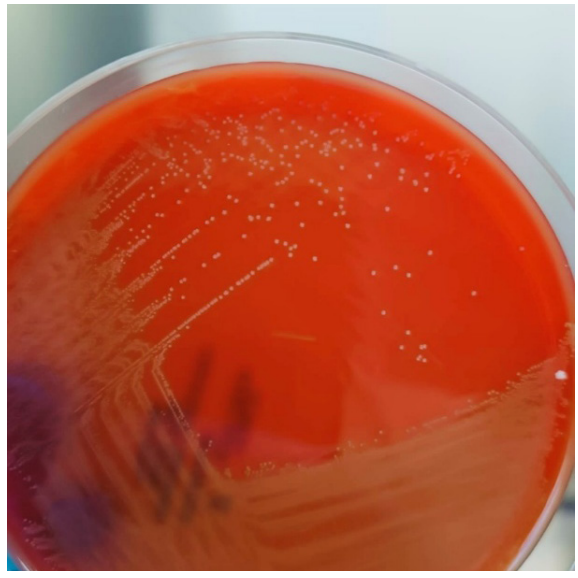


Figure 1.
Enlarged, swollen, dark spleens removed from laying hens with erysipelas.

From eight samples, small, round, greenish colonies measuring 1–2 mm in diameter were recovered on blood agar plates, exhibiting alpha-hemolysis (Figure 2a). These colony characteristics were consistent with those of *E. rhusiopathiae*. No bacterial growth was observed on MacConkey agar. Colonies compatible with *Escherichia coli* or *Pasteurella multocida* were not observed, thereby ruling out systemic bacterial diseases, such as avian colibacillosis and fowl cholera. Catalase and oxidase tests were negative in all eight samples, as expected for *E. rhusiopathiae*. Gram staining revealed Gram-positive, slender rods that tended to decolorize, consistent with this species (Figure 2b). Identification using the Vitek® 2 Compact system confirmed the isolates as *E. rhusiopathiae* with 99% accuracy. No growth of other bacterial pathogens was observed.

(a)



(b)

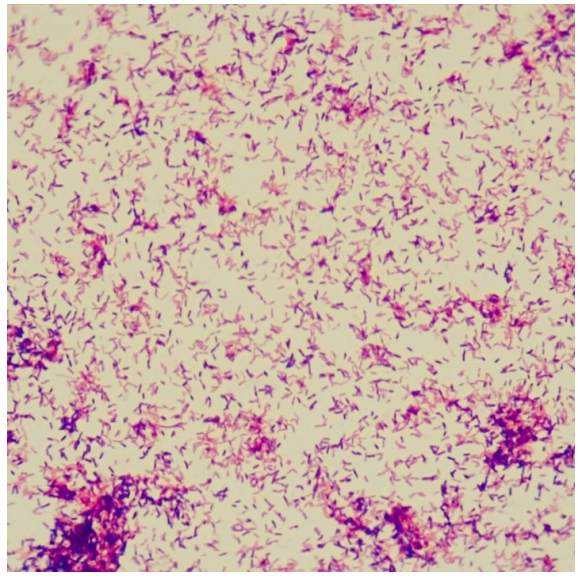


Figure 2.
Isolation of *E. rhusiopathiae*. (a) Small round of 1-2 mm, green-colored alpha hemolysis on blood agar plates. (b) Magnification, 100X. Gram-positive, rod-shaped bacteria that tended to decolorize.

Testing for NDV and AIV using a hemagglutination assay yielded negative results, ruling out systemic viral diseases. The absence of other primary viral pathogens further supports *E. rhusiopathiae* as the primary etiological agent responsible for the observed lesions and increased mortality in this study.

Microscopic examination revealed severe disruption of the splenic architecture, characterized by marked lymphoid depletion within follicles, resulting in a substantial reduction in the white pulp throughout the parenchyma. Multiple foci of fibrinoid necrosis associated with blood vessels were also observed, along with severe vascular congestion, findings consistent with a systemic bacterial infection such as erysipelas (Eriksson & Swayne, 2020; Eriksson *et al*., 2025, Figure 3).

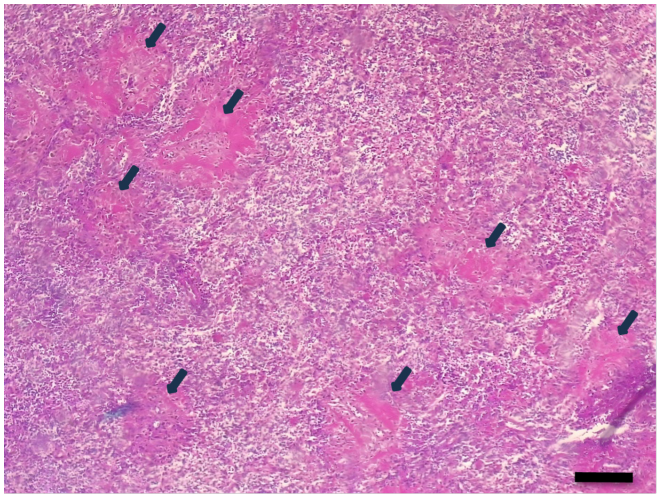


Figure 3.
Magnification, 10X. Histopathology of the spleen showing disruption of splenic histoarchitecture in a hen with erysipelas. Multiple foci of fibrinoid necrosis associated with the blood vessels were observed (black arrows). H&E. Bar = 200µm.

PCR amplification of a 937 bp fragment confirmed the identity of *E. rhusiopathiae*. Multiplex PCR further classified the strain as serotype 1b based on the amplification of a 1357 bp fragment (Figure 4). Considering the farm history, gross lesions, histopathological findings, biochemical identification, and molecular confirmation, we diagnosed an outbreak of erysipelas caused by *E. rhusiopathiae* in a cage-free commercial layer farm in Chile.

The *E. rhusiopathiae* isolate analyzed was susceptible to amoxicillin (≤ 0.25 µg/mL), penicillin (≤ 0.06 µg/mL), ceftiofur (≤ 0.25 µg/mL), florfenicol (8 µg/mL), and erythromycin (≤ 0.12 µg/mL). Intermediate susceptibility was observed for clindamycin (≤ 0.5 µg/mL), whereas resistance was detected against enrofloxacin (> 2 µg/mL), tylosin (≤ 2.5 µg/mL), oxytetracycline (> 8 µg/mL), and tetracycline (> 8 µg/

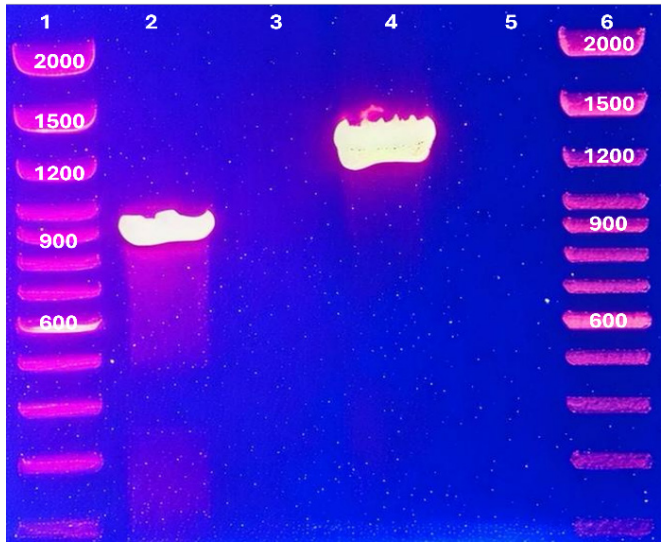


Figure 4.
Detection and serotyping of *Erysipelothrix rhusiopathiae* using PCR. Lane 1: 100 bp DNA ladder; Lane 2: *E. rhusiopathiae* identification amplicon; Lane 3: negative control for identification PCR; Lane 4: Multiplex PCR amplicon for serotyping; Lane 5: negative control for multiplex PCR; Lane 6: 100 bp DNA ladder.

mL). As previously described, *E. rhusiopathiae* is intrinsically resistant to aminoglycosides, vancomycin, sulfonamides, trimethoprim, and polymyxins (Datri *et al*., 2023). These findings indicate that the isolated strain exhibits resistance to three distinct classes of antimicrobials—fluoroquinolones, macrolides, and tetracyclines— thereby meeting the criteria for classification as a multidrug-resistant (MDR) strain. Despite this, the isolate remained highly susceptible to β-lactam antibiotics, including penicillin, ampicillin, and ceftiofur, consistent with previous studies (Dec *et al*., 2023; Dec *et al*., 2024; Chang *et al*., 2024; Kerek *et al*., 2025). Accordingly, β-lactams remain the drug of choice for treating erysipelas. The outbreak was successfully controlled after seven days of therapy using amoxicillin administered via drinking water at 15 mg/kg body weight daily for five days, consistent with the results of the antimicrobial susceptibility test. However, emerging penicillin resistance reported in field isolates (Hess *et al*., 2023; Bobrek & Gawel, 2023) raises concerns regarding the future reliability of β-lactams as first-line agents. The resistance to enrofloxacin, tylosin, tetracycline, and oxytetracycline observed in this study aligns with previous studies (Dec *et al*., 2024; Chang *et al*., 2024; Kerek *et al*., 2025). Together, these resistance patterns narrow the range of effective therapeutic options during outbreaks and underscore the need for ongoing antimicrobial susceptibility monitoring based on a broader isolate collection.

Each of today's poultry housing systems, including conventional cages, enriched cages, cage-free or free-range

systems, pasture systems, multitiered aviaries, mobile poultry units, and organic systems, offers distinct advantages and disadvantages. Cage systems allow efficient space utilization and reduce pathogen transmission but compromise animal welfare because of confinement (McMullin, 2022). In contrast, cage-free or free-range systems prioritize animal welfare and promote natural behaviors; however, they are associated with a greater environmental impact and an increased risk of infectious disease outbreaks. Consequently, the extent to which these systems truly ensure high welfare standards is debatable if birds are periodically affected by preventable infectious diseases. Furthermore, such outbreaks also pose a heightened public health risk (Bist *et al*., 2024).

Erysipelas outbreaks have been documented in nearly all poultry species; however, the most recent cases have involved laying hens, particularly in Europe, where outbreaks have frequently been linked to cage-free systems with suboptimal biosecurity and inadequate control of biological vectors (Eriksson *et al*., 2025). As *E. rhusiopathiae* is a ubiquitous bacterium capable of surviving for extended periods in soil, these production systems inherently pose an elevated risk to both birds and humans (Opriessnig & Coutinho, 2019). Additionally, numerous wild mammalian and avian species can serve as reservoirs of *E. rhusiopathiae* and act as sources of infection in poultry (Wang *et al*., 2010). Therefore, free-range or cage-free systems without effective access control substantially increase the risk of diseases, such as erysipelas. Elevated biosecurity standards are recommended for organic farms implementing these systems, including robust hygienic barriers and efficient biological vector control (Eriksson *et al*., 2013).

To date, 17 serotypes of *E. rhusiopathiae* have been described. In the present case, the strain was identified as serotype 1b, which has also been reported in isolates from aquatic birds (Dec *et al*., 2024), pigs (Morimoto *et al*., 2022), and laying hens (Hess *et al*., 2023). This cross-species distribution highlights the ability of *E. rhusiopathiae* to circulate among diverse animal hosts, underscoring the potential for interspecies transmission and the need for comprehensive surveillance across production systems and wildlife reservoirs.

Several commercial vaccines, primarily based on serotypes 1a or 2, are available to protect pigs and poultry (Morimoto *et al*., 2022). The serotype identified in the present case was 1b, and the affected birds had not been vaccinated against erysipelas, as vaccination is not routinely implemented in laying hens. When commercial vaccines are unavailable, autogenous vaccines represent a viable alternative for preventing future outbreaks (Opriessnig *et al*., 2020). Although the use of autogenous vaccines is restricted to the farm from which the microorganism was isolated, vaccinating subsequent pullet flocks against erysipelas, either prior to or upon arrival at the facility, is advisable (Opriessnig *et al*., 2020). Despite biosecurity measures, a defining characteristic of *E. rhusiopathiae* is its ability to persist in soil and farm environments, creating the potential for disease re-emergence in future flocks (Eriksson *et al*., 2014).

To our knowledge, this is the first documented case of *Erysipelothrix rhusiopathiae* infection in laying hens in Chile. However, the present study had several important limitations. The small number of isolates precluded drawing robust conclusions about the circulating strains. The absence of genomic sequencing limits the ability to assess genetic diversity, virulence factors, antimicrobial resistance determinants, and potential epidemiological links to strains reported worldwide. Similarly, the lack of pathogenicity assessment restricts the interpretation of the clinical relevance and potential impact of the detected strain. Collectively, these limitations underscore a critical gap in national surveillance. Despite these constraints, this study provides an important contribution to the understanding of erysipelas in Chile, documenting its emergence in cage-free laying flocks and offering the first microbiological, histopathological, and molecular confirmation of an outbreak in the country. The identification of serotype 1b highlights the need to consider cross-species transmission dynamics and aligns Chile with global epidemiological patterns.

CONCLUSIONS

To properly evaluate the emergence and dissemination of erysipelas within the Chilean poultry sector, particularly in cage-free systems where the risk is inherently higher, active and systematic surveillance is urgently required. Increasing the number of recovered isolates will allow for a more comprehensive characterization of circulating serotypes and genomic lineages, support evidence-based prevention and control strategies, and strengthen diagnostic capacity for early detection and response to future outbreaks.

As a re-emerging disease in poultry, erysipelas represents a growing concern in the poultry industry. Organic farms and operations employing cage-free or free-range systems should implement active surveillance, reinforce biosecurity measures to prevent the introduction and spread of *E. rhusiopathiae*, reduce the risk of human exposure, and consider integrating erysipelas vaccination into routine health programs. These actions would help reconcile animal welfare objectives with production systems that maintain appropriate sanitary conditions.

DECLARATIONS

Acknowledgements
This study was funded by the Avian Pathology Laboratory, Department of Animal Pathology, Faculty of Veterinary and Animal Sciences, Universidad de Chile.

Conflict of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Authors' contributions

Conceptualization: L.C., F.N., and H.H.; methodology: L.C. and F.N.; investigation: L.C. and H.H.; writing—original draft preparation: L.C.; writing—review and editing: L.C.; F.N.; and H.H. All authors have read and agreed to the published version of the manuscript.

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