

RAPID COMMUNICATION

Outbreak of Swine Erysipelas in a Semi-Intensive Wild Boar Farm in Spain

D. Risco¹, P. F. Llario², R. Velarde³, W. L. García¹, J. M. Benítez¹, A. García¹, F. Bermejo¹, M. Cortés¹, J. Rey¹, J. H. de Mendoza¹ and L. Gómez⁴

¹ Unidad de Patología Infecciosa, Departamento de Sanidad Animal, Facultad de Veterinaria, UEX, Cáceres, Spain

² Unidad de Biología y Etología, Departamento de Anatomía, Biología Celular y Zoología, Facultad de Veterinaria, UEX, Cáceres, Spain

³ Servei d'Ecopatologia de Fauna Salvatge, Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain

⁴ Unidad de Histología y Anatomía Patológica, Departamento de Medicina Animal, Facultad de Veterinaria, UEX, Cáceres, Spain

Keywords:

Erysipelothrix rhusiopathiae; wild boar; swine erysipelas; panuveitis; vasculitis

Correspondence:

D. Risco. Unidad de Patología Infecciosa, Departamento de Sanidad Animal, Facultad de Veterinaria, UEX. Avda. Universidad sn. C.P. 10003 Cáceres, Spain.
Tel.: +34927257114;
Fax: +34927257110;
E-mail: riscope@unex.es

Received for publication December 1, 2010

doi:10.1111/j.1865-1682.2011.01234.x

Summary

Swine erysipelas (SE) is a disease caused by the bacterium *Erysipelothrix rhusiopathiae* and is one of the best-known and most serious diseases affecting domestic pigs. However, few studies exist concerning the susceptibility of wild boars to this disease and the role of this species as a reservoir. This study investigates and describes an outbreak of SE that occurred on a semi-intensive wild boar breeding farm housing 40 boars in Extremadura (SW Spain) on 11–18 February 2010. Seven animals died, of which four were examined post-mortem. Of these, three (two females and one male) were approximately 3 months old, and one was 1 year old (male). Lesions were consistent with acute septicaemia, consisting of cutaneous erythema/cyanosis and petechial haemorrhages in kidneys, urinary bladder, lungs and meninges. The 1-year-old male also had proliferative polyarthritis. Histopathology confirmed the presence of disseminated intravascular coagulation and vasculitis. Additionally, a bilateral acute panuveitis with concurrent necrotizing vasculitis and diffuse corneal oedema, neither of which have been described before in this disease, were found in the 3-month-old male boar. *E. rhusiopathiae* was isolated from all four animals in pure cultures from several tissues. Of these four animals, antibodies against *E. rhusiopathiae*, using an indirect ELISA test, were only detected in the 1-year-old male boar with polyarthritis. Posteriorly, of nine live adults tested for antibodies, four (including an adult male with polyarthritis) were positive.

Introduction

Swine erysipelas (SE) is a disease found all over the world that has an important economic impact on pig farming (Wood and Henderson, 2006). The cause of SE is *Erysipelothrix rhusiopathiae* (*E. rhusiopathiae*), a small Gram-positive, facultative anaerobic, non-spore-forming and non-acid-fast bacillus (Brooke and Riley, 1999). Although SE is the best-known disease caused by *E. rhusiopathiae*, this microorganism also causes disease in other domestic animals, particularly in turkeys and sheep (Wang et al., 2010). This pathogen is also a zoonotic agent, and infec-

tions in humans may result in cutaneous erysipeloid lesions, endocarditis and septicaemia (Brooke and Riley, 1999).

The source of infection in an outbreak of erysipelas is seldom known. The most important reservoir of *E. rhusiopathiae* is domestic pigs, which may act as apparently healthy carriers. Up to 10–50% of healthy swine harbour the organism in their oropharynx (tonsils) (Okolo, 1986; Takahashi et al., 1987), and infected or subclinically diseased animals act as a source from which other individuals can be infected. Bacterial shedding has been demonstrated to occur in nasal secretions, saliva and

faeces in both healthy and sick pigs (Laber et al., 2002). Nevertheless, pigs are not the only reservoir of *E. rhusiopathiae*, and over 30 species of wild birds and at least 50 species of wild mammals are also known to harbour this pathogen (Wang et al., 2010).

The most common routes of infection are via the ingestion of contaminated food or water and skin wounds (Laber et al., 2002). Pigs between 3 months and 3 years old are most susceptible to erysipelas (Wood and Henderson, 2006), and there is evidence to suggest that environmental and stress factors can predispose animals to the disease. For example, sudden changes in nutrition, environmental temperature and fatigue are commonly linked to the appearance of SE (Wood and Henderson, 2006). As well, parasitic infestations have been reported to increase the severity of clinical SE (Wood and Henderson, 2006).

Three clinical forms of SE have been described in the domestic pig: acute, subacute and chronic (Conklin and Steele, 1979). The acute form begins with sudden deaths in the group and lesions consistent with septicaemia such as diffuse areas of cutaneous erythema/cyanosis or the appearance of well-defined rhomboid-shaped red-purple lesions. In the subacute forms, pigs do not usually show any signs of disease, while chronic forms are characterized by the appearance of arthritis and endocarditis (Wood and Henderson, 2006; Wang et al., 2010).

Domestic pigs and wild boar belong to the same species and are susceptible to similar types of pathogens (Yamamoto et al., 1999), including *E. rhusiopathiae* (Lipowski, 2003). In Spain, serological studies indicate that 5% of wild boars possess antibodies against *E. rhusiopathiae* (Vicente et al., 2002, Closa-Sebastià et al., 2011), although cases of SE in this species have yet to be fully described. The isolation in Japan of *E. rhusiopathiae* from farmed wild boars with acute septicaemic erysipelas was mentioned in a study by Yamamoto et al. (1999), but the lesions were not described.

Currently, many wild boar populations in south-central Spain are subject to management practices, such as fencing, feeding and translocation. These measures may lead to both a rise in wild boar population densities and potentially an increase in the risk of exposure to infectious pathogens (Gortazar et al., 2006). As farmed wild boar numbers and stocking rates increase, diseases such as SE may well assume greater economic importance in areas such as Japan (Yamamoto et al., 1999). Infected wild boars may become a hazard for the domestic pig-rearing industry, as this disease could be transmitted from one population to another. Furthermore, the presence of SE in wild boars could become a public health hazard because of its zoonotic character (Wang et al., 2010).

This work describes an outbreak of SE in a semi-intensive wild boar breeding farm in Spain and is accompanied by a pathological and microbiological study of the affected animals.

Materials and Methods

Study site

The outbreak was declared in a wild boar population located in Puebla de la Reina (NE Badajoz Province, Extremadura, SW Spain). This area has a continental thermo-Mediterranean climate, with hot dry summers and mild and moderately wet winters. The vegetation consists mainly of scrubland (genus *Cistus* sps, e.g. *C. ladanifer*) and evergreen oak forests (*Quercus suber*). February 2010, the month in which the outbreak occurred, was cold and extremely wet. According to the Spain's State Meteorological Agency (AEMET) of the Ministry of Environment and Rural and Marine Affairs, the mean temperature and rainfall in February were 9.8°C and 166.6 l per square metre in 2010 compared to historic values of 10.1 and 54.2, respectively.

The home range of this population is surrounded by a fence and divided into two very different parts. The first consists of a 'free-ranging area' of about 500 ha in which approximately 120 wild boars live with almost no restrictions on their mobility and feed naturally. This population is composed mostly of 1- to 2-year-old boars (70%); the remaining animals are older. There are similar numbers of males and females because the area is not hunted and the birth/sex ratio, which is about 50 : 50, is maintained. These data have been obtained through an analysis of the photographs taken by four cameras (HCO Scoutguard SG550-V Camo; HCO Outdoor Products, Norcross, GA, USA) located near drinking points. The cameras are checked on a weekly basis.

The second area consists of a fenced-off enclosure of about 5 ha within the first area with physical barriers that prevent animals from dispersing. This area is a semi-intensive breeding farm, although no barn or any other kind of shelter is provided for the boars. The outbreak occurred in this second area where at the time there were 40 animals, 28 adults, mostly 1–3 years old (20 sows and eight boars) and 12 young animals between 3 months and 1 year old (seven males and five females). Within this area, there are three other small fenced-off areas with a system of coordinated doors to allow the separation and manipulation of the animals when required. In these small fenced-off areas, there are several feeders. Supplementary food is provided every day in this area and is based on commercial fodder specifically designed for this species and these age groups. Young animals are fed a growth-promoting diet (JB-2, Iniciativas Alimentarias S.A.,

INALSA, Torralba de Calatrava, Ciudad Real, Spain), which is provided in feeders that are designed exclusively for the young animals and which the adults cannot access. Adult animals have a maintenance diet (JB-4, Iniciativas Alimentarias S.A., INALSA, Torralba de Calatrava), which is also supplied in separate feeders. All animals are identified with plastic ear-tags when they are 3 months old.

Only the officially recommended vaccines are given to the pigs in the farm. All animals were vaccinated against Aujeszky's disease with a commercial inactivated vaccine (Aukipra-BK, Hipra, Amer, Spain). Hygienic conditions were poor in the farm, as there was no systematic plan for the disinfection and disposal of organic waste. Farm workers check the semi-intensive breeding installations every day and carry out maintenance and animal-feeding work.

Clinical signs and treatment

In the week 11–18 February 2010, seven wild boars were found dead by the farm workers next to the feeders. No clinical signs were observed prior to the deaths, and no important health problems had been detected on the farm. The treatment given to the surviving boars consisted of oxytetracyclin (200 ppm) and flubendazole (30 ppm) added to the feed for 10 days starting on 15 February. An adult male wild boar with clinical signs of tarsal and carpal arthritis, observed 3 weeks after the beginning of the outbreak, was successfully treated with long-acting penicillin (no attempt was made to isolate bacteria from the affected joints). No further cases were detected on the farm, and 3 months later, all animals were vaccinated with a commercial vaccine (PARVO-SUIN-MR[®], Hipra, Spain).

Pathologic examination

A post-mortem examination was performed on four dead animals, and multiple tissue samples were processed for histopathology. Briefly, tissues were placed in 10% buffered formalin, trimmed and embedded in paraffin, sectioned at 3–4 μm and stained with haematoxylin and eosin. Sections of the urinary bladder, kidney and eye were also stained with Gram's stain.

Bacterial isolation and identification

Tissue samples from liver, spleen, kidney, lung, heart, brain and the synovial membrane of the carpal joint collected during necropsy were cultured on blood agar. The plates were incubated for 24 h at 37°C in aerobic conditions. To identify the microorganisms obtained, Gram's stain and routine biochemical tests (catalase and oxidase tests) were

applied. Identification was confirmed with the use of API Coryne[®] galleries (BioMérieux, Marcy l'Etoile, France).

Antibody detection

A blood sample was collected from the heart of the necropsied animals. Three weeks after the beginning of the outbreak, blood samples were obtained from nine adult animals (five males and four females) in the semi-intensive breeding farm. Blood was collected from the orbital venous sinus (as described in Wood and Henderson, 2006). Samples were centrifuged at 1200 g for 15 min, and the serum was then removed and stored at –20°C until tested for antibodies against *E. rhusiopathiae*. A commercial indirect enzyme-linked immunosorbent assay (ELISA) test was used (Ingezim Mal Rojo; Ingenasa S.A., Madrid, Spain) according to the manufacturer's protocol.

Results

Pathologic examination

Seven of the 40 wild boars housed in the restricted area in the breeding farm died suddenly, of which four were studied. Of these, three (two females and one male) were approximately 3 months old and one was 1 year old (male), and all were in good physical condition. All had lesions consistent with acute septicaemia with diffuse-to-irregular patches of ventral cutaneous erythema and/or cyanosis, generalized internal congestion and petechial-to-ecchymotic haemorrhages in the kidneys, urinary bladder, lungs, pulmonary artery and meninges. The 3-month-old male boar had bilateral corneal opacity (oedema) (Fig. 1), while the 1-year-old boar had a moderate proliferative polyarthritis. A large number of intestinal nematodes such as *Ascaris suum* and *Trichuris suis* were identified in all four animals.

All four boars studied had similar histopathological lesions. Histopathology confirmed the presence of



Fig. 1. Wild boar. Eye. Diffuse corneal oedema.

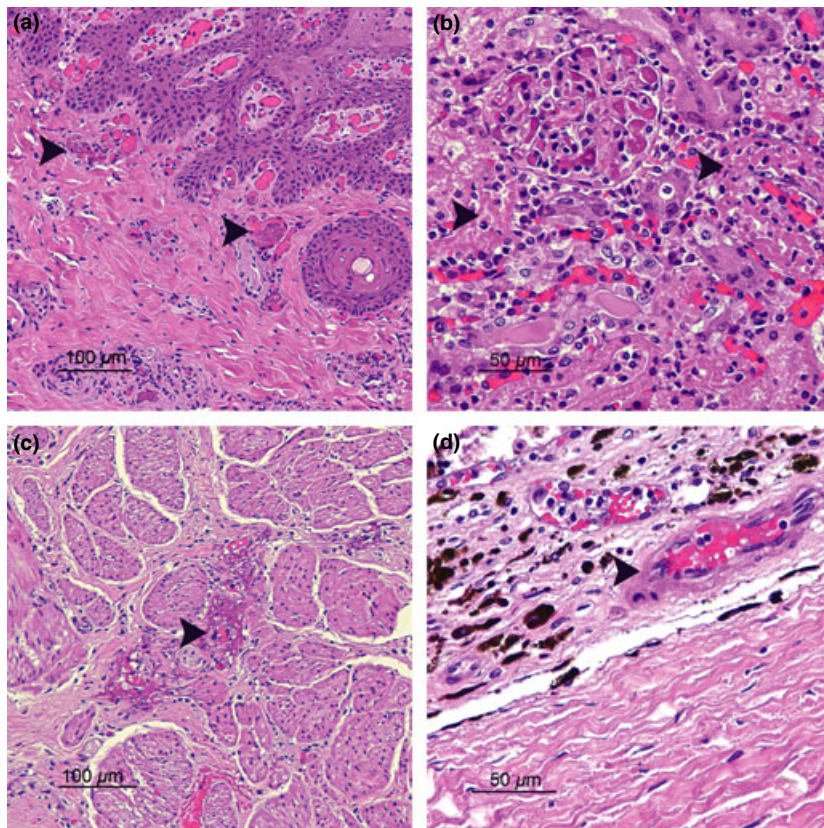


Fig. 2. Septicaemic lesions in a wild boar with swine erysipelas. (a) Skin, marked hyperaemia and fibrin thrombi (arrows); (b) Kidney, fibrin thrombi in glomerular capillaries, tubular necrosis (arrows) with mild lymphocytic interstitial nephritis; (c) Urinary bladder, fibrinoid necrosis of small blood vessels with intravascular bacterial clumps (arrow); and (d) Choroid, fibrinoid necrosis of small blood vessel (arrow). HE stain.

disseminated intravascular coagulation, septic fibrinous microthrombi and vasculitis with swollen endothelial cells invaded by bacteria, along with some leucocytic infiltration and marked fibrinoid necrosis of the vessel walls, intramural haemorrhages, and perivascular fibrin extravasation and haemorrhages. These lesions were especially remarkable in the kidneys, the urinary bladder and on the skin (Figs 2a, b and c). In the kidneys, there were fibrin thrombi in the capillaries of all glomeruli, multifocal interstitial haemorrhages and subacute interstitial nephritis with predominantly mononuclear cells. In the lungs, diffuse congestion, marked interstitial lymphocytic infiltration, thrombosis in small vessels and oedema in the alveoli were the main findings. A few mild mononuclear inflammatory infiltrates were detected in the myocardium, and there were a few foci of fibrinohaemorrhagic necrosis in the spleen. Bilaterally, the ocular lesions in one of the 3-month-old wild boars were consistent with septicaemia, characterized by marked diffuse corneal oedema, diffuse subacute mild panuveitis with mainly mononuclear cells, lymphocytes and plasma cells, and marked fibrinoid vasculitis (Fig. 2d). A circumferential cortical cataract with Morgagnian globules was

also present in both eyes. Gram-stained sections of the urinary bladder, kidneys and ocular globe confirmed the presence of Gram-positive bacteria in the lumen of the vessels and within the cytoplasm of endothelial cells.

Bacterial isolation and identification

A pure growth of tiny grey, alpha-haemolytic, catalase-negative, oxidase-negative colonies consistent with *E. rhusiopathiae* was cultured from all samples (lung, spleen, brain, liver, kidney and heart) taken from all four animals studied, as well as from the carpal synovial membrane of the 1-year-old boar with proliferative arthritis. Microscopic observation showed Gram-positive coccobacilli. These bacteria were identified as *E. rhusiopathiae* using API Coryne[®] galleries.

Antibody detection

In the serum samples from the four dead wild boars, antibodies against *E. rhusiopathiae* were only detected in the 1-year-old male boar with polyarthritis. Of the nine

live adults tested for antibodies, four (including an adult male with polyarthritis) were positive.

Discussion

Although serological studies indicate that at least 5% of free-ranging wild boars have had contact with *E. rhusiopathiae* in Spain (Vicente et al., 2002; Closa-Sebastià et al., 2011), to date, no complete lesional studies of clinical cases of SE in wild boar have been carried out. This article describes an outbreak of SE affecting wild boars in a Mediterranean area. Clinical signs were not observed in any of the animals prior to death, and lesions were consistent with the acute form of the disease in the three 3-month-old wild boars examined and with the chronic form (with proliferative polyarthritis) in the 1-year-old boar. Both macroscopic and microscopic lesions were similar to those described in domestic pigs (Wood and Henderson, 2006). The absence of the characteristic cutaneous lesions ('diamond-skin' lesions) previously described in SE could be due to the acute development of the disease in these wild boars, because these lesions appear as early as the second day (but usually by the third day) after exposure to the microorganism (Wood and Henderson, 2006). These skin lesions were not detected in the rest of the animals on the farm, although if present they would probably have been difficult to see because of the boars' thick hair coat.

Ocular lesions, as seen in one of the 3-month-old wild boars, have never previously been described in cases of SE. In human medical literature, a recent case of bilateral endogenous endophthalmitis was associated with infection by *E. rhusiopathiae* and is thought to be the first report of *E. rhusiopathiae* causing this type of lesion (Elvy et al., 2008). In veterinary literature, two such cases have been described: a horse with bilateral uveitis (Seahorn et al., 1989) and a calf with purulent panophthalmitis with cataracts and focal retinal detachment (Kiluge and Perl, 1992). However, in both of these cases, the animals suffered mixed infections of *E. rhusiopathiae* and *Streptococcus* sp., and the role of *E. rhusiopathiae* in these ocular lesions was not totally clarified. In our case, the presence of vasculitis in the uveal tissue, as seen in other tissues, and the detection of Gram-positive coccobacillus in the vessels suggest that these bacteria were involved as the aetiological agent of the lesions. Corneal oedema occurs under three circumstances, which are not mutually exclusive: corneal ulceration, corneal endothelial dysfunction and vascular leakage from corneal neovascularization (Peiffer et al., 1999). The lack of any peripheral vascularization or evidence of corneal epithelial damage suggests, in our case, that a corneal endothelial injury is the most likely reason for the diffuse corneal oedema. However,

the endothelial layer was not present in the slides, and the mild degree of autolysis prevented us from differentiating between artefactual separation and genuine disappearance.

In our study, antibodies were only detected in the dead 1-year-old boar with lesions consistent with the chronic form of the disease. Three weeks after the beginning of the outbreak, nine of the adults were tested for antibodies, of which four resulted positive. Only one of these animals showed clinical signs of possible active infection with arthritis, but no attempt was made to isolate the bacteria from the joints and the diagnosis remains presumptive. The prevalence of antibodies against *E. rhusiopathiae* before the outbreak in the farm was not known, and so we were unable to differentiate between recent or past contact with the bacteria.

Many of the predisposing factors that may have predisposed these animals to an outbreak of SE such as poor hygienic conditions, extreme temperatures and high rates of parasite infestation (Wood and Henderson, 2006) were present on this farm. Wild boar behaviour such as bathing, which helps control parasites and thermoregulation (Rossell et al., 2001), is probably another risk factor favouring transmission of the infection. It is known that *E. rhusiopathiae* resists for a long time in aquatic environments (Wang et al., 2010), so these communal bathing areas might be important sources of infection.

The appearance of outbreaks of SE on wild boar farms could affect the prevalence of SE in nearby pig farms. The possibility of transmission will be especially high in extensive farms with poor containment measures and in which both wild boar and pigs share the same terrain (Parra et al., 2003). Further surveys aimed at examining whether this pathogen is carried by apparently healthy farmed wild boars, and freshly captured/hunted boars in the wild will help clarify the role of the wild boar as a possible reservoir for this infectious agent.

The presence of wild boars with SE could also represent a hazard for certain human groups (farm workers, hunters, butchers, veterinarians and even by taxidermists). Human infection can arise from contact with infected animals, their secretions or with contaminated material used for carcass (meat) processing (Wood, 1975). The penetration of this microorganism usually occurs through a cut in the skin (McGinnes and Spindle, 1934), although infections caused by the ingestion of contaminated food have also been described (Hunter, 1975).

We can conclude that the lesions found in the studied wild boars with SE did not differ from the acute form of the disease known in domestic pigs, where sudden death and cutaneous erythema are the most common outcomes. However, this study also provides a detailed pathologic description of this disease in farmed wild boars and

represents the first time that *E. rhusiopathiae* has been associated with ocular lesions in suid species.

Acknowledgement

We thank Dr. Brian Wilcock (OVC; University of Guelph) for a thorough revision of the ocular pathology. R. Velarde is supported by the sub-programme *Personal Técnico de Apoyo* of the MICINN (Spain) and the European Social Fund. D. Risco is supported by the programme *Formación del Profesorado* (FPU) of the Spanish Ministry of Education.

References

- Brooke, C.J., and T.V. Riley, 1999: *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *J. Med. Microbiol.* 48, 789–799.
- Closa-Sebastià, F., E. Casas-Díaz, R. Cuenca, S. Lavín, G. Mentaberre, and I. Marco, 2011: Antibodies to selected pathogens in wild boar (*Sus scrofa*) from Catalonia (NE Spain). *Eur. J. Wildl. Res.* (doi 10.1007/s10344-010-0491-9).
- Conklin, R.H., and J.H. Steele, 1979: *Erysipelothrix* infections. In: Steele, J.H. (ed.), *CRC Handbook. Series in Zoonoses*, pp. 327–337. CRC press, Boca Raton.
- Elvy, J., I. Hanspal, and P. Simcock, 2008: A case of *Erysipelothrix rhusiopathiae* causing bilateral endogenous endophthalmitis. *J. Clin. Pathol.* 61, 1223–1224.
- Gortazar, C., P. Acevedo, F. Ruiz-Fons, and J. Vicente, 2006: Disease risks and overabundance of game species. *Eur. J. Wildl. Res.* 52, 81–87.
- Hunter, D., 1975: *The Diseases of Occupations*. English University Press, London.
- Kiluge, J.P., and S. Perl, 1992: *Erysipelothrix rhusiopathiae* septicaemia-polyserositis and streptococcal encephalitis in a calf. *J. Vet. Diagn. Invest.* 4, 196–197.
- Laber, K.E., S.A. Bingel, J.A. Goodrich, A.C. Smith, and M.M. Swindle, 2002: Biology and diseases of swine. In: Fox, J.G., L.C. Anderson, F.M. Loew, and F. Quimby (eds), *Laboratory Animal Medicine*, pp. 956–1005. Department of Comparative Medicine, Medical University of South Carolina, Charleston.
- Lipowski, A., 2003: European wild boar (*Sus scrofa* L.) as a reservoir of infectious diseases for domestic pigs. *Med. Weter.* 59, 861–863.
- McGinnes, G.F., and F. Spindle, 1934: Erysipeloid condition among workers in a bone button factory due to the bacillus of swine erysipelas. *Am. J. Public Health* 24, 32–35.
- Okolo, M.I.O., 1986: Isolation of *Erysipelothrix rhusiopathiae* from apparently healthy pigs reared under intensive and free range systems of management. *Microbios* 47, 29–35.
- Parra, A., P. Fernández-Llario, A. Tato, J. Larrasa, A. García, J.M. Alonso, M. Hermoso De Mendoza, and J. Hermoso De Mendoza, 2003: Epidemiology of *Mycobacterium bovis* infections of pigs and wild boars using a molecular approach. *Vet. Microbiol.* 97, 123–133.
- Peiffer, R.L., B.P. Wilcock, R.R. Dubielzig, J.A. Render, and H.E. Whiteley, 1999: Fundamentals of veterinary ophthalmic pathology. In: Gelatt K.N. (ed.), *Veterinary Ophthalmology* pp. 355–425. Lippincott Williams & Wilkins, Philadelphia.
- Rossell, C., P. Fernández-Llario, and J. Herrerros, 2001: Jabalí (*Sus scrofa* Linnaeus 1758). *Galemys* 13, 1–25.
- Seahorn, T.L., G.W. Brumbaugh, G.K. Carter, and R.L. Wood, 1989: *Erysipelothrix rhusiopathiae* bacteremia in a horse. *Cornell Vet.* 79, 151–156.
- Takahashi, T., T. Sawada, and M. Muramatsu, 1987: Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. *J. Clin. Microbiol.* 25, 536–539.
- Vicente, J., L. León-Vizcaino, C. Gortázar, M.J. Cubero, M. González, and P. Martín-Atance, 2002: Antibodies to selected viral and bacterial pathogens in European wild boars from southcentral Spain. *J. Wildl. Dis.* 38, 649–652.
- Wang, Q., B.J. Chang, and T.V. Riley, 2010: *Erysipelothrix rhusiopathiae*. *Vet. Microbiol.* 140, 405–417.
- Wood, R.L., 1975: *Erysipelothrix* infection. In: Hubbert, W.T., W.F. McCulloch, and P.R. Scurrenberger (eds), *Diseases Transmitted from Animals to Man*, pp. 271–281. Charles C. Thomas Limited, Springfield.
- Wood, R.L., and L.M. Henderson, 2006: Erysipelas. In: Straw, B.E., J.J. Zimmerman, S. D’Allaire, and D.J. Taylor (eds), *Diseases of Swine*, pp. 629–639. Iowa State University Press, Ames.
- Yamamoto, K., M. Kijima, T. Takahashi, H. Yoshimura, O. Tani, T. Kojyou, Y. Yamawaki, and T. Tanimoto, 1999: Serovar, pathogenicity and antimicrobial susceptibility of *Erysipelothrix rhusiopathiae* isolates from farmed wild boars (*Sus scrofa*) affected with septicemic erysipelas in Japan. *Res. Vet. Sci.* 67, 301–303.