臨床的に「ボケ」と診断されたアユの鰓病変の病理組織

誌名	魚病研究
ISSN	0388788X
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発行元	日本魚病学会
巻/号	46巻2号
巻号補足	
掲載ページ	p. 59-61
発行年月	2011年6月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

AgriKnowledge

魚病研究 Fish Pathology, 46 (2), 59–61, 2011. 6
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Histopathology of Gill Lesions of Ayu *Plecoglossus altivelis* Clinically Diagnosed with '*Boke*' Disease

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(Received August 4, 2010)

ABSTRACT—We investigated the histopathological features of the gill lesions of diseased ayu *Plecoglossus altivelis* that had been diagnosed with '*Boke*'. The features were classified into three types: one characterized by atypical gill epithelial cells, one characterized by numerous long Gram-negative rods, and one characterized by both atypical gill epithelial cells and long rods. TEM revealed the presence of poxvirus-like virus in the atypical gill epithelial cells. Our results indicated that '*Boke*' was composed of different kinds of diseases. We propose 'atypical cellular gill disease (ACGD)' as the new name of novel gill disease in ayu.

Key words: *Boke*, gill lesion, histopathology, *Plecoglossus altivelis*, ayu, atypical cellular gill disease, ACGD

Since 1998, several severe episodes of mortality have been recorded among cultured populations of ayu *Plecoglossus altivelis* in Japan. During these outbreaks, the diseased fish showed appetite reduction, a spinning motion and/or poor swimming ability, and a tendency to congregate near the surface of the water, which were associated with a sudden increase in the mortality rate of > 90% in some cases. Based on these clinical signs, local aquaculturists referred to the condition as '*Boke*', which is a Japanese word that is generally used to indicate disorientation and senility in humans. A previous histopathological examination revealed that the disease was characterized by proliferative branchitis with enlarged and atypical epithelial cells in the gills and was not accompanied with any pathological abnormality in other internal organs[1]. Abundant poxvirus-like particles were also observed within the cytoplasm of the atypical epithelial cells. However, the clinical signs and gross features of the novel gill disease, characterized by pathognomonic atypical gill epithelial cells, were difficult to distinguish from those of other epizootic gill diseases such as bacterial gill disease (BGD)[2,3] of ayu. We therefore suspected that local aquaculturists, who might not have made a diagnosis based on histopathology, might have used the term '*Boke*' to describe a number of different clinical conditions in the ayu, including the novel gill disease with pathognomonic atypical gill epithelial cells, which was characterized by appetite reduction, abnormal swimming ability and pale gill coloration among other traits, and was associated with severe mortality. We therefore examined the histopathological features of the gill lesions of diseased ayu that had been diagnosed with '*Boke*' disease by local aquaculturists, focusing on the clinical signs and gross features.

Materials and Methods

Fish

In total, 63 fish were collected between 2003 and 2004 from Tochigi (five outbreaks including 10, 12, 10, 7 and 9 fish, respectively, between May and July, 2003), Miyazaki (one outbreak including 5 fish in May, 2003) and Yamanashi (one outbreak including 10 fish in June, 2004). These fish had originated from artificial seeds produced by prefectural fisheries stations using brood fish captured in each prefecture, and had been diagnosed with '*Boke*' disease by local aquaculturists based on their clinical signs and gross features. The fish were euthanized using an overdose of FA 100 (Dainippon Sumitomo Co. Ltd., Tokyo, Japan). The sampled individuals ranged from 9.5 to 136.8 g in body weight.

Histopathology

All of the fish were fixed in 10% phosphate-buffered formalin solution. Gills were removed from the fixed fish, decalcified in 10% ethylenediaminetetraacetic acid (EDTA) with 5% formalin, and processed routinely for histology. Sections were stained with haematoxylin and eosin (H & E), Giemsa or Gram. For electron microscopy, the gills of three fish from each disease outbreak (seven outbreaks, 21 fish in total) were fixed in McDowell's and Trump's 4F:1G fixative (pH 7.2–7.4), postfixed in 1% osmium tetraoxide (OsO_4) in 0.1 M phosphate buffer (pH 7.2–7.4), dehydrated through a graded ethanol series, transferred to 100% acetone and finally embedded in Spurr's resin[4]. Thin sections (80 nm) were cut on a Reichert Ultracut-E ultramicrotome,

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stained with methanolic uranyl acetate and Reynolds' lead citrate, and observed under a Hitachi H-7100 transmission electron microscope (TEM).

Results

Gross features

The sampled fish showed gross features including appetite reduction, a spinning motion and/or poor swimming ability, and a tendency to congregate near the surface of the water, which were associated with a sudden increase of the mortality rate to around 70%. The gills of the fish examined were pale and swollen, and bore petechiae.

Histopathology

Based on the histopathological features of the gill lesions, the sampled fish were classified into three types as follows: those that presented with proliferative branchitis characterized by enlarged and atypical gill epithelial cells, which frequently contained large vacuoles and/or basophilic inclusions in the cytoplasm as well as swollen nuclei (Fig. 1A); those that presented with apparent proliferation of 'typical' gill epithelial cells resulting in gill lamellae fusion and clubbing of gill filaments associated with numerous long Gram-negative rods (Fig. 1B); and those that presented with proliferative branchitis with enlarged and atypical gill epithelial cells associated with numerous long Gram-negative rods (Fig. 1C). In the present article, we refer to the first type as the atypical cellular (Ac) type, the second type as the long rods (Lr) type, and the third type as the mixed (Mx) type. In total, 34 fish (54%) of one outbreak (5 fish) in Miyazaki and three outbreaks (10, 12 and 7 fish, respectively) in Tochigi were classified as Ac-type, 10 fish (15.9%) of one outbreak in Yamanashi were classified as Lr-type and 19 fish (30.1%) of two outbreaks (10 and 9 fish, respectively) in Tochigi were classified as Mx-type. In Lr-type and Mx-type fish, long rods were observed attached to the surface of the gill lamellae and in the small pockets between the lamellae that resulted from their fusion. A slight inflammatory response involving lymphocytes was detected within the gill lesions of all types. TEM examination of the gill lesions revealed the presence of numerous virus-like particles in the cytoplasm of the atypical epithelia in the Ac- and Mx-types (18 fish in total, Fig. 1D). These particles were shaped like cocoons or smiling mouths, with adjacent small, rounded structures. The major axis length of the virions, calculated from the measurements of 50 particles, ranged from 200 to 300 nm. The virus-like particles were not observed in the proliferative 'typical' gill epithelial cells of the Lr-type fish (3 fish in total).

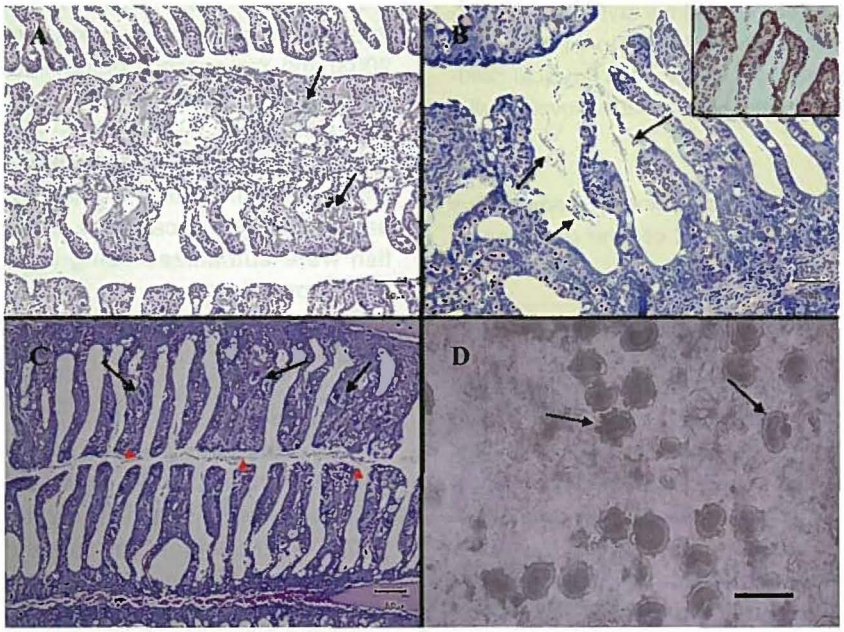


Fig. 1. Histopathological (A–C) and TEM (D) features of the gills of diseased ayu. A: The Ac type was characterized by enlarged and atypical gill epithelial cells (arrows). H & E, Bar = 50 μm. B: The Lr type was characterized by the proliferation of 'typical' gill epithelial cells associated with numerous long rods (arrows). Giemsa, Bar = 30 μm. Boxed part of B shows Gram-negative long rods. Gram, Bar = 10 μm. C: The Mx type was characterized by proliferative branchitis associated with enlarged and atypical gill epithelial cells (arrows) and numerous long rods (red arrow heads). H & E, Bar = 50 μm. D: Numerous virus-like particles (arrows) with a cocoon-like or smiling-mouth shape were observed in the cytoplasm of the gill epithelia in the Ac and Mx types. Bar = 500 nm.

Discussion

The histopathological features of the diseased fish examined in the present study included enlarged and atypical epithelial cells in the Ac- and Mx-types, which corresponded with those described previously by Wada *et al.*[1]. In addition, the morphological features of the virus-like particles detected in the atypical epithelial cells resembled those of the family Poxviridae[5]. Based on these observations, the Ac- and Mx-types might be identified as the novel gill disease associated with poxvirus-like virus reported by Wada *et al.*[1]. However, the Mx-type disease was also characterized by the presence of numerous long Gram-negative rods, which might play a role in the pathogenesis of the novel gill disease characterized by atypical gill epithelial cells as a factor that exacerbates the pathophysiology. The clinical signs, gross features and histopathological features in gills of the Lr-type corresponded with those of BGD in ayu caused by *Flavobacterium branchiophilum*[2]. However, we were unable to confirm that the Lr-type disease was BGD, because we did not attempt to isolate and identify the long rods observed in the Lr- and Mx-types in this study. More detailed bacteriological examinations, including the isolation and identification of the long rods, as well as artificial transmission studies, are required to make an accurate diagnosis of the Lr-type, and to confirm the relationship between the long rods and the novel gill disease with atypical epithelial cells. The sampled fish of each disease outbreak in the present study showed a single disease type; however, more detailed study with many samples should be required to determine whether each outbreak was composed of some disease types or a single disease type.

Our results confirmed that the diagnostic term '*Boke*' had been used by the local aquaculturists to describe not only the novel gill disease characterized by pathognomonic enlarged atypical gill epithelial cells (Ac-type)[1], but also other gill diseases characterized by numerous long Gram-negative rods with or without atypical gill epithelial cells (Mx- and Lr-types). The most prominent histopathological feature of all three types was proliferative change of the atypical or typical gill epithelial cells resulting in fusion of the lamellae and clubbing of the gill filaments. This histological change could cause considerable hypoxia due to a significant decrease of the available respiratory surface. The gill epithelial cells are considered to be a major portal site for the excretion of nitrogenous waste products, such as ammonia and urea, into the surrounding water[6], and they also help to maintain the acid–base balance in combination with osmoregulation[7]. Proliferative changes of the gill epithelial cells causing a significant decrease of the available functional surface, as observed in all three types among the present samples, could thus cause an accumulation of nitrogen metabolites, an acid–base imbalance and a deficiency of osmoregulation. We therefore suggested that there might be no notable difference in the pathophysiology caused by the Ac-, Lr- and Mx-types in the present samples, due to the significant decrease of the available functional surface of the gills resulting from the proliferative changes of the epithelial cells. This led us to propose that the clinical signs generated by the pathophysiology were common among all three types of disease described as '*Boke*'. We also suspected that the clinical signs and gross features would be insufficient tools with which to distinguish these three types. The histopathological examination of gills is a readily available tool that can allow an objective diagnosis of atypical gill epithelial cells to be made, and can clearly distinguish the three types; however, this method is time consuming and thus cannot provide the rapid diagnosis required to control the disease by the local aquaculture industry. Novel diagnostic tools that can rapidly detect the presence of atypical gill epithelial cells and/or long Gram-negative rods are thus required for the clinical control of these diseases.

In the present study, it was revealed that '*Boke*' was composed of different kinds of diseases relating to poxvirus-like virus and/or long Gram-negative rods. We therefore would propose atypical cellular gill disease (abbreviated to ACGD) in ayu as the new disease name of the novel gill disease characterized by pathognomonic enlarged atypical gill epithelial cells and associated with poxvirus-like virus.

Acknowledgement

This research was funded by Japan Fisheries Resource Conservation Association and Ministry of Agriculture, Forestry and Fisheries.

References

1) Wada, S., O. Kurata, K. Hatai, H. Ishii, K. Kasuya and Y. Watanabe (2008): *Fish Pathol.*, **43**, 89–91. 2) Miyazaki, T. and Y. Jo (1986): *Fish Pathol.*, **21**, 207–208. 3) Wakabayashi, H. and T. Iwado (1985): In "Fish and shellfish pathology" (ed. by A. E. Ellis). Academic Press, London, pp. 153–160. 4) Dykstra, M. J. (1993): In "A manual of applied techniques for biological electron microscopy" (ed. by M. J. Dykstra). Plenum Press, New York, pp. 12–16. 5) Moss, B. (2001): In "Fields virology, 4th ed., Vol. 2" (ed. by D. M. Knipe and P. M. Howley). Lippincott Williams and Wilkins, Philadelphia, pp. 2849–2883. 6) Stoskopf, M. K. (1993): In "Fish medicine" (ed. by M. K. Stoskopf). W. B. Saunders Company, Philadelphia, pp. 113–131. 7) Speare, D. J. and H. W. Ferguson (2006): In "Systemic pathology of fish, 2nd ed." (ed. by H. W. Ferguson). Scotian Press, London, 2562 p.

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「ボケ」と診断されたアユの鰓病変を病理組織学的に類別した。その結果，鰓病変は異型細胞が特徴的な型，グラム陰性長桿菌が特徴的な型，およびそれらの混合型の3種に類別された。電顕観察の結果，異型細胞内にはポックスウイルス様粒子が観察された。結果より「ボケ」は異なる疾病より成ることが示された。異型細胞を伴うアユの新規の鰓病に対し，異型細胞性鰓病（ACGD）という病名を提案する。

魚病研究，**46**（2），59−61（2011）