ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Red mark syndrome (RMS) in farmed rainbow trout: First report of outbreaks in Bosnia and Herzegovina

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:
Galeotti, M., Volpatti, D., Byadgi, O., Beraldo, P., Orioles, M., Sarti, M., et al. (2021). Red mark syndrome (RMS) in farmed rainbow trout: First report of outbreaks in Bosnia and Herzegovina. JOURNAL OF FISH DISEASES, 44(5), 627-631 [10.1111/jfd.13336].

Availability:
This version is available at: https://hdl.handle.net/11585/790760 since: 2021-01-23

Published:
DOI: http://doi.org/10.1111/jfd.13336

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

01 December 2024

This is the final peer-reviewed accepted manuscript of:

Marco Galeotti, Donatella Volpatti, Omkar Byadgi, Paola Beraldo, Massimo Orioles, Massimo Sarti, Sara Ciulli, Gian Enrico Magi, (2021). Red mark syndrome (RMS) in farmed rainbow trout: First report of outbreaks in Bosnia and Herzegovina. Journal of Fish Diseases 2021; 44: 627–631. https://doi.org/10.1111/jfd.13336

The final published version is available online at: https://doi.org/10.1111/jfd.13336

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

Red mark syndrome (RMS) in farmed rainbow trout: First report of outbreaks in Bosnia and Herzegovina

Marco Galeotti[1], Donatella Volpatti[1], Omkar Byadgi[1], Paola Beraldo[1], Massimo Orioles[1], Massimo Sarti[2], Sara Ciulli[3], Gian Enrico Magi[4]

[1]Veterinary Pathology Unit, University of Udine, Udine, Italy

[2]Skretting Italia S.p.A., Verona, Italy

[3]Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna, Cesenatico, Italy

[4]School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, Italy

Correspondence

Marco Galeotti, Veterinary Pathology Unit, DI4A, University of Udine, Italy.

Email: marco.galeotti@uniud.it

Abstract

Red mark syndrome (RMS) is a non-lethal inflammatory skin disorder spreading in farmed adult rainbow trout (Oncorhynchus mykiss) and reported worldwide. The aetiology is still uncertain, but positive correlation was found between Midichloria-like organism and RMS-affected fish. Here, we describe the first cases of RMS in Bosnia and Herzegovina. The outbreaks under study occurred in two intensive farms during the late winter and spring of 2020. Affected fish showed signs of disease ascribable to RMS, confirmed by pathological and molecular examination.

Keywords

Bosnia and Herzegovina, dermatitis, MLO, rainbow trout, red mark syndrome, Rickettsiales

Introduction

In recent years, among the emerging skin diseases of farmed trout (Oncorhynchus mykiss), red mark syndrome is one of the most reported. It was originally described in U.K. (Ferguson et al., 2006; Noguera, 2008; Oidtmann & Noguera, 2008; Verner-Jeffreys et al., 2006, 2008) and USA (Olson et al., 1985; LaPatra et al. 1994; Lloyd et al., 2008), then reported in Finland (Bruno et al., 2007), Austria, Switzerland (Schmidt-Posthaus et al., 2009), Italy (Galeotti et al., 2011), Turkey (Kubilay et al., 2014), Iran (Sasani et al., 2016) and Chile (Sandoval et al., 2016), more recently also signalled in Slovenia (Galeotti, Ronza, et al., 2017) and Denmark (Schmidt et al., 2018).

RMS is an inflammatory skin disorder affecting adult rainbow trout, whose classical signs have been well characterized according to the criteria of Oidtmann et al. (2013). The possible involvement of an infectious agent has been suggested since early studies and then confirmed through cohabitation transmission trials (Verner-Jeffreys et al., 2008) as well as through the detection of positivity in affected tissues for a Rickettsia-like organism (RLO)-related DNA (Lloyd et al., 2008; Metselaar et al., 2010). More recently, a Midichloria-like organisms (MLO)-related DNA (Cafiso et al., 2016; Metselaar et al., 2020) was demonstrated in affected trout, and also intracytoplasmic microorganisms resembling Rickettsiales were detected by transmission electron microscopy (TEM) in tissues of symptomatic rainbow trout in Italy and Slovenia, strengthening the hypothetical role of a MLO as possible causative agent of RMS (Galeotti, Manzano, et al., 2017; Galeotti, Ronza, et al., 2017). Considering the spread of RMS in many countries around the world, it is of the outmost importance to report every single outbreak in “newly” affected geographical areas in order to trace and define precisely the propagation of this disease in trout aquacultures. The

present contribution describes the first occurrence of RMS outbreaks in Bosnia and Herzegovina commercial trout farms.

Bosnia and Herzegovina freshwater aquaculture sector consists of approximately 100 small-sized farms and 5–6 larger ones (authors personal communication). The total annual production varies in between 3,000 and 4,000 tons, and rainbow trout is the most important farmed species (FAO, 2020).

Traditionally, up to now the small-size farms have used primarily their own broodstocks, but this tendency has decreased in recent years. Currently, many of them purchase fingerlings from larger farms or small hatcheries in Bosnia and Herzegovina. Conversely, all the specialized large-scale producers buy the eggs from USA, Spain or Italy. The water provision for these aquaculture plants derives mostly from local rivers.

The present outbreaks occurred in two large intensive rainbow trout farms (here referred as farm A and farm B), during the late winter and spring of 2020. In farm A, fish were reared up to commercial size in concrete raceways supplied by river water (T 8,5° in winter–9,5°C in summer) deriving from nearby snowfields and fed with a commercial pelleted diet. Rearing density was about 40–50 Kg/m3. The affected individuals, at a percentage of 20%–25%, were kept in several basins. Their size was approximately 250–300 g. In farm B, located in a geographical area quite distant from the previous one, fish were similarly reared up to commercial size in concrete raceways supplied by river water (T 8° in winter–11°C in summer) and fed with a commercial pelleted diet. Rearing density was about 25 Kg/m3. The affected fish (30%–35%) were distributed in a limited number of basins within the whole farm. Their size was approximately 300 g. Mortality was absent in both farms. Recorded individuals showed signs of disease ascribable to RMS, according to the criteria proposed by Oidtmann et al. (2013). Concerning the farm A, the disease already occurred in the previous years with peaks in prevalence, sometimes as high as 50%. In the farm B, RMS already

occurred in a sporadic but not impactful form. Currently, the disease appeared with a more severe course in both farms.

Regarding the sanitary situation, both surveyed farms are free from SEV and IHN, as reported by the Croatian Veterinary Institute (Lab for Fish and Molluscs diseases). Sleeping disease and furunculosis are observed instead, whereas ERM (caused by biotype 1 and 2 Yersinia ruckeri) is recorded every year. Seventeen symptomatic fish were sampled in Farm A, and eighteen in Farm B, then killed with an overdose of MS222 (500 mg/L). All individuals were submitted to necropsy, and digital images were captured in order to document the macroscopic lesions.

For the histological examination, tissue samples obtained from skin were fixed in 4% neutral buffered formaldehyde and then processed by an automatic histoprocessor (TISBE, Diapath, Italy) to be embedded in paraffin (ParaplastPlus, Diapath). Serial 5-µm sections were stained with haematoxylin–eosin (HE). Three hyperplastic spleens collected from farm B trout were used to prepare smears and impressions, and stained with Giemsa and Macchiavello stain as previously described (Culling, 1974). Slides were observed under optical microscope, and relevant digital histological images were captured.

The DNA extraction and purification was carried out in the same seventeen symptomatic fish sampled in Farm A and eighteen symptomatic fish sampled in Farm B. Five healthy fish as control group were collected from a commercial rainbow trout farm, which is a RMS-free facility, in order to be used as reference samples. The tissues (skin/muscle, spleen and head kidney) were hygienically dissected and cut to ≤ 0.5 cm in any single dimension, before the immersion in ethanol for DNA extraction.

All samples including infected and controls underwent DNA extraction using the QIAamp DNA Mini kit (Qiagen) according to the manufacturer's instructions for animal tissue, and eluted DNA was stored at − 20°C. Samples were lysed (3 hr), before washing and elution were completed. Total

genomic DNA was assessed for yield and quality using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific).

The specific MLO DNA was amplified using PCR according to Galeotti, Manzano, et al. (2017) with few modifications. PCR amplification of the MLO DNA using the primer was carried out in a final volume of 50 µl. First, PCR was amplified using RLO1/RLO2 (Lloyd et al., 2008) primer in a Bio-Rad thermo cycler (Bio-Rad Laboratories Inc., CA, USA, http://www.bio-rad.com/) with a reaction mixture containing 100 ng DNA, 100 ng of each primer and 1.25 units of Taq DNA polymerase (Thermo Fisher scientific, USA, https://www.thermofisher.com/) under the following conditions: the amplification protocol: the time of denaturation at 95°C was increased from 2 to 5 min, 35 cycles of 95°C for 30 s, and the annealing temperature was increased from 57°C to 69°C for 30 s, 72°C for 30 s instead of 60 s and a final extension at 72°C for 10 min. For each PCR, water has been used as a blank (negative control) to verify that no contaminations were present during the amplification step.

Second, nested PCR assay was performed using a primer pair, RiFCfw 5'-AAGGCAACGATCTTTAGTTGG-3' and RiFCrev 5'-CCGTCATTATCTT CCCCACT-3', within the Amplicon obtained by the first step using the primers RLO1 and RLO2. The amplification was obtained using 2 µl of the first step as template following the protocol: 95°C denaturation for 5 min, 35 cycles of 95°C for 45 s, 54°C for 45 s, 72°C for 45 s and a final extension at 72°C for 7 min.

Subsequently, 5 µl of the PCR product was analysed by 2% agarose gel electrophoresis stained with ethidium bromide and visualized with an UV transilluminator. PCR product molecular weight was determined using a 50-bp DNA ladder (Thermo Fisher Scientific, Pittsburgh, PA, USA). To identify the type MLO isolate used in the present study, PCR products were then purified using a QIAquick Purification kit (Qiagen, https://www.qiagen.com/it/) and directly sequenced using the RiFCfw 50/RiFCrev AO18SF/R primer set (Eurofins Genomics). Using a BLAST search, the sequences obtained were compared with those published in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

In fish deriving from both farms, gross skin lesions, single or multiple, consisted in small to large areas, ranging from 1x1 to 3x4 cm, flat or protruding, round/oval shaped, pink or pale red, sometimes displaying scattered petechial haemorrhages, sometimes with a desquamation in the central part of the lesion, often covered by serous/fibrin exudate (Figures 1 and 2).

In the farm A, these lesions were single, and flank located in 7 out of 17 individuals, and multiple in 10 out of 17 individuals. In the farm B, the lesions were single, and flank located in 7 out of 18 individuals, and multiple in 11 out of 18 individuals. Spleens showed increased volume and rough surface in 2 out of 17 individuals in farm A, instead 8 out of 18 individuals in farm B. Once cut, the organ showed congestion. No lesions were detectable in the other organs.

Histology revealed a severe skin inflammatory disease affecting all the skin layers sometimes extending to the underlying muscular tissues (Figures 3 and 4). The inflammatory infiltrate was composed by numerous lymphocytes and macrophages mainly localized within the stratum compactum and spongiosum. In the latter, the scale pockets were often cancelled or obscured by the inflammatory cells and numerous haemorrhages were also present. The epidermis appeared variably eroded and/or infiltrated by inflammatory cells, whereas the hypodermis and, in some cases, the deep muscular tissue appeared heavily infiltrated by the same inflammatory cells. Cytological examination of spleen smears revealed a mixed population of cells, including monocytes containing numerous intracytoplasmic oval to round, 0.5–1.5 μm diameter, microorganisms that stained red with Macchiavello's method (Figure 5). The three spleen specimens submitted to this stain resulted also positive by PCR for MLO.

The amplification of 188 bp (RMS/MLO) after nested PCR was considered as positive in this study. From the Farm A, skin/ muscle analysis indicated 11 positive out of 17 symptomatic trout (64.70%). Whereas from Farm B, 16 out of 18 individuals were positive (88.88%). No amplification of MLO DNA was recorded in skin/muscle samples from the control RMS-free farm. Five fish out of 11 positive from Farm A resulted positive in all the investigated tissues:

skin/muscle, spleen and head kidney. In Farm B out of 16 positives, 11 indicated amplification in all the tissues analysed.

According to the standardized method proposed by Oidtmann et al. (2013) for the description of rainbow trout skin disorders of uncertain aetiology, it is possible to refer both episodes to RMS.

It has been reported in the literature that RMS could be transmitted through water and contaminated eggs (Verner-Jeffreys et al., 2008). Given that the farm A water supply comes via river from nearby snowfields, it is unlikely that RMS could have spread through the aquatic route. On the other hand, the farm B water supply comes from river water, which is connected with other trout farms; in this case, a water-based transmission might be possible. This river is tributary of the Sava river stretch entering Croatia. Moreover, both farms purchase variable percentages of eggs from external sources (personal communication of farm managers), specifically farm A for about 50%, whereas farm B for 100%. The authors hypothesize that the disease could have spread through contaminated eggs, especially in the case of farm A for the reason mentioned above. Contaminated eggs represent a further risk factor of infection in the case of farm B, in association with the possible entrance of the pathogen by the river water eventually contaminated.

RMS has been sporadically but unofficially reported for many years in Bosnia and Herzegovina. Trout farming production in this country has gradually increased in the last 15 years and RMS seems to be more frequently reported in the last five years, since the activity of many intensive trout farms has started. The spreading of RMS from Bosnia and Herzegovina to other nearby countries like Croatia is certainly possible, considering the presence of many transboundary waters.

Considering the cases reported in this article in Bosnia and Herzegovina and the recent descriptions of RMS in Slovenia (Galeotti, Ronza, et al., 2017) and Denmark (Schmidt et al., 2018), the authors think RMS will be spreading quickly throughout all Europe.

The relative Bosnia's geographical, orographical and economical isolation makes the infection through egg's contamination likely, even though no further speculations are possible due to the uncertain aetiology and pathophysiology of RMS, and to the absence of the literature evidences on MLO/RLO molecular detection in trout eggs. Therefore, in the future a sanitary control of the eggs entering the farms by means of molecular analysis for MLO would be useful.

Acknowledgements

The authors wish to thank Pierluigi Bagatella (Department of Agricultural, Food, Environmental and Animal Science, Veterinary Pathology Unit, University of Udine) for the laboratory technical assistance.

Conflict of interest

None.

Data availability statement

Data available on request from the authors.

References

Bruno, D., Crumlish, M., LaPatra, S., Noguera, P., & Verner-Jeffreys, D. (2007). Workshop on salmonid skin diseases. In: Proceedings 15th International Conference on Fish and Shellfish Diseases of the European Association of Fish Pathologists, Grado.

Cafiso, A., Sassera, D., Serra, V., Bandi, C., McCarthy, U., & Bazzocchi, C. (2016). Molecular evidence for a bacterium of the family Midichloriaceae (order Rickettsiales) in skin and organs of the rainbow trout Oncorhynchus mykiss (Walbaum) affected by red mark syndrome. Journal of Fish Diseases, 39, 497–501.

Culling, C. F. A. (1974). Handbook of histopathological and histochemical techniques (pp. 402–403). (3rd ed.). Butterworth. FAO (2020). National Aquaculture Sector Overview Bosnia and Herzegovina. http://www.fao.org/fishe ry/count rysec tor/naso_bosni a/en

Ferguson, H. W., Girons, A., Rizgalla, G., LaPatra, S., Branson, E. J., Mackenzie, K., Davies, M., Collins, R. O., Diab, A., & Crumlish, M. (2006). Strawberry disease in rainbow trout in Scotland: Pathology and association with Flavobacterium psychrophilum. Veterinary Record, 158, 630–632. https://doi.org/10.1136/vr.158.18.630

Galeotti, M., Giavenni, R., Volpatti, D., Beraldo, P., & Feist, S. W. (2011). Red mark syndrome/cold water strawberry disease: emergence in Italy and histopathological investigations. In: Proceedings 15th International Conference of the European Association of Fish Pathologists, Split, Croatia.

Galeotti, M., Manzano, M., Beraldo, P., Bulfon, C., Rossi, G., Volpatti, D., & Magi, G. E. (2017). Ultrastructural and biomolecular detection of Rickettsiales-like organisms in tissues of rainbow trout with Red Mark Syndrome. Journal of Fish Diseases, 40, 907–917. https://doi.org/10.1111/jfd.12571

Galeotti, M., Ronza, P., Beraldo, P., Bulfon, C., Magi, G. E., Manzano, M., & Volpatti, D. (2017). First report of Red Mark Syndrome (RMS) in farmed rainbow trout in Slovenia. Journal of Fish Diseases, 40, 1935–1939. https://doi.org/10.1111/jfd.12652

Kubilay, A., Ciftci, S., Yildirim, P., Didinen, I., Metin, S., Demirkan, T., Ozen, M. R., & Oidtmann, B. (2014). First observation of Red Mark Syndrome (RMS) in cultured rainbow trout (Oncorynchus

mykiss Walbaum, 1972) in Turkey. Bulletin of European Association of Fish Pathologists, 34, 95–101.

LaPatra S.E., Groff J.M., Lauda K.A., Munn B. & Jones G.R. (1994). Dermatitides in cultured rainbow trout. In: Proceedings International Symposium on Aquatic Animal Health, September 4–8, 1994.

Lloyd, S. J., LaPatra, S. E., Snekvik, K. R., St-Hilaire, S., Cain, K. D., & Call, D. R. (2008). Strawberry disease lesions in rainbow trout from southern Idaho are associated with DNA from a Rickettsia-like organism. Diseases of Aquatic Organisms, 82, 111–118. https://doi.org/10.3354/dao01969

Metselaar, M., Thompson, K. D., Gratacap, R. M. L., Kik, M. J. L., LaPatra, S. E., Lloyd, S. J., Call, D. R., Smith, P. D., & Adams, A. (2010). Association of red-mark syndrome with a Rickettsia-like organism and its connection with strawberry disease in the USA. Journal of Fish Diseases, 33, 849–858. https://doi.org/10.1111/j.1365-2761.2010.01187.x

Metselaar, M., Thompson, K. D., Paley, R., Green, D. M., Verner-Jeffreys, D., Feist, S., & Adams, A. (2020). Investigating the involvement of a Midichloria -like organism (MLO) in red mark syndrome in rainbow trout Oncorhynchus mykiss. Aquaculture, 528, 735485. https://doi.org/10.1016/j.aquac ulture.2020.735485

Noguera, P. (2008). Red Mark Syndrome (RMS). Fish Farmer, 31, 38. Oidtmann, B., LaPatra, S. E., Verner-Jeffreys, D., Pond, M., Peeler, E. J., Noguera, P. A., Bruno, D. W., St-Hilaire, S., Schubiger, C. B., Snekvik, K., Crumlish, M., Green, D. M., Metselaar, M., Rodger, H., Schmidt-

Posthaus, H., Galeotti, M., & Feist, S. W. (2013). Differential characterization of emerging skin diseases of rainbow trout - a standardized approach to capturing disease characteristics and development of case definitions. Journal of Fish Diseases, 36, 921–937. https://doi.org/10.1111/jfd.12086

Oidtmann, B., & Noguera, P. (2008). Red Mark Syndrome/Cold water strawberry disease-Epidemiological study. Finfish News, 6, 10–11.

Olson, D. P., Beleau, M. H., Busch, R. A., Roberts, S., & Krieger, R. I. (1985). Strawberry disease in rainbow trout, Salmo gairdneri Richardson. Journal of Fish Diseases, 8, 103–111. https://doi.org/10.1111/j.1365-2761.1985.tb011 91.x

Sandoval, C., Infante, J., Abad, J., Ferguson, H. W., Paredes, E., Valdebenito, S., Yáñez, A. J., Ilardi, P., & Avendaño-Herrera, R. (2016). Case Report: Strawberry Disease in Farmed Chilean Rainbow Trout. J Aquatic Animals Health., 28(1), 1–10. https://doi.org/10.1080/08997659.2015.1114534

Sasani, F., Shokrpoor, S., Rahmati-Holasoo, H., & Zargar, A. (2016). Appearance of Red Mark Syndrome (RMS) in cultured rainbow trout (Oncorynchus mykiss Walbaum, 1972) in Iran. Bulletin of European Association of Fish Pathologists, 36(2), 90–94.

Schmidt, J., Thompson, K., & Padros, F. (2018). Emerging skin diseases in aquaculture. Bull. Eur. Assoc. Fish Pathol., 38, 122–129.

Schmidt-Posthaus, H., Bergmann, W., Knüsel, R., Heistinger, H., & Licek, E. (2009). Appearance of red mark syndrome/cold water strawberry disease in Switzerland and Austria. Diseases of Aquatic Organisms, 88, 65–68. https://doi.org/10.3354/dao02152

Verner-Jeffreys, D., Algoet, M., Feist, S., Bateman, K., Peeler, E., & Branson, E. (2006). Studies on Red Mark Syndrome. Finfish News, 1, 19–22.

Verner-Jeffreys, D. W., Pond, M. J., Peeler, E. J., Rimmer, G. S. E., Oidtmann, B., Way, K., Mewett, J., Jeffrey, K., Bateman, K., Reese, R. A., & Feist, S. W. (2008). Emergence of cold water strawberry disease of rainbow trout Oncorynchus mykiss in England and Wales: Outbreak investigations and transmission studies. Diseases of Aquatic Organisms, 79, 207–218. https://doi.org/10.3354/dao01916

How to cite this article: Galeotti M, Volpatti D, Byadgi O, et al. Red mark syndrome (RMS) in farmed rainbow trout: First report of outbreaks in Bosnia and Herzegovina. J Fish Dis. 2021;44:627–631. https://doi.org/10.1111/jfd.13336

Sasani, F., Shokrpoor, S., Rahmati-Holasoo, H., & Zargar, A. (2016). Appearance of Red Mark Syndrome (RMS) in cultured rainbow trout (Oncorynchus mykiss Walbaum, 1972) in Iran. Bulletin of European Association of Fish Pathologists, 36(2), 90–94.

Schmidt, J., Thompson, K., & Padros, F. (2018). Emerging skin diseases in aquaculture. Bull. Eur. Assoc. Fish Pathol., 38, 122–129. Schmidt-Posthaus, H., Bergmann, W., Knüsel, R., Heistinger, H., & Licek, E. (2009). Appearance of red mark syndrome/cold water strawberry disease in Switzerland and Austria. Diseases of Aquatic Organisms, 88, 65–68. https://doi.org/10.3354/dao02152

Verner-Jeffreys, D., Algoet, M., Feist, S., Bateman, K., Peeler, E., & Branson, E. (2006). Studies on Red Mark Syndrome. Finfish News, 1, 19–22.

Verner-Jeffreys, D. W., Pond, M. J., Peeler, E. J., Rimmer, G. S. E., Oidtmann, B., Way, K., Mewett, J., Jeffrey, K., Bateman, K., Reese, R. A., & Feist, S. W. (2008). Emergence of cold water strawberry disease of rainbow trout Oncorynchus mykiss in England and Wales: Outbreak investigations and transmission studies. Diseases of Aquatic Organisms, 79, 207–218. https://doi.org/10.3354/dao01916

Figure 1 RMS gross skin lesions in symptomatic rainbow trout of farm A. Multifocal to coalescing round shaped, raised and pale to pink-red lesions

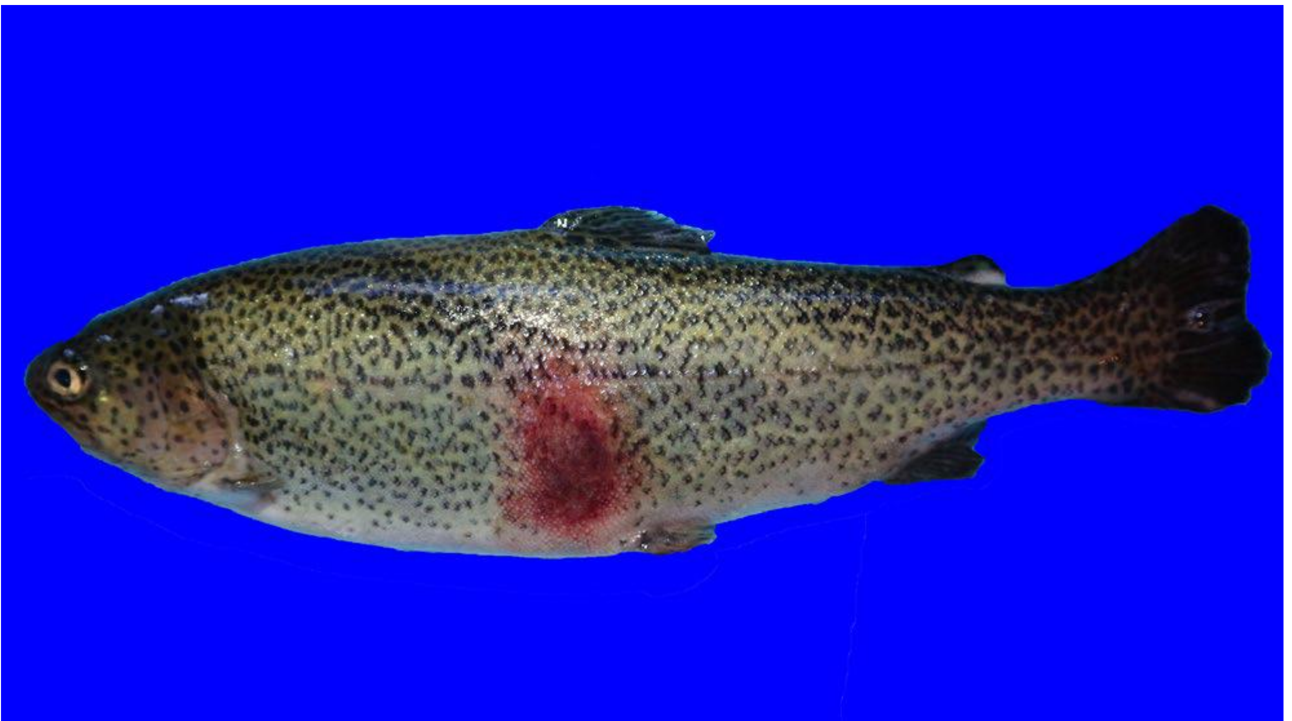

Figure 2 RMS gross skin lesion in symptomatic rainbow trout of farm B characterized by large, locally extensive, oval-shaped and raised haemorrhagic areas

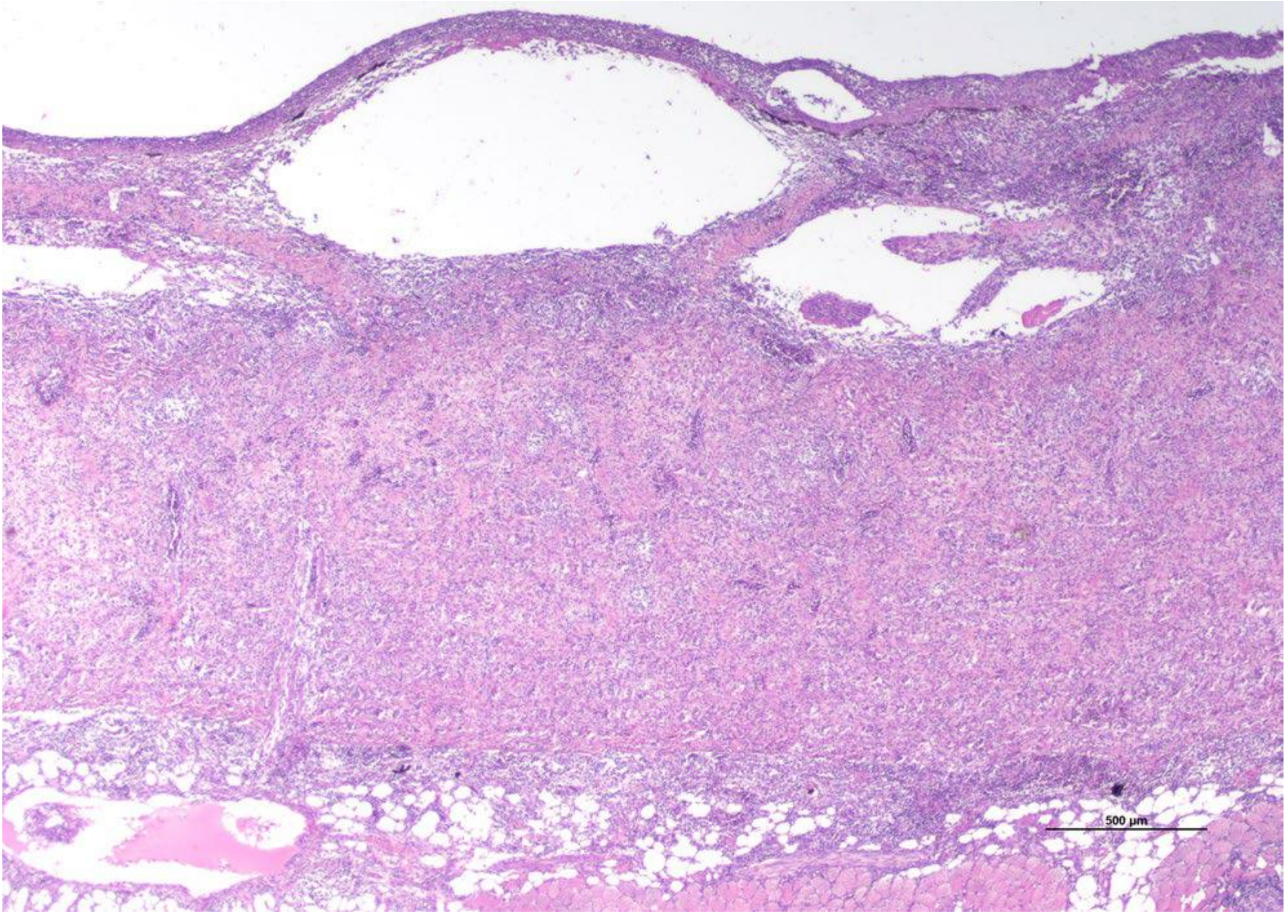

Figure 3 RMS microscopic skin lesions in symptomatic rainbow trout of farm A. Histology reveals inflammation in all skin layers and the underlying muscular tissue. The epidermis is thinned, and the scale pockets appear severely infiltrated by lymphocytes and monocytes/macrophages with complete scale resorption. The stratum compactum of the derma and the hypodermis are thickened by the presence of inflammatory infiltrate (H&E)

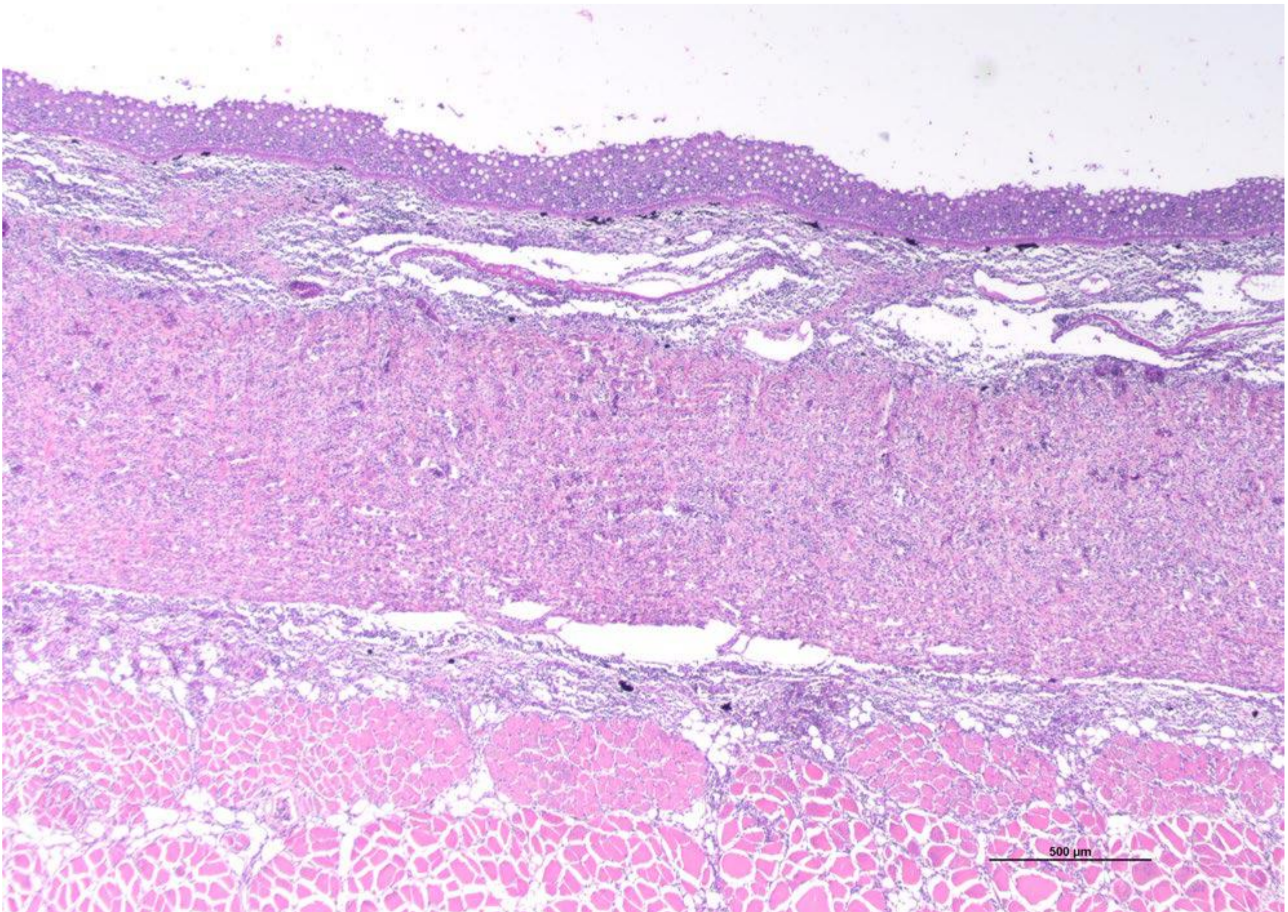

Figure 4 RMS microscopic skin lesions in symptomatic rainbow trout of farm B. Histopathological examination reveals inflammation extending from dermis to the deep muscular tissue. The stratum spongiosum of the derma appears severely oedematous and expanded by lymphocytes and monocytes infiltrating scales pockets. The scales are still present. The stratum compactum and the hypodermis are thickened and heavily infiltrated by inflammatory cells (H&E)

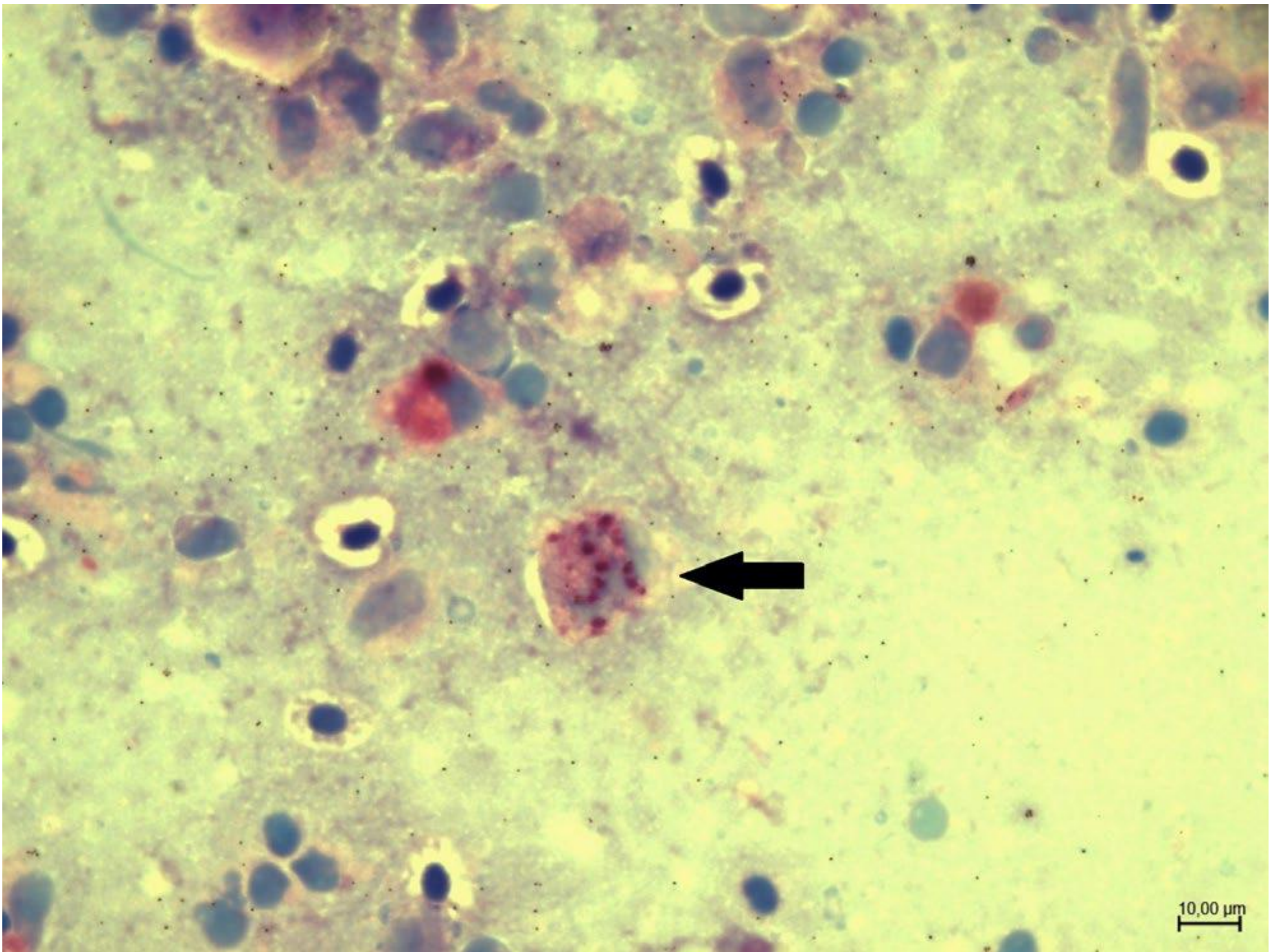

Figure 5 Spleen smear cytological examination reveals mononuclear cells containing numerous intracytoplasmic oval to round microorganisms (arrow) (Macchiavello stain)