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Isolation of *Serratia rubidaea* from a mixed infection after a horse bite

MIRTA R. LITTERIO[1]*, SOLANGE ARAZI[2], CLAUDIA HERNÁNDEZ[1], HORACIO LOPARDO[1]

Servicios de [1]Microbiología y [2]Control Epidemiológico e Infectología, Hospital de Pediatría "Prof. Dr. Juan P. Garrahan". Combate de los Pozos 1881 (1245) Ciudad Autónoma de Buenos Aires, Argentina.
**Correspondence. E-mail: mirtalitterio@gmail.com*

ABSTRACT

Horse bite infections are very rarely reported in the medical literature. Here we present a case of a severe facial infection in a 2-year-old boy after a horse bite, from which *Serratia rubidaea* and *Enterobacter cloacae* were isolated. Some pieces of grass were found inside the wound and were removed before performing a surgical toilet. The presence of these two gram-negative bacteria associated with a horse bite infection, as well as other organisms such as anaerobes, *Pseudomonas*, gram-positive cocci, *Actinobacillus* spp., previously described in other works, should be taken into account when selecting the antibiotics for prophylactic treatment of farm animal bites.

Key words: *Serratia rubidaea,* horse bite, pediatrics

RESUMEN

Aislamiento de *Serratia rubidaea* de una infección mixta posterior a la mordedura de un caballo. Los casos de infecciones por mordedura de caballo son muy infrecuentemente comunicados en la literatura. Aquí se presenta un caso de infección facial grave posterior a la mordedura de un caballo en un niño de 2 años. De esa herida se aislaron *Serratia rubidaea* y *Enterobacter cloacae*. Algunos trozos de pasto se encontraron dentro de la herida y se extrajeron antes de realizar la limpieza quirúrgica. La presencia de esos dos bacilos gram negativos como microorganismos asociados a una infección posterior a una mordedura de caballo, del mismo modo que la de otros microorganismos descritos en la literatura (anaerobios, *Pseudomonas*, cocos gram positivos, *Actinobacillus* spp.), debe tomarse en cuenta a la hora de elegir antibióticos para realizar un tratamiento profiláctico de pacientes que sufran una mordedura por animales de granja.

Palabras clave: *Serratia rubidaea,* mordedura, caballo, pediatría

Animal bites frequently cause only minor injury in humans. Only 1 to 2 % of victims require hospitalization. Most of these injuries are produced by dogs and cats because they share their habitat with humans (6). Infection rates are variable depending on the animal, the nature of the wound, the treatment and the delay in seeking medical care. Most primary infections are polymicrobial, reflecting the oral microbiota of the animal. However, few species may penetrate into deep tissues, including bones and joints, producing bacteremia. Horse bite infections are very rarely reported in the medical literature.

Here we present a case of severe facial infection in a 2-year-old boy after a horse bite, from which *Serratia rubidaea* and *Enterobacter cloacae* were isolated.

A previously healthy 2-year-old boy was bitten on his left cheek by a horse. He was admitted to hospital, where a surgical toilet was performed. Some pieces of grass were found inside the wound and removed before performing the toilet. The wound was sutured and prophylactic ampicillin-sulbactam was administered. Rabies and tetanus vaccines were applied. The patient was discharged on oral amoxicillin-clavulanic acid treatment. Three days later, he was readmitted due to worsening of the wound, around which swelling and erythema were observed. A purulent discharge in the absence of fever subsequently appeared. This purulent material was removed and cultured at the microbiology laboratory. Another surgical toilet followed by suturing was performed and the patient was discharged again on oral amoxicillin-clavulanic acid (50 mg/kg/d). Outcome was good and the patient was followed-up by the department of plastic surgery.

Following the current guidelines of our laboratory for the initial procedures of samples obtained by punction-aspiration or biopsy, the purulent material from the wound was cultured in the following media: chocolate agar incubated at 35 °C in 5 % CO_2, sheep blood agar and thioglycolate broth both incubated in air, anaerobic 5 % laked blood agar and brain heart infusion, supplemented with vitamin K, hemin, and yeast extract in anaerobiosis. Two different isolates (*E. cloacae* and *S. rubidaea*) were observed in anaerobic conditions, while only one of them (*E. cloacae*) was isolated in aerobically incubated media. Identification was performed by conventional tests, api20E and Vitek 1 (bioMèrieux, Argentina). *E. cloacae* and *S. rubidaea* were identified using all three methods. Key tests to suspect *Serratia* spp. were: DNase positive, gelatinase positive and lipase positive in egg-yolk agar media. The strain did not show pigment and produced acid from adonitol, L-arabinose and D-arabitol. Vitek 1 (biochemical profile number 7624757230, 99 % probability) and API 20E (biochemical profile number 120716357, 88 % probability), confirmed our initial suspicion. Our results were also confirmed by the Argentinean Reference Center of Bacteriology (Servicio de Bacteriología Especial, INEI-ANLIS "Dr. Carlos G. Malbrán").

Susceptibility tests were performed with the disk diffusion method following the CLSI guidelines (2) and with the Etest (bioMèrieux, Argentina). The organism was susceptible to colistin, ampicillin, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, cefoxitin, cefotaxime, cefepime, ceftazidime, imipenem, meropenem, gentamicin, amikacin, aztreonam, ciprofloxacin, trimethoprim-sulfamethoxazole, and resistant to cephalotin.

Susceptibility to colistin initially determined using the disk diffusion method (14 mm) was confirmed with the Etest (0.5 µg/ml).

S. rubidaea is unusually recovered from clinical samples in microbiology laboratories. The pieces of grass inside the wound may be the source of *S. rubidaea* considering its ability to colonize vegetables. We would like to highlight the isolation of this organism for the following reasons: 1) its infrequent occurrence in samples from human infections, 2) because microbiologists should be aware that animal bite infections may be due not only to oropharyngeal-colonizing organisms, but also to skin-colonizing organisms or, as in the present case, to organisms found in the environment, and 3) the unusual susceptibility of the *S. rubidaea* isolate to colistin, a drug that showed to be ineffective against organisms of the genus *Serratia* with the exception of *Serratia fonticola* (12).

The literature cited in PubMed related to horse bite infections was reviewed and 42 references were found. Only seven of them described cases of patients bitten by horses. The infection was due to mixed organisms in four cases, *Actinobacillus* species were involved in three cases, whereas *Enterobacteriaceae* were present in four (including the present report). Only two cases were due to *Pasteurella* spp., anaerobic bacteria were isolated in only one case, and two species of viridians streptococci were found (1, 3-5, 7, 9, 10) (Table 1) in another case. Two other cases involving both a donkey and a zebra, were reported (Table 1). One reported a case of infection due to *Streptococcus agalactiae* and *Pseudomonas aeruginosa* after a zebra bite (13). The other was a case of *Staphylococcus hyicus* infection after a donkey bite (8).

Actinobacillus lignieresii and other *Actinobacillus* spp., gram-positive aerobic cocci, *Pseudomonas, Enterobacteriaceae*, *Pasteurella,* and anaerobic bacteria could be the causal agents of infections after horse bites.

The genus *Serratia* includes several species that are seldom a cause of primary infections, being instead frequently involved in nosocomial human infections. *S. rubidaea*, a red-pigmented species, has been mainly described as an environmental colonizer. The habitats of *S. rubidaea* are not well known. It has been isolated from coconuts and vegetables but not from water, insects, or other animals (11). Its clinical significance in human infections cannot be excluded since it has been reported in association with some cases of invasive diseases (11). As animal bite wounds may be infected by bacteria colonizing the victim's skin, by organisms living in the offender's mouth, as well as by those coming from the environment, we suspect that the grass extracted from the wound could have been the source of this organism.

Susceptibility to amoxicillin and resistance to first- and second-generation cephalosporins have previously been described for this species (12).

Taking into account that the genus *Serratia* is almost uniform in its natural resistance to colistin, we highlight the susceptibility to colistin of this isolate. A large study describing natural antimicrobial susceptibilities of unusual *Serratia* species failed to include data about colistin or polymyxin B (12). As information about natural resistance to colistin in *S. rubidaea* is lacking, we are not able to determine if this case was an exception or if *S. rubidaea* is naturally susceptible to polymyxins.

The good outcome of the patient in the present work in spite of using an ineffective drug for one of the two isolated pathogens (amoxicillin-clavulanic acid) could be explained either by the fact that the patient underwent extensive surgical toilet or because *E. cloacae* would only be colonizing the zone around the wound.

The presence of these two gram-negative bacteria as organisms associated with a horse bite infection, as well as other organisms previously described in other works (anaerobes, *Pseudomonas*, gram-positive cocci, *Actinobacillus* spp.) (Table 1), should be taken into consideration when selecting the antibiotics to be use in the prophylactic treatment of farm animal bites.

Table 1. Wound infections produced by horses and similar animals

Animal	Bacteria	Age and sex	Underlying disease	Reference
Horse	*Streptococcus anginosus* + *Streptococcus mutans*	ND (1)	ND	Marrie *et al.* 1979 (7)
Horse	*Actinobacillus lignieresii* + *Escherichia coli* + anaerobes	13 y, M	No	Dibb *et al.* 1981 (4)
Horse	*Pasteurella* sp.	ND	ND	Dibb *et al.* 1981 (3)
Horse	*Yersinia* sp.	ND	ND	Räisänen *et al.* 1989 (10)
Horse	*Actinobacillus lignieresii* + *Actinobacillus equuli*-like	22 y, M	No	Peel *et al.* 1991 (9)
Horse	*Actinobacillus suis* + *Escherichia coli*	35 y, M	No	Peel *et al.* 1991 (9)
Horse	*Actinobacillus lignieresii*	59 y, M	No	Benaoudia *et al.* 1994 (1)
Horse	*Pasteurella caballi*	56 y, M	ND	Escande *et al.* 1997(5)
Donkey	*Staphylococcus hyicus* subsp. *hycus*	20 y, F	No	Osterlund *et al.* 1997 (8)
Zebra	*S. anginosus* + *Pseudomonas aeruginosa*	44 y, F	No	Toovey *et al.* 2004 (13)
Horse	*Serratia rubidaea* + *Enterobacter cloacae*	2 y, M	No	This study

(1) ND: not determined

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