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# First report of lesions resembling red mark syndrome observed in wild-caught common dab (*Limanda limanda*)

Maaïke Vercauteren  | Annemie Decostere | Koen Chiers

Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

**Correspondence**

Vercauteren Maaïke Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.

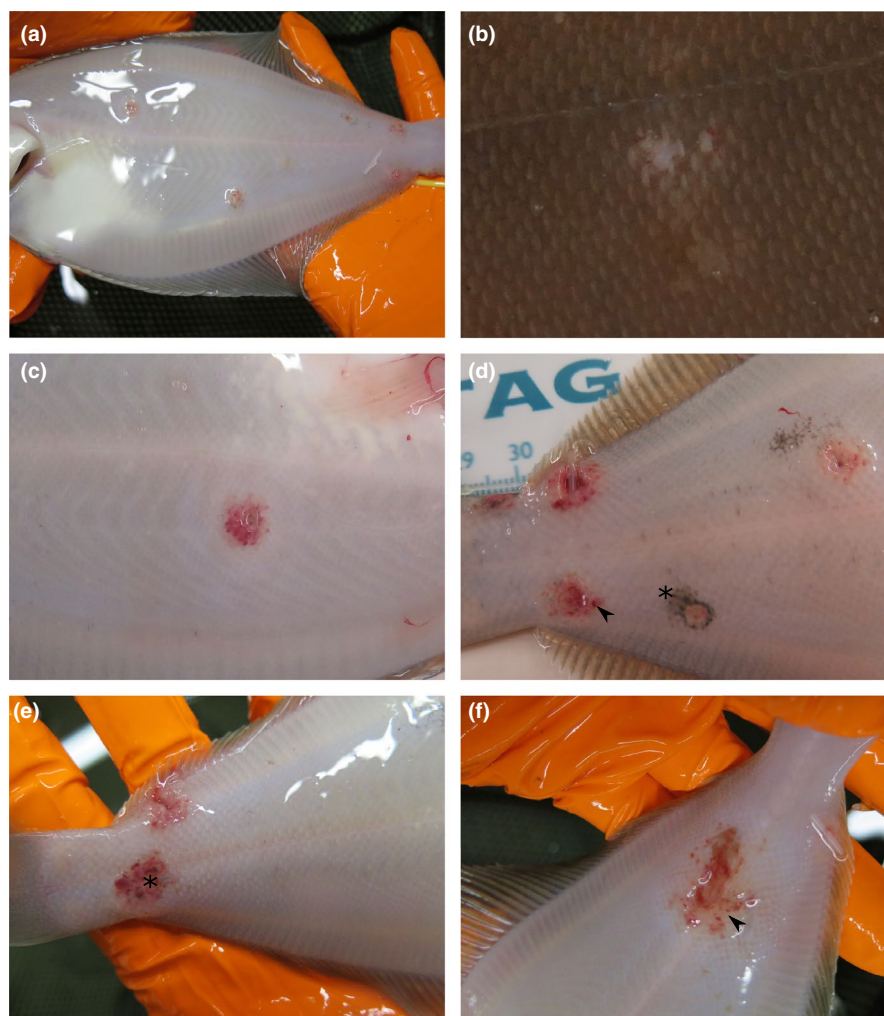
Email: Maaïke.Vercauteren@ugent.be

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**KEYWORDS:** common dab (*Limanda limanda*), dermatitis, red mark syndrome, scale resorptionRed mark syndrome (RMS) or cold water strawberry disease is a skin disease observed in farmed rainbow trout (*Oncorhynchus mykiss*).

The disease was described for the first time in Scotland (Ferguson et al., 2006; Verner-Jeffreys et al., 2006, 2008) and was, thereafter,



**FIGURE 1** Macroscopic lesions encountered during the experiment. (a) Multifocal, raised, irregular skin lesions observed at the start of the experimental trial. (b) Slightly bulging approximately 3-day-old lesion on the pigmented side showing loss of pigmentation. (c) Irregular haemorrhagic skin lesion (at least 15 days old). (d) Occasionally observed pigmentation (\*) or haemorrhages (arrow) around the lesions (at least 15 days old). (e) Ulceration development (\*) in the centre of the lesion (at least 15 days old). (f) Small signs of healing, recognized as reduced haemorrhages, in the lesions found at 21 days [Colour figure can be viewed at [wileyonlinelibrary.com](https://wileyonlinelibrary.com)]

reported in various European countries (Ferguson et al., 2006; Fleury, Vuillaume, & Sochon, 1985; Galeotti, Ronza, et al., 2017), Turkey (Kubilay et al., 2014), Iran (Sasani, Shokrpour, Rahmati-Holasoo, & Zagar, 2016) and Chile (Sandoval et al., 2016). The disease is very similar to UK strawberry disease and might be regarded as the same (Metselaar et al., 2010; Oidtmann et al., 2013).

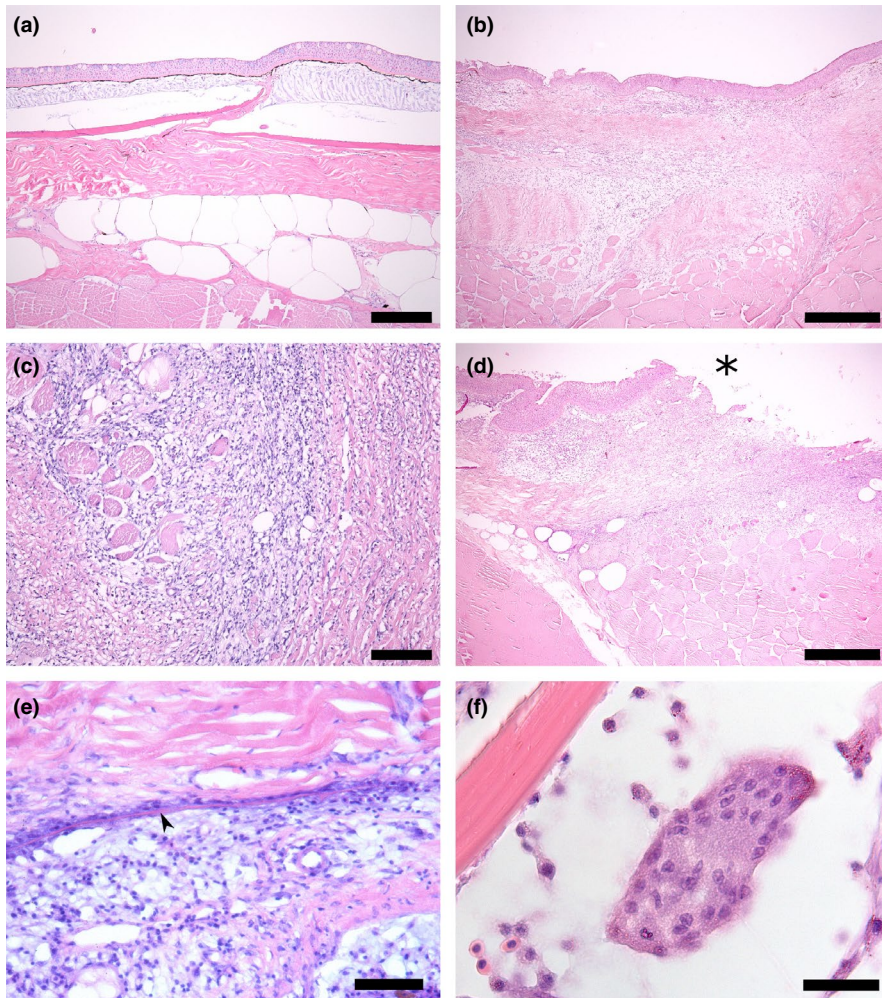
Red mark syndrome is characterized by small (5 mm to some centimetres), bright red, raised, haemorrhagic lesions mainly developing at the lateral side of the rainbow trout (Oidtmann et al., 2013). Microscopically, acute dermal inflammation, sometimes extending to the epidermis and hypodermis (Metselaar et al., 2010), necrosis of connective tissue (McCarthy, Casadei, Wang, & Secombes, 2013) and resorption of scales are described (Oidtmann et al., 2013). Other occasionally reported abnormalities are myocarditis, inflammation of the intestinal muscle, splenic congestion and peritonitis (Oidtmann et al., 2013). In most cases, skin lesions heal spontaneously with no effect on body condition or behaviour (Cafiso et al., 2016; Ferguson et al., 2006). However, in fish farming industry, this disease can cause great economic losses due to the downgrading of the product (Cafiso et al., 2016; Oidtmann et al., 2013). The aetiology of RMS has not yet been indisputably elucidated although in recent research *Rickettsia*-like organisms (RLO), intracellular pathogens, were

found in the lesions (Galeotti, Manzano, et al., 2017; Metselaar et al., 2010). Cafiso et al. (2016) provided molecular evidence for the involvement of *Midichloriaceae* (order Rickettsiales, Montagna et al., 2013). Due to their intracellular nature, RLO are difficult to isolate on artificial media explaining the difficulties to pinpoint their involvement in RMS-lesion development (Cvitanich, Garate, & Smith, 1991; Makrinos & Bowden, 2017).

To our knowledge, hitherto, RMS has only been reported in cultured freshwater rainbow trout. The present report describes abnormal changes in the skin in marine common dab (*Limanda limanda*) caught in the Belgian part of the North Sea having various common features with lesions associated with RMS.

The fish (21–81 months) were part of an experimental challenge study whereby the pathogenesis of a *Vibrio tapetis* infection was investigated in common dab (Vercauteren et al., 2019). These fish were caught at sea and, after an acclimatization period of 18 days, stocked in five groups of 12 animals per experimental unit (1 × 1 × 0.5 m; 450 L). From this point onwards, the fish were caught and clinically inspected every 2 days for 21 days. The temperature of the water was 15 ± 1°C.

At the beginning of the experiment, three fish, from different experimental units, presented with a few (1–5) multifocal, raised,



**FIGURE 2** Histological analysis of normal skin of common dab (a) and skin presenting with lesions sharing various similarities with RMS (b–f). (a) Normal skin of common dab comprising epidermis, basement membrane, dermis and scales located in scale pockets. HE, scale bar = 200 µm. (b) Histopathology of lesions in common dab similar as shown in Figure 1c, with marked inflammation in the dermis. Note the loss of scales and the intact epidermis. HE, scale bar = 500 µm. (c) Marked inflammatory infiltration in stratum spongiosum and compactum of the dermis and underlying hypodermis and muscular tissue. HE, scale bar = 50 µm. (d) Loss of epidermis (\*) observed at the centre of some lesions as shown in Figure 1e. In the edge of the lesions, scale resorption and dermatitis are observed. HE, scale bar = 500 µm. (e) Detail of scale resorption site (arrowhead) with dermal inflammation. HE, scale bar = 50 µm. (f) Presence of a multinucleated osteoclast (\*) in the scale pocket. HE, scale bar = 20 µm [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 1** Attributes of red mark syndrome (RMS) in rainbow trout as described by Oidtmann et al. (2013) compared to skin lesions observed in common dab. (a) Epidemiology; (b) Clinical signs, (c) Gross presentation and (d) Histopathological features. A full description of RMS in rainbow trout can be found in Oidtmann et al. (2013)

	RMS in rainbow trout	Skin lesions in common dab
<b>(a) Epidemiology</b>		
Aetiology	Currently unknown involvement of <i>Rickettsia</i> -like organism has been suggested	Unknown
Species affected	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Common dab ( <i>Limanda limanda</i> )
Age/size of fish	>100 gr	33 and 81 months old between 71 and 139 g
Freshwater/marine	Freshwater	Marine
Spreading within units	Yes	Yes
Spreading within farm	Yes, depending on farm layout and water connectivity	Not applicable
Incubation period	Long incubation period	Not available
Prevalence farm level	Variable usually < 50%	Not applicable
Prevalence fish level	5%–80% usually 10%–30%	11.67%
Geographic spread	Scotland, England, Switzerland, Austria, Italy, Serbia, France, Spain, Germany, Finland, Slovenia	Belgian part of the North Sea
Country and year first observation	England, 2001	Belgium, 2016
Temperature range	<17–18°C	15 ± 1°C
Season	Variable (temperature related)	Caught in October
<b>(b) Clinical signs</b>		
Behaviour	Normal	Normal
Condition	Generally in good condition	Generally in good condition
Mortality	No	No
<b>(c) Gross appearance</b>		
Affected area	Flanks and ventrum, between pectoral fins, head and fins not usually affected	Present on the whole body of the fish, with no specific predilection site
Distribution pattern	Few to several focal lesions (depending on stage)	Few focal lesions.
Shape	Elongated to ovoid, variable shape	Round, irregular edges, raised
Size	5 mm up to 2 cm (depending on stage)	<1 cm
Colour	Opaque (early stage) to bright red	Pale to haemorrhagic
Scale loss	Yes	Yes, but not grossly visible
Ulceration	Rare, related to secondary infections	Occasionally
Healed lesion	Pale yellow/grey, reduced redness	Not observed (reduced haemorrhages)
<b>(d) Histopathological features</b>		
Epidermis	Largely unaffected, lymphocytic infiltrate, rare ulceration in the centre of the lesion	Unaffected to slight hyperplasia, mononuclear infiltrate, occasional ulceration in centre of lesion
Dermis	Severe inflammatory response, especially focused to scale pockets, generally throughout the dermis, haemorrhages	Severe inflammation of both stratum spongiosum and compactum, scales pockets affected, haemorrhages
Hypodermis and muscle	Occasionally affected, inflammation	Rarely affected
Scales	Lifting and osteoclastic resorption, severe inflammation of scale pockets	Osteoclastic resorption, moderate-to-severe inflammation of scale pockets

irregular lesions (0.2–1 cm diameter) on the skin (Figure 1a). Two fish developed lesions between 5 and 9 days and two at 18 days after the start of the experiment, leading to a total of 12% affected fish. Merely, all fish were part of the sham-treated control group, except from one challenged fish. The observed skin lesions ranged from pale bulging zones (Figure 1b) to raised irregular haemorrhagic

skin lesions (Figure 1c). In two lesions, dark pigmentation or haemorrhages were observed surrounding the lesion (Figure 1d). Lesions were present on both the pigmented and non-pigmented side of the fish with no specific predilection site. Some lesions evolved to a small ulceration with associated haemorrhages (Figure 1e). In two cases, haemorrhages diminished (Figure 1f). The fish were

satisfactorily eating, gained weight and behaved as expected. At the end of the experiment, fish were killed using an overdose of tricaine methanesulfonate (500 mg/L). Samples of skin lesions, liver, spleen, kidney and heart were collected for histological examination. All tissues were processed according to standard histological techniques, sectioned (5 µm) and stained. Skin samples were decalcified prior to processing (2 hr, Rapid decalcifier, J.T. Baker, Avantor, Poland).

Upon histological examination, a lympho-monocytic inflammatory infiltration was noted in all examined skin lesions, both in the stratum spongiosum and compactum (Figure 2b and c), and extended occasionally to the hypodermis and muscular tissue. The inflammation was mainly focused to the scale pockets with partial or complete scale resorption (Figure 2e) and occasional presence of multinucleated osteoclasts (Figure 2f). In three skin samples, a mild epidermal hyperplasia and/or mononuclear inflammation was observed. Occasionally, an ulceration was present in the lesion (Figure 2d). Histological analysis of the internal organs did not reveal any abnormalities.

Table 1 shows a comparison between the attributes of RMS in rainbow trout as described by Oidtmann et al. (2013) and the features in common dab as observed in the present study.

Based on the attributes as proposed by Oidtmann et al. (2013), one may state that the lesions observed in wild-caught common dab show marked analogies with RMS described in cultured rainbow trout. These comprise epidemiology (prevalence on fish level and temperature range) clinical signs (impact on condition, behaviour and mortality) and histopathological features (Table 1). The most striking similarities are the outspoken dermatitis and scale resorption. The latter phenomenon in RMS is mainly attributed to the multinucleated osteoclasts, cells of the monocyte-macrophage lineage, presumably stimulated by lipopolysaccharide and interleukin-1 of the causative agent (McCarthy et al., 2013). In some of the lesions observed in common dab, osteoclasts were also observed in the scale pockets. Although differences are found regarding the gross clinical presentation (Table 1), more research is necessary to demonstrate whether these are specific for dab.

*Rickettsia*-like organisms, the presumed causative agent of RMS in rainbow trout, were isolated in various marine host species such as oysters (Sun & Wu, 2004), juvenile sea bass (*Dicentrarchus labrax*) (Comps, Raymond, & Plassiart, 1996) and grouper (*Epinephelus melanostigma*) (Chen, Wang, Tung, Thompson, & Adams, 2000). *Midichloriaceae*, which were opted by Cafiso et al. (2016) as a possible causative agent, are also known to be able to infect a wide range of hosts, from ciliate protists to marine organisms (Cafiso et al., 2016). Therefore, in both cases, an infection of a marine host by RLO or *Midichloriaceae* is plausible. Further research is warranted to further elucidate the aetiology of the skin lesions in dab whereby specific immunohistochemical staining (Metselaar et al., 2010), transmission electron microscopy (Galeotti, Manzano, et al., 2017) or PCR analysis (Galeotti, Manzano, et al., 2017) may prove useful.

In conclusion, this case description is the first report on lesions that share various similarities with RMS observed in wild-caught dab. Further research is necessary to identify the causative agent(s)

of the lesions and confirm or refute the hypothesis of it being similar to the RMS aetiological spectrum.

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## CONFLICT OF INTEREST

The authors do not have financial and non-financial competing interests.

## ORCID

Maike Vercauteren  <https://orcid.org/0000-0002-7618-143X>

## DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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