

DESCRIPTION

THERAPEUTIC AGENT FOR DISEASES MEDIATED BY MICROSPORIDIA AND MYXOSPOREA BOTH PARASITIC IN MARINE FISHES

TECHNICAL FIELD

[0001] The present invention relates to an agent for controlling parasites present in marine fishes and a method for controlling such parasites. More particularly, this invention relates to an agent and a method for controlling Beko disease caused by microsporidia parasitic in marine fishes, or myxosporean diseases through oral medication.

BACKGROUND ART

[0002] The causative parasite of Beko disease in yellowtail is *Microsporidium seriolae*. Those microsporidia which have been determined to be of a new species but whose suprageneric taxonomic positions are difficult to identify can be, in principle, placed in the collective genus *Microsporidium*. This principle is also applied to the case of the causative parasite of Beko disease in yellowtail. The parasitism of this parasite in yellowtail was first reported in 1982, and then this parasite was observed to be parasitic in gold striped amberjack and greater amberjack. This disease is characterized in that white irregular aggregates of small parasitic cysts with a size of about several millimeters to one centimeter are formed on the lateral muscles of an affected fish. When spore formation within cysts is completed, followed by the disruption of the cysts, surrounding muscles are lysed, so that the body surface of the affected area appears to be depressed. Therefore, the formation of asperities on the body surface is the reason why this disease is called "Beko" disease. This parasite may inhabit a wide area of the trunk of a fish, so that the fish may in some cases become severely emaciated, resulting in death. If the affected area is localized and besides there is no secondary infection with bacteria, etc., cysts are lysed, microsporidia escape out of the body, and then the resulting wound heals spontaneously. However, it is found that not all cysts are lysed and not all microsporidia escape out of the body, and that some microsporidian cysts may remain present in the trunk even after a long period of time has

passed before shipment -- this is one of the causes of reducing the commercial value of fishes. At present, no therapeutic agent is developed against the disease at issue, and damage caused by this disease still persists.

[0003] It is known that not only yellowtail but also other fish species such as red sea bream and Pacific bluefin tuna are infected by Beko disease caused by microsporidia.

[0004] The causative parasite of the brain myxosporean disease in yellowtail is *Kudoa yasunagai*. This parasite was discovered in 1980 from the brains of infected Japanese seabass and striped beakfish cultured in Nagasaki prefecture, which exhibited abnormal swimming behavior. Due to its having spores that generally contain seven shell valves and seven polar capsules, this parasite was originally described as the new species *Septemcapsula yasunagai*. However, as a result of subsequent molecular phylogenetic analysis, the family *Septemcapsulidae* and the genus *Septemcapsula* were deleted, and this species was transferred to the genus *Kudoa*. A fish infected by this disease exhibits a characteristic swimming behavior in which it swivels around while flexing its body. In some affected yellowtails, their trunks may become curved. White small spherical cysts are observed around the brain. There is no effective method to control this disease.

[0005] Other known myxosporean diseases observed in other fish species besides yellowtail include a myxosporean disease in tuna, olive flounder, etc., also called Kudoosis, which is caused by myxosporidian parasites of the genus *Kudoa*, and an enteric myxosporean disease in tiger puffer (also called tiger puffer emaciation disease), which is caused by myxosporidian parasites of the genus *Enteromyxum* or *Leptotheca*.

[0006] Benzimidazole-based agents are known as antiparasitic agents. In Japan, mebendazole is approved as a therapeutic agent for enterobiasis; albendazole is approved as a therapeutic agent for cysticercosis; flubendazole is approved as a pharmaceutical product for animal use to treat nematodes of the orders *Strongylida* and *Ascaridida*; and febantel and fenbendazole are approved as pharmaceutical products for animal use to treat nematodes and cestodes. For fishery purposes, febantel is approved for use in tiger puffer.

[0007] There has been a report on a study investigating the effects of albendazole against

Loma salmonae, a microsporidian parasite living in rainbow trout (NPL 1). Another report has been published on a study investigating the effects of flubendazole, mebendazole, oxibendazole, parbendazole, and triclabendazole against *Gyrodactylus* sp., a monogenean parasite living in rainbow trout (NPL 2). Yet another report has been issued describing a study of the *in vitro* efficacy of flubendazole, mebendazole, oxibendazole, parbendazole, and triclabendazole against *Philasterides dicentrarchi*, a ciliate parasitic in turbot and sea bass (NPL 3). Further, there was another report on a study that evaluated the effects of albendazole, mebendazole, and fenbendazole on *Glugea anomala*, a microsporidian parasite living in three-spined stickleback (NPL 4). Furthermore, it has been reported that benzimidazole-based drugs are effective against *Heterobothrium okamotoi*, a monogenean parasitic in tiger puffer (PTL 1).

CITATION LIST

PATENT LITERATURE

[0008] PTL 1: WO 02/005649

NON PATENT LITERATURES

[0009] NPL 1: D. J. Speare, *et al.*, *J. Comp. Path.* 121, 241-248, 1999. "A Preliminary Investigation of Alternatives to Fumagillin for the Treatment of *Loma salmonae* Infection in Rainbow Trout".

NPL 2: J. L. Tojo, M. T. Santamarina, *Disease of Aquatic Organisms*, 33, 187-193, 1998. "Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. II: *Gyrodactylus* sp."

NPL 3: R. Iglesias, *et al.*, *Disease of Aquatic Organisms*, 49, 191-197, 2002. "Antiprotozoals effective *in vitro* against the scuticociliate fish pathogen *Philasterides dicentrarchi*".

NPL 4: Schmahl G., Benini J., *Parasitol Res.*, 84(1), 41-49, 1998. "Treatment of fish parasites. 11. Effects of different benzimidazole derivatives (albendazole, mebendazole, fenbendazole) on *Glugea anomala*, Moniez, 1887 (Microsporidia): ultrastructural aspects and efficacy studies."

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0010] An object of the present invention is to provide, for example, an oral administration agent for controlling Beko disease or myxosporean diseases in marine fishes (*esp.*, cultured fishes), and a method for controlling said diseases using said agent.

SOLUTION TO PROBLEM

[0011] In search of an oral administration agent effective against Beko disease which has become an important problem with yellowtail aquaculture, the present inventors had explored different existing antiparasitic agents and natural product-derived substances for animal use. As a result, the inventors found that some of benzimidazole-based drugs commercially available as antiparasitic agents for animal use are effective against this disease, and thus completed the present invention.

[0012] The gist of the present invention resides in a therapeutic agent for diseases caused by microsporidia or myxosporidia parasitic in fishes, and the like, as defined in (1) to (24) below.

- (1) A therapeutic agent for Beko disease or a myxosporean disease in a marine fish, the therapeutic agent comprising, as an active ingredient, any of albendazole, febantel, fenbendazole, oxfendazole, mebendazole, and flubendazole.
- (2) The therapeutic agent for Beko disease as set forth in (1), wherein the Beko disease is caused by a parasite which is a microsporidian parasite belonging to the genus *Microsporidium* or the genus *Spraguea*.
- (3) The therapeutic agent for a myxosporean disease as set forth in (1), wherein the myxosporean disease is caused by a parasite which is a myxosporidian parasite belonging to any of the genus *Kudoa*, the genus *Enteromyxum*, and the genus *Leptotheca*.
- (4) The therapeutic agent as set forth in any of (1) to (3), wherein the marine fish is a fish of the order *Perciformes*, the order *Pleuronectiformes* or the order *Tetraodontiformes*.
- (5) The therapeutic agent as set forth in (4), wherein the fish of the order *Perciformes* is a fish belonging to the genus *Seriola*, the family *Sparidae*, or the genus *Thunnus*, wherein the

fish of the order *Pleuronectiformes* is a fish belonging to the family *Paralichthyidae*; or wherein the fish belonging to the order *Tetraodontiformes* is a fish belonging to the family *Tetraodontidae*.

(6) The therapeutic agent as set forth in (5), wherein the fish belonging to the genus *Seriola* is any of yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gold striped amberjack (*Seriola lalandi*), Almaco jack (*Seriola rivoliana*), Guinean amberjack (*Seriola carpenteri*), lesser amberjack (*Seriola fasciata*), samsonfish (*Seriola hippos*), fortune jack (*Seriola peruana*), and banded rudderfish (*Seriola zonata*); wherein the fish belonging to the family *Sparidae* is any of Okinawa seabream (*Acanthopagrus sivicolus*), king soldier bream (*Argyrops bleekeri* Oshima), yellowback seabream (*Dentex tumifrons*), crimson seabream (*Evynnis tumifrons*), red seabream (*Pagrus major*), blackhead seabream (*Acanthopagrus schlegelii*), and goldlined seabream (*Rhabdosargus sarba*, *Sparus sarba*); wherein the fish belonging to the genus *Thunnus* is any of Pacific bluefin tuna (*Thunnus orientalis*), Atlantic bluefin tuna (*Thunnus thynnus*), southern bluefin tuna (*Thunnus maccoyii*), bigeye tuna (*Thunnus obesus*), albacore tuna (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), longtail tuna (*Thunnus tonggol*), and blackfin tuna (*Thunnus atlanticus*); wherein the fish belonging to the family *Paralichthyidae* is any of olive flounder (*Paralichthys olivaceus*), California flounder (*Paralichthys californicus*), summer flounder (*Paralichthys dentatus*), fivespot flounder (*Pseudorhombus pentophthalmus*), cinnamon flounder (*Pseudorhombus cinnamomeus*), ocellated flounder (*Pseudorhombus dupliciocellatus*), largetooth flounder (*Pseudorhombus arsius*), and pygmy whiff (*Tarphops oligolepis*); or wherein the fish belonging to the family *Tetraodontidae* is tiger puffer (*Takifugu rubripes*) or purple puffer (*Takifugu porphyreus*).

(7) The therapeutic agent as set forth in any of (1) to (6), wherein the therapeutic agent is intended for orally administering the active ingredient at a dose of 5 to 100 mg/kg fish body weight per day.

(8) A therapeutic agent for Beko disease in a marine fish, the therapeutic agent comprising, as an active ingredient, any of albendazole, febantel, fenbendazole, oxfendazole, and

mebendazole.

- (9) A therapeutic agent for Beko disease or a myxosporean disease in a marine fish, the therapeutic agent comprising albendazole as an active ingredient.
- (10) A therapeutic agent for a myxosporean disease, comprising albendazole or flubendazole as an active ingredient.
- (11) A method for treating Beko disease or a myxosporean disease in a marine fish, the method comprising orally administering an effective dose of any of albendazole, febantel, fenbendazole, oxfendazole, mebendazole, and flubendazole to the marine fish.
- (12) The method for treating Beko disease as set forth in (11), wherein the Beko disease is caused by a parasite which is a microsporidian parasite belonging to the genus *Microsporidium* or the genus *Spraguea*.
- (13) The method for treating a myxosporean disease as set forth in (11), wherein the myxosporean disease is caused by a parasite which is a myxosporidian parasite belonging to any of the genus *Kudoa*, the genus *Enteromyxum*, and the genus *Leptotheca*.
- (14) The method as set forth in any of (11) to (13), wherein the marine fish is a fish of the order *Perciformes*, the order *Pleuronectiformes* or the order *Tetraodontiformes*.
- (15) The method as set forth in (14), wherein the fish of the order *Perciformes* is a fish belonging to the genus *Seriola*, the family *Sparidae*, or the genus *Thunnus*; wherein the fish of the order *Pleuronectiformes* is a fish belonging to the family *Paralichthyidae*; or wherein the fish of the order *Tetraodontiformes* is a fish belonging to the family *Tetraodontidae*.
- (16) The method as set forth in (15), wherein the fish belonging to the genus *Seriola* is any of yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gold striped amberjack (*Seriola lalandi*), Almaco jack (*Seriola rivoliana*), Guinean amberjack (*Seriola carpenteri*), lesser amberjack (*Seriola fasciata*), samsonfish (*Seriola hippos*), fortune jack (*Seriola peruana*), and banded rudderfish (*Seriola zonata*); wherein the fish belonging to the family *Sparidae* is any of Okinawa seabream (*Acanthopagrus sivicolus*), king soldier bream (*Argyrops bleekeri* Oshima), yellowback seabream (*Dentex tumifrons*), crimson seabream (*Evynnis tumifrons*), red seabream (*Pagrus major*), blackhead seabream (*Acanthopagrus*

schlegelii), and goldlined seabream (*Rhabdosargus sarba*, *Sparus sarba*); wherein the fish belonging to the genus *Thunnus* is any of Pacific bluefin tuna (*Thunnus orientalis*), Atlantic bluefin tuna (*Thunnus thynnus*), southern bluefin tuna (*Thunnus maccoyii*), bigeye tuna (*Thunnus obesus*), albacore tuna (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), longtail tuna (*Thunnus tonggol*), and blackfin tuna (*Thunnus atlanticus*); wherein the fish belonging to the family *Paralichthyidae* is any of olive flounder (*Paralichthys olivaceus*), California flounder (*Paralichthys californicus*), summer flounder (*Paralichthys dentatus*), fivespot flounder (*Pseudorhombus pentophthalmus*), cinnamon flounder (*Pseudorhombus cinnamomeus*), ocellated flounder (*Pseudorhombus dupliciocellatus*), largetooth flounder (*Pseudorhombus arsius*), and pygmy whiff (*Tarphops oligolepis*); or wherein the fish belonging to the family *Tetraodontidae* is tiger puffer (*Takifugu rubripes*) or purple puffer (*Takifugu porphyreus*).

(17) The method as set forth in any of (11) to (16), wherein any of albendazole, febantel, fenbendazole, oxfendazole, mebendazole, and flubendazole is orally administered at a dose of 5 to 100 mg/kg fish body weight per day.

(18) Use of any of albendazole, febantel, fenbendazole, oxfendazole, mebendazole, and flubendazole for the production of a medicament for the treatment of Beko disease or a myxosporean disease in a marine fish.

(19) The use as set forth in (18), wherein the Beko disease is caused by a parasite which is a microsporidian parasite belonging to the genus *Microsporidium* or the genus *Spraguea*.

(20) The use as set forth in (18), wherein the myxosporean disease is caused by a parasite which is a myxosporidian parasite belonging to any of the genus *Kudoa*, the genus *Enteromyxum*, and the genus *Leptotheca*.

(21) The use as set forth in any of (18) to (20), wherein the marine fish is a fish of the order *Perciformes*, the order *Pleuronectiformes* or the order *Tetraodontiformes*.

(22) The use as set forth in (21), wherein the fish of the order *Perciformes* is a fish belonging to the genus *Seriola*, the family *Sparidae*, or the genus *Thunnus*; wherein the fish of the order *Pleuronectiformes* is a fish belonging to the family *Paralichthyidae*; or wherein

the fish of the order *Tetraodontiformes* is a fish belonging to the family *Tetraodontidae*.

(23) The use as set forth in (22), wherein the fish belonging to the genus *Seriola* is any of yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gold striped amberjack (*Seriola lalandi*), Almaco jack (*Seriola rivoliana*), Guinean amberjack (*Seriola carpenteri*), lesser amberjack (*Seriola fasciata*), samsonfish (*Seriola hippos*), fortune jack (*Seriola peruana*), and banded rudderfish (*Seriola zonata*); wherein the fish belonging to the family *Sparidae* is any of Okinawa seabream (*Acanthopagrus sivicolus*), king soldier bream (*Argyrops bleekeri* Oshima), yellowback seabream (*Dentex tumifrons*), crimson seabream (*Evynnis tumifrons*), red seabream (*Pagrus major*), blackhead seabream (*Acanthopagrus schlegelii*), and goldlined seabream (*Rhabdosargus sarba*, *Sparus sarba*); wherein the fish belonging to the genus *Thunnus* is any of Pacific bluefin tuna (*Thunnus orientalis*), Atlantic bluefin tuna (*Thunnus thynnus*), southern bluefin tuna (*Thunnus maccoyii*), bigeye tuna (*Thunnus obesus*), albacore tuna (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), longtail tuna (*Thunnus tonggol*), and blackfin tuna (*Thunnus atlanticus*); wherein the fish belonging to the family *Paralichthyidae* is any of olive flounder (*Paralichthys olivaceus*), California flounder (*Paralichthys californicus*), summer flounder (*Paralichthys dentatus*), fivespot flounder (*Pseudorhombus pentophthalmus*), cinnamon flounder (*Pseudorhombus cinnamomeus*), ocellated flounder (*Pseudorhombus dupliciocellatus*), largetooth flounder (*Pseudorhombus arsius*), and pygmy whiff (*Tarphops oligolepis*); or wherein the fish belonging to the family *Tetraodontidae* is tiger puffer (*Takifugu rubripes*) or purple puffer (*Takifugu porphyreus*).

(24) The use as set forth in any of (18) to (23), wherein the medicament is used for orally administering any of albendazole, febantel, fenbendazole, oxfendazole, mebendazole, and flubendazole at a dose of 5 to 100 mg/kg fish body weight per day.

[0013] Also, the gist of the present invention resides in an agent for controlling microsporidia or myxosporidia parasitic in fishes of the genus *Seriola*, as defined in (A1) to (A5) below.

(A1) An agent for controlling a microsporidian or myxosporidian parasite living in a fish of

the genus *Seriola*, the agent comprising a benzimidazole-based drug as an active ingredient.

(A2) The agent as set forth in (A1), wherein the microsporidian or myxosporidian parasite is any of *Microsporidium seriolae*, a microsporidian parasite belonging to the genus *Spraguea*, *Kudoa yasunagai*, *Myxobolus acanthogobii*, *Kudoa shiomitsui*, *Kudoa pericardialis*, and *Kudoa amamiensis*.

(A3) The agent as set forth in (A1) or (A2), wherein the benzimidazole-based drug is any of albendazole, febantel, flubendazole, triclabendazole, fenbendazole, oxfendazole, and thiabendazole.

(A4) The agent as set forth in any of (A1) to (A3), wherein the fish of the genus *Seriola* is any of yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gold striped amberjack (*Seriola lalandi*), Almaco jack (*Seriola rivoliana*), Guinean amberjack (*Seriola carpenteri*), lesser amberjack (*Seriola fasciata*), samsonfish (*Seriola hippos*), fortune jack (*Seriola peruana*), and banded rudderfish (*Seriola zonata*).

(A5) The agent as set forth in any of (A1) to (A4), wherein the agent is intended for orally administering the benzimidazole-based drug at a dose of 0.5 to 500 mg/kg fish body weight per day.

[0014] Further, the gist of the present invention resides in a method for controlling parasites, as defined in (A6) to (A10) below.

(A6) A method for controlling a microsporidian or myxosporidian parasite living in a fish of the genus *Seriola*, the method comprising administering a benzimidazole-based drug.

(A7) The method as set forth in (A6), wherein the microsporidian or myxosporidian parasite is any of *Microsporidium seriolae*, a microsporidian parasite of the genus *Spraguea*, *Kudoa yasunagai*, *Myxobolus acanthogobii*, *Kudoa shiomitsui*, *Kudoa pericardialis*, and *Kudoa amamiensis*.

(A8) The method as set forth in (A6) or (A7), wherein the benzimidazole-based drug is any of albendazole, febantel, flubendazole, triclabendazole, fenbendazole, oxfendazole, and thiabendazole.

(A9) The method as set forth in any of (A6) to (A8), wherein the fish of the genus *Seriola*

is any of yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gold striped amberjack (*Seriola lalandi*), Almaco jack (*Seriola rivoliana*), Guinean amberjack (*Seriola carpenteri*), lesser amberjack (*Seriola fasciata*), samsonfish (*Seriola hippos*), fortune jack (*Seriola peruana*), and banded rudderfish (*Seriola zonata*).

(A10) The method as set forth in any of (A6) to (A9), wherein the benzimidazole-based drug is orally administered at a dose of 0.5 to 500 mg/kg fish body weight per day.

ADVANTAGEOUS EFFECT OF INVENTION

[0015] According to the present invention, Beko disease or myxosporean diseases -- which are severely problematic parasitic diseases caused by parasites living in widely cultured marine fishes, especially fishes belonging to the genus *Seriola*, the family *Sparidae*, the genus *Thunnus*, the family *Paralichthyidae*, or the family *Tetraodontidae* -- can be effectively treated by oral medication.

BRIEF DESCRIPTION OF DRAWING

[0016] [FIG. 1] FIG. 1 shows the test schedule used in Example 7, according to which administration of a test diet is repeated for 2 cycles during the rearing period.

DESCRIPTION OF EMBODIMENTS

[0017] In the therapeutic agent or parasite-control agent of the present invention, the active ingredient is a drug that is classified as a benzimidazole-based drug and which is effective for Beko disease or myxosporean diseases. The benzimidazole-based drug is a drug having the backbone of benzimidazole, and is known as a parasiticide or fungicide. Examples of the benzimidazole-based drug effective for Beko disease include, but are not limited to, albendazole (methyl N-(5-propylsulfanyl-1H-benzimidazol-2-yl)carbamate), febantel (methyl (NE)-N-[[2-[(2-methoxyacetyl)amino]-4-phenylsulfanylanilino]-(methoxycarbonylamino) methylidene]carbamate), fenbendazole (methyl N-(5-phenylsulfanyl-1H-benzimidazol-2-yl)carbamate), oxfendazole (methyl N-[5-(benzenesulfinyl)-1H-benzimidazol-2-yl]carbamate), and mebendazole (methyl [5-(benzoyl)benzimidazol-2-yl]carbamate). Febantel is known as a prodrug, and is metabolized into two active agents: fenbendazole and oxfendazole. Examples of the benzimidazole-based drug effective for myxosporean

diseases include, but are not limited to, albendazole, and flubendazole (methyl N-[5-(4-fluorobenzoyl)-1H-benzimidazol-2-yl]carbamate).

[0018] In one mode, the therapeutic agent or parasite-control agent of the present invention comprises any of albendazole, febantel, fenbendazole, oxfendazole, and mebendazole as an active ingredient, and is intended for the treatment of Beko disease in marine fishes. In a preferred mode, the therapeutic agent or parasite-control agent of this invention comprises albendazole as an active ingredient, and is intended for the treatment of Beko disease in marine fishes.

[0019] In another mode, the therapeutic agent or parasite-control agent of the present invention comprises albendazole or flubendazole as an active ingredient, and is intended for the treatment of myxosporean diseases.

[0020] Benzimidazole-based drugs effective for Beko disease, especially albendazole, not only exhibit an inhibitory effect on cyst formation through eliminating the causative parasite of Beko disease before cyst formation, but also have a therapeutic effect on diseased fishes having cysts formed therein.

[0021] The parasites on which the therapeutic agent or parasite-control agent of the present invention can have an antiparasitic effect are microsporidian parasites responsible for Beko disease, or myxosporidian parasites responsible for myxosporean diseases, both of which are parasitic in fishes belonging to the category of marine fishes. Examples of the microsporidian parasites responsible for Beko disease include, but are not limited to, microsporidian parasites belonging to the genus *Microsporidium* (*Microsporidium* sp.) or the genus *Spraguea*. Specific examples thereof include *Microsporidium seriolae* which is parasitic in yellowtail, and microsporidian parasites of the genus *Spraguea* which is responsible for microsporidian encephalomyelitis. Examples of the myxosporidian parasites responsible for myxosporean diseases include myxosporidian parasites belonging to the genus *Kudoa* which is responsible for Kudoosis, and myxosporidian parasites belonging to the genus *Enteromyxum* or the genus *Leptotheca* which are responsible for enteric myxosporean diseases. Specific examples thereof include *Kudoa yasunagai* which is

responsible for brain myxosporean diseases, *Myxobolus buri* which is responsible for myxosporean scoliosis, *Kudoa shiomitsui* and *Kudoa pericardialis* which are responsible for cardiac Kudoosis, *Kudoa amamiensis* which is responsible for Kudoosis Amami, *Kudoa hexapunctata* which is known to be parasitic in tuna, *Kudoa septempunctata* which is known to be parasitic in olive flounder, and *Enteromyxum leei*, *Enteromyxum fugu* and *Leptotheca fugu* which are known to be parasitic in puffer.

[0022] The marine fishes targeted by the present invention are fishes having any of the aforementioned parasites living therein. Such marine fishes include fishes belonging to the order *Perciformes* -- for example, fishes belonging to the order *Perciformes*, family *Carangidae*, genus *Seriola*, to the order *Perciformes*, family *Sparidae*, or to the order *Perciformes*, family *Scombridae*, genus *Thunnus*.

[0023] Examples of the fishes belonging to the genus *Seriola* include yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gold striped amberjack (*Seriola lalandi*), Almaco jack (*Seriola rivoliana*), Guinean amberjack (*Seriola carpenteri*), lesser amberjack (*Seriola fasciata*), samsonfish (*Seriola hippos*), fortune jack (*Seriola peruana*), and banded rudderfish (*Seriola zonata*). In a preferred mode, the therapeutic agent or parasite-control agent of the present invention is used particularly for commonly cultured fishes such as yellowtail, greater amberjack, gold striped amberjack, and Almaco jack.

[0024] Examples of the fishes belonging to the family *Sparidae* include Okinawa seabream (*Acanthopagrus sivicolus*), king soldier bream (*Argyrops bleekeri* Oshima), yellowback seabream (*Dentex tumifrons*), crimson seabream (*Evynnis tumifrons*), red seabream (*Pagrus major*), blackhead seabream (*Acanthopagrus schlegelii*), and goldlined seabream (*Rhabdosargus sarba*, *Sparus sarba*).

[0025] Examples of the fishes belonging to the genus *Thunnus* include Pacific bluefin tuna (*Thunnus orientalis*), Atlantic bluefin tuna (*Thunnus thynnus*), southern bluefin tuna (*Thunnus maccoyii*), bigeye tuna (*Thunnus obesus*), albacore tuna (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), longtail tuna (*Thunnus tonggol*), and blackfin tuna (*Thunnus atlanticus*).

[0026] Examples of the fishes belonging to the family *Paralichthyidae* include olive flounder (*Paralichthys olivaceus*), California flounder (*Paralichthys californicus*), summer flounder (*Paralichthys dentatus*), fivespot flounder (*Pseudorhombus pentophthalmus*), cinnamon flounder (*Pseudorhombus cinnamoneus*), ocellated flounder (*Pseudorhombus dupliciocellatus*), largetooth flounder (*Pseudorhombus arsius*), and pygmy whiff (*Tarphops oligolepis*).

[0027] Examples of the fishes belonging to the family *Tetraodontidae* include tiger puffer (*Takifugu rubripes*), and purple puffer (*Takifugu porphyreus*).

[0028] The therapeutic agent or parasite-control agent of the present invention can take effect through oral medication. The therapeutic agent or parasite-control agent of this invention can also be administered by injection or by immersion bath treatment in which a fish is immersed in a solution containing a drug dissolved therein.

[0029] The therapeutic agent or parasite-control agent of the present invention is orally administered to any species of fishes at a dose ranging, for example, from 5 to 100 mg, preferably from 10 to 50 mg, or from 10 to 40 mg, per kg fish body weight per day. The duration of administration of said agent of this invention is 1 to 20 days, preferably 3 to 10 days.

[0030] The therapeutic agent or parasite-control agent of the present invention may comprise any of the compounds mentioned above as active ingredients, either alone or optionally in combination with other substances including auxiliary components such as carrier, stabilizer, solvent, excipient, and diluent. Further, said agent of this invention can be of any form commonly used for such compounds, such as powder, granule, tablet, and capsule. When intended for use in fishes sensitive to the taste and smell of a compound, said agent of this invention can be further processed by coating or other means to thereby prevent a decrease in the palatability of a diet and make it harder for the compound to leak out.

[0031] In the case of fishes, oral drugs are commonly used in admixture with diets. When the therapeutic agent or parasite-control agent of the present invention is added to a diet, it is

preferable to use a diet produced taking into account the nutrients and physical properties required for each fish species. Examples of commonly used diets include pelletized diets made from a mixture of fish flour, bran, starch, minerals, vitamins, fish oil, etc., and pelletized diets made from a mixture of frozen fish such as frozen sardine and a powder diet (mash) composed of fish flour supplemented with vitamins, etc. Since the daily diet intake is substantially determined by the species and size of fish, the therapeutic agent or parasite-control agent of this invention is added to a diet in an amount calculated to comply with the aforementioned dosage regimen. A daily dose of said agent of this invention may be administered at a time or in divided portions. Since the therapeutic agent of this invention is used in admixture with a fish diet, it is preferable to make said agent into a pharmaceutical preparation suitable to ensure that an appropriate concentration of the active ingredient is added to a daily dose of diet ingested by a fish. More specifically, it is preferable to use a pharmaceutical preparation made to ensure that the active ingredient is present therein at a concentration of 1 to 50 wt.%, preferably 5 to 30 wt.%, more preferably 10 to 20 wt.%.

[0032] Hereunder, working examples of the present invention will be described, but this invention is by no means limited to these examples.

Example 1

[0033] <Anthelmintic effect of albendazole against Beko disease>

Juvenile fishes were produced from fertilized yellowtail eggs using sand-filtered, UV-sterilized seawater at an onshore rearing facility. The produced juvenile fishes were transferred to an offshore sea cage, reared for several days, and transferred back to the onshore facility. By means of this rearing in a sea cage, the juvenile yellowtails were naturally infected with the causative parasite of Beko disease. The juvenile yellowtails transferred again to the onshore facility were divided into two segments: control segment and oral albendazole-treated segment, and the fishes in each segment were housed in a 200 L tank. Sand-filtered, UV-sterilized seawater was injected into the tank for each segment at a rate of 2.4 L/min. On the last day of acclimation, the fishes were measured for weight. After acclimation, the fishes were fed a test diet for 10 consecutive days. The dose of

albendazole administration was 40 mg/kg fish body weight/day, given once daily. The albendazole-medicated diet was prepared by the following procedure: specified amounts of a commercially available diet and albendazole were placed in a polyethylene bag, and a 2-fold diluted spreading agent, Eswee 30 (low saccharified reduced starch syrup, produced by B Food Science Co., Ltd.), was added thereto in an amount of 4% of the diet weight, which was then followed by mixing of the mixture. The control diet was prepared by adding the diluted spreading agent Eswee 30 alone to a commercial diet in an amount of 4% of the diet weight, followed by mixing of the mixture. After administration of a test diet for 10 days, the fishes were fed a commercially available diet in an amount of 2% of the fish body weight. The test was repeated for four rounds. Table 1 shows, for each of the test rounds, the days of offshore sea-cage rearing, water temperature during offshore sea-cage rearing, acclimation period after transfer to the onshore facility, test fish body weight at the start of the test, feeding rate for oral drug administration, rearing test period, water temperature during the rearing test, and number of test fishes. Additionally, the fishes in the control segment used at the fourth round of test alone were housed and reared in a 500 L tank, with sand-filtered, UV-sterilized seawater being injected at a rate of 4.8 L/min.

[0034] Upon completion of the rearing test, all the fishes in both segments were captured and observed by autopsy to determine the presence of cysts on the lateral muscles. Those fishes observed to have cysts were regarded as diseased fishes. Evaluation was done by comparing the control segment with the oral albendazole-treated segment in terms of incidence rate (number of diseased fishes / number of test fishes $\times$ 100).

[0035] [Table 1]

Item	1st round	2nd round	3rd round	4th round
Days of offshore sea-cage rearing	10 days	10 days	7 days	7 days
Average water temperature during offshore sea-cage rearing	23.0°C	18.0°C	20.5°C	21.0°C
Acclimation period after transfer to onshore rearing facility	7 days	2 days	2 days	2 days
Average fish body weight at the start of the test	16.1 g	12.1 g	42.4 g	13.2 g
Feeding rate (% of fish body weight)	1.5%	1.5%	1.5%	2.0%
Days of rearing test	36 days	39 days	42 days	37 days
Average water temperature during rearing test	20.0°C	19.6°C	21.1°C	22.4°C
Number of test fishes (control segment)	30	46	20	112
Number of test fishes (albendazol-treated segment)	30	30	20	30

[0036] Results and discussion

In the control segment, diseased fishes were observed in all the four rounds of test. On the other hand, no fish with cysts was observed in the oral albendazole segment in any of the four rounds of test, resulting in a Beko disease incidence rate of 0% (Table 2). Therefore, it was confirmed that oral administration of albendazole eliminates the causative parasite of this disease that invades the fish body, and inhibits the formation of cysts by this parasite.

[0037] [Table 2]

	1st round	2nd round	3rd round	4th round
Control segment	3.3%	2.2%	5.0%	2.7%
Albendazole-treated segment	0%	0%	0%	0%

Example 2

[0038] <Anthelmintic effects of benzimidazole-based drugs against Beko disease -- 1>

In Example 1, it was found that albendazole is effective for eliminating the causative parasite of Beko disease. Thus, different benzimidazole-based drugs were investigated for their anthelmintic effects against the causative parasite of Beko disease. Juvenile fishes produced in the same way as in Example 1 were transferred to an offshore sea cage, reared for 10 days, and transferred back to an onshore rearing facility. By means of this rearing in a sea cage, the juvenile yellowtails were naturally infected with the causative parasite of Beko disease. The juvenile yellowtails transferred again to the onshore facility were divided into 10 groups each consisting of 40 fishes (2 groups each in the control segment, the albendazole-treated segment, the febantel-treated segment, the triclabendazole-treated segment, and the flubendazole-treated segment), and the fishes in each group were housed in a 200 L tank. Water injection into the tank was done under the same conditions as in Example 1. On the last day of acclimation, the test fishes were measured for weight and determined to weigh about 10 g. After acclimation, the test fishes were fed a test diet for 10 consecutive days. The dose of administration of the benzimidazole-based drugs was 40 mg/kg fish body weight/day, given once daily. The benzimidazole-based drug-medicated diets and the control diet were prepared by the same procedure as in Example 1. After administration of a test diet for 10 days, the fishes were fed a commercially available diet in an amount of 2% of the fish body weight. The test was repeated for two rounds. The rearing periods in the first and second rounds of test were 37 and 40 days, respectively. The water temperature was held at about 20.5°C during the rearing periods.

[0039] Upon completion of the rearing test, all the fishes in all segments were captured and observed by autopsy to determine the presence of cysts on the lateral muscles. Those fishes observed to have cysts were regarded as diseased fishes. Evaluation was done by comparing the control segment with each of the benzimidazole-based drug-treated segments in terms of incidence rate (number of diseased fishes / number of test fishes × 100), number of cysts in diseased fish, and cyst length.

[0040] Results and discussion

In the albendazole- and febantel-treated segments, no fish with cysts was observed in either of the two rounds of test, resulting in a Beko disease incidence rate of 0% (Tables 3, 4). Therefore, these results not only reproduced the ability of albendazole to eliminate the causative parasite of this disease and to inhibit the formation of cysts by this parasite, but also demonstrated the anthelmintic effect of febantel against this parasite. On the other hand, diseased fishes were observed in the triclabendazole- and flubendazole-treated segments in both of the two rounds of test, and these results exhibit a tendency that the incidence of this disease was slightly higher in these treatment segments than in the control segment. These treatment segments also showed comparable values for number of cysts in diseased fish and cyst length to the control segment. The above results suggested that these two drugs may have no ability to eliminate the causative parasite of this disease but rather may promote the incidence of this disease. It was revealed that not all benzimidazole-based drugs exert an anthelmintic effect against the causative parasite of this disease.

[0041] [Table 3]

Test segment	Incidence rate	Number of cysts in diseased fish	Cyst length
Control segment	2.5% (2 of 80 fishes)	6.5±3.5 cysts per fish	9.6±5.6 mm
Albendazole-treated segment	0% (0 of 80 fishes)	--	--
Febantel-treated segment	0% (0 of 80 fishes)	--	--
Triclabendazole-treated segment	3.8% (3 of 80 fishes)	7.7±4.9 cysts per fish	9.7±3.1 mm
Flubendazole-treated segment	6.3% (5 of 80 fishes)	5.6±4.0 cysts per fish	11.1±3.6 mm

[0042] [Table 4]

Test segment	Incidence rate	Number of cysts in diseased fish	Cyst length
Control segment	2.5% (2 of 80 fishes)	4.5±3.5 cysts per fish	11.8±3.1 mm
Albendazole-treated segment	0% (0 of 80 fishes)	--	--
Febantel-treated segment	0% (0 of 80 fishes)	--	--
Triclabendazole-treated segment	3.8% (3 of 80 fishes)	5.3±2.1 cysts per fish	8.3±3.7 mm
Flubendazole-treated segment	5.0% (4 of 80 fishes)	4.5±1.3 cysts per fish	11.4±3.9 mm

Example 3

[0043] <Anthelmintic effects of benzimidazole-based drugs against Beko disease -- 2>

Following Example 2, one other benzimidazole-based drug, mebendazole, was also investigated for its anthelmintic effect against the causative parasite of Beko disease.

Juvenile fishes produced in the same way as in Example 1 were transferred to an offshore sea cage, reared for 11 days, and transferred back to an onshore rearing facility. By means of this rearing in a sea cage, the juvenile yellowtails were naturally infected with the causative parasite of Beko disease. The juvenile yellowtails transferred again to the onshore facility were divided into 6 groups each consisting of 40 fishes (2 groups each in the control segment, the albendazole-treated segment, and the mebendazole-treated segment), and the fishes in each group were housed in a 200 L tank. Water injection into the tank was done under the same conditions as in Example 1. On the last day of acclimation, the test fishes were measured for weight and determined to weigh about 10 g. After acclimation, the test fishes were fed a test diet for 10 consecutive days. The dose of administration of the benzimidazole-based drugs was 40 mg/kg fish body weight/day, given once daily. The benzimidazole-based drug-medicated diets and the control diet were prepared by the same procedure as in Example 1. After administration of a test diet for 10 days, the fishes were fed a commercially available diet in an amount of 2% of the fish body weight. The rearing

period was 40 days. The water temperature was held at about 20.1°C during the rearing period.

[0044] Upon completion of the rearing test, all the fishes in all segments were captured and observed by autopsy to determine the presence of cysts on the lateral muscles. Those fishes observed to have cysts were regarded as diseased fishes. Evaluation was done by the same procedure as in Example 2.

[0045] Results and discussion

In the albendazole-treated segment, no fish with cysts was observed, resulting in a Beko disease incidence rate of 0% (Table 5). Therefore, this result reproduced the ability of albendazole to eliminate the causative parasite of this disease and to inhibit the formation of cysts by this parasite. The incidence rate, number of cysts in diseased fish, and cyst length in the mebendazole-treated segment were all lower than those in the control segment. From this, it was found that mebendazole exerted an anthelmintic effect against this parasite, but the effect of mebendazole was considered inferior to that of albendazole.

[0046] It was demonstrated that even benzimidazole-based drugs vary from each other in their anthelmintic effect.

[0047] [Table 5]

Test segment	Incidence rate	Number of cysts in diseased fish	Cyst length
Control segment	17.5% (14 of 80 fishes)	8.3±5.1 cysts per fish	13.2±5.3 mm
Albendazole-treated segment	0% (0 of 80 fishes)	--	--
Mebendazole-treated segment	7.5% (6 of 80 fishes)	6.2±1.8 cysts per fish	10.8±3 mm

Example 4

[0048] <Effects of albendazole and febantel on diet consumption by yellowtails>

The results obtained in Examples 1-4 revealed that the benzimidazole-based drugs, albendazole and febantel, have a high anthelmintic effect against *Microsporidium seriolae*, which is responsible for Beko disease in yellowtail. In order that these drugs can be utilized

in yellowtail culture, it is desired that administration of these drugs should have no or little side effects that adversely affect diet consumption by fishes. The tests in the previous examples were conducted at a water temperature of 20°C or higher, but no adverse effect was observed on diet consumption. Thus, this example investigated whether administration of albendazole and febantel at a water temperature of about 18°C adversely affects diet consumption. Juvenile yellowtails were divided into 6 groups each consisting of 10 fishes (2 groups each in the control segment, the albendazole-treated segment, and the febantel-treated segment), and the fishes in each group were housed in a 200 L tank. Water injection into the tank was done under the same conditions as in Example 1. On the last day of acclimation, the test fishes were measured for weight and determined to weigh about 73 g. After acclimation, the test fishes were fed a drug-medicated diet for 10 consecutive days, followed by rearing on a commercially available diet for 20 days, and then the fishes were fed the drug-medicated diet again for 10 consecutive days, followed by rearing on the commercially available diet for 29 days. The dose of administration of albendazole and febantel was 40 mg/kg fish body weight/day, given once daily. The drug-medicated diets and the control diet were prepared by the same procedure as in Example 1. The amount of diet fed was 1.5% of the fish body weight. The water temperature was held at about 18.3°C during the rearing period.

[0049] Upon completion of the rearing test, all the fishes in all segments were captured and measured for body weight and length.

[0050] Results and discussion

The albendazole-treated segment, during the first round of drug administration, showed a slight decrease in diet consumption activity and required more time to eat up the diet than the control segment. However, no decrease in diet consumption activity was observed during subsequent feeding on a commercial diet and subsequent drug administration, and the albendazole-treated segment was comparable in body weight and length to the control segment upon completion of the test (Table 6). On the other hand, the febantel-treated segment did not eat up all the specified amount of the drug-medicated diet

and left about 20 percent of the diet during the first round of drug administration. Even after the diet was switched from the drug-medicated diet to a commercial diet, about 20% of the diet was left over for 10 days. During the second round of administration of the drug-medicated diet, the febantel-treated segment showed no decrease in diet consumption activity, but when the drug administration was completed and switched to rearing on a commercial diet, this segment showed a decrease in diet consumption activity for 10 days; so, the diet was given in two divided doses daily to allow the fishes to eat up all the specified amount of the diet. The body weights and lengths of the fishes in this treatment segment measured upon completion of the test were evidently lower than those in the control and albendazole-treated segments. Therefore, it was demonstrated that administration of febantel at low water temperatures reduces the diet consumption activity of yellowtails. From the results obtained in this example, it was surmised that febantel is more severe in side effects on fishes than albendazole.

[0051] [Table 6]

Test segment	Body weight at the start of test	Body weight at the end of test	Body length at the end of test
Control segment	74.2±6.4 g	130.7±12.5 g	20.2±0.7 cm
Albendazole-treated segment	73.3±9.7 g	130.5±34.2 g	20.4±1.6 cm
Febantel-treated segment	72.8±10.1 g	110.2±11.5 g	19.2±0.8 cm

Example 5

[0052] <Investigation of the dose of albendazole required to eliminate the causative parasite of Beko disease>

Juvenile fishes produced in the same way as in Example 1 were transferred to an offshore sea cage, reared for 11 days, and transferred back to an onshore rearing facility. By means of this rearing in a sea cage, the juvenile yellowtails were naturally infected with the causative parasite of Beko disease. The juvenile yellowtails transferred again to the onshore facility were divided into 12 groups each consisting of 40 fishes (2 groups each in

the control segment treated with 0 mg/kg fish body weight of albendazole, the albendazole-treated segment at 5 mg/kg fish body weight, the albendazole-treated segment at 10 mg/kg fish body weight, the albendazole-treated segment at 20 mg/kg fish body weight, the albendazole-treated segment at 30 mg/kg fish body weight, and the albendazole-treated segment at 40 mg/kg fish body weight), and the fishes in each group were housed in a 200 L tank. Water injection into the tank was done under the same conditions as in Example 1. On the last day of acclimation, the test fishes were measured for weight and determined to weigh about 10 g. After acclimation, the test fishes were fed a test diet for 10 consecutive days. Administration of a test diet was performed once daily. The albendazole-medicated diets and the control diet were prepared by the same procedure as in Example 1. After administration of a test diet for 10 days, the fishes were fed a commercially available diet in an amount of 2% of the fish body weight. The rearing period was 40 days. The water temperature was held at about 20.1°C during the rearing period.

[0053] Upon completion of the rearing test, all the fishes in all segments were captured and observed by autopsy to determine the presence of cysts on the lateral muscles. Those fishes observed to have cysts were regarded as diseased fishes. Evaluation was done by the same procedure as in Example 2.

[0054] Results and discussion

The fishes in the albendazole-treated segment at 5 mg/kg fish body weight were observed to have cysts formed by the causative parasite of Beko disease, although the number of cysts formed was smaller than in the control segment (Table 7). On the other hand, no fish with cysts formed by this parasite was observed in the albendazole-treated segments at 10 mg/kg fish body weight or more. Therefore, the minimum effective dose of albendazole required to eliminate the causative parasite of Beko disease was considered to be about 10 mg/kg fish body weight.

[0055] [Table 7]

Test segment	Incidence rate	Number of cysts in diseased fish	Cyst length
Control segment (treated with 0 mg/kg fish body weight of albendazole)	17.5% (14 of 80 fishes)	8.3±5.1 cysts per fish	13.2±5.3 mm
5 mg/kg albendazole-treated segment	6.3% (5 of 80 fishes)	5.4±4.0 cysts per fish	11.0±5.9 mm
10 mg/kg albendazole-treated segment	0% (0 of 80 fishes)	--	--
20 mg/kg albendazole-treated segment	0% (0 of 80 fishes)	--	--
30 mg/kg albendazole-treated segment	0% (0 of 80 fishes)	--	--
40 mg/kg albendazole-treated segment	0% (0 of 80 fishes)	--	--

Example 6

[0056] <Investigation of the number of days of albendazole administration required to eliminate the causative parasite of Beko disease>

Juvenile yellowtails captured in nature were introduced into a sea cage at a fish farm and reared for 21 days. By means of this rearing in a sea cage, the juvenile yellowtails were naturally infected with the causative parasite of Beko disease. The juvenile yellowtails transferred again to the onshore facility were divided into 4 groups (90 fishes in the control segment treated with 0 mg/kg fish body weight of albendazole for 10 days, 70 fishes in the albendazole-treated segment at 40 mg/kg fish body weight for 3 days, 70 fishes in the albendazole-treated segment at 40 mg/kg fish body weight for 6 days, and 70 fishes in the albendazole-treated segment at 40 mg/kg fish body weight for 10 days). The fishes in each segment were housed in a 200 L tank. Water injection into the tank was done under the same conditions as in Example 1. Feeding on a test diet was started after acclimation for 2 days. Administration of a test diet was performed once daily. The test fishes weighed about 8 g at the start of the test. The albendazole-medicated diets and the control diet were prepared by the same procedure as in Example 1. After administration of a test diet, the

fishes were fed a commercially available diet in an amount of 3% of the fish body weight. The rearing period was 22 days. The water temperature was held at about 20°C for the first 10 days of the rearing period, and held at 22°C subsequently until the end of the rearing period.

[0057] Upon completion of the rearing test, all the fishes in all segments were captured and observed by autopsy to determine the presence of cysts on the lateral muscles. Those fishes observed to have cysts were regarded as diseased fishes. Evaluation was done by the same procedure as in Example 2.

[0058] Results and discussion

No diseased fish was observed in the albendazole-treated segment at 40 mg/kg for 10 days. On the other hand, there were some, albeit a few, diseased fishes observed in the albendazole-treated segments at 40 mg/kg for 3 or 6 days, but the incidence rate, number of cysts in diseased fish, and cyst length in these segments were evidently lower than those in the control segment -- these results showed that administration of albendazole even for such short periods as 3 or 6 days exhibited an anthelmintic effect on the causative parasite of Beko disease. Additionally, one fish each in the albendazole-treated segments for 3 or 10 days lost its weight and died because of refusal to eat diets. Since these fishes had not eaten any diets from the time of grouping, the death of these fishes was presumably affected by transportation and environmental changes.

[0059] [Table 8]

Test segment	Incidence rate	Number of cysts in diseased fish	Cyst length
Control segment	26.7% (24 of 90 fishes)	6.2±6.0 cysts per fish	10.4±8.5 mm
Albendazole-treated segment at 40 mg/kg for 3 days	4.3% (3 of 69 fishes)	4.0±2.6 cysts per fish	7.3±2.2 mm
Albendazole-treated segment at 40 mg/kg for 6 days	2.9% (2 of 70 fishes)	2.0±1.4 cysts per fish	3.0±1.0 mm
Albendazole-treated segment at 40 mg/kg for 10 days	0% (0 of 80 fishes)	--	--

Example 7

[0060] <Therapeutic effect of albendazole on fishes developing Boko disease>

Juvenile fishes captured in nature were introduced into a sea cage at a fish farm and reared for 50 days. By means of this rearing in a sea cage, the juvenile yellowtails were naturally infected with the causative parasite of Boko disease. The juvenile yellowtails transferred to an onshore facility were divided into 10 groups each consisting of 28 fishes (2 groups each in the control segment treated with 0 mg/kg fish body weight of albendazole for 10 days, the albendazole-treated segment at 10 mg/kg fish body weight for 3 days, the albendazole-treated segment at 10 mg/kg fish body weight for 10 days, the albendazole-treated segment at 40 mg/kg fish body weight for 3 days, and the albendazole-treated segment at 40 mg/kg fish body weight for 10 days). The fishes in each group were housed in a 200 L tank. Water injection into the tank was done under the same conditions as in Example 1. For the purpose of gaining an understanding of the incidence status of Boko disease at the start of the test, 29 fishes were investigated by autopsy to determine the number of cysts on the lateral muscles and the cyst length. Feeding on a test diet was started after acclimation for 3 days. Administration of a test diet was performed once daily. The test fishes weighed about 21 g at the start of the test. The albendazole-medicated diets and the

control diet were prepared by the same procedure as in Example 1. After administration of a test diet, the fishes were fed a commercially available diet in an amount of 2% of the fish body weight. In this test, the test diet was administered for 2 cycles during the rearing period. The test schedule is shown in FIG. 1. The water temperature was held at 22°C during the rearing period.

[0061] Upon completion of the rearing test, all the fishes in all segments were captured and observed by autopsy to determine the presence of cysts on the lateral muscles. Those fishes observed to have cysts were regarded as diseased fishes. Evaluation was done by the same procedure as in Example 2.

[0062] Results and discussion

All the albendazole-treated segments showed decreased values for incidence rate, number of cysts in diseased fish, and cyst length as compared to those values at the start of the test -- these results revealed that albendazole exerts an anthelmintic effect even on diseased fishes previously observed to have cysts. The reason for an increased number of diseased fishes in the control segment at the end of the test was considered to be because some parasites had not initially reached the cyst formation stage but subsequently formed cysts, and this demonstrated once again the inhibitory effect of albendazole on cyst formation. Also, it was shown that even administration of 10 mg/kg fish body weight of albendazole for 3 days exerts a therapeutic effect and an inhibitory effect on cyst formation. Additionally, there were several fishes which were reported to die in each of the test segments. The dead fishes were all thin, and the cause of death of these fishes was presumably due to refusal to eat diets as in the cases of death in Example 6.

[0063] [Table 9]

Test segment	Incidence rate	Number of cysts in diseased fish	Cyst length
At the start of test	17.2% (5 of 29 fishes)	9.2±2.4 cysts per fish	11.2±6.4 mm
Control segment	38.5% (20 of 52 fishes)	7.6±7.3 cysts per fish	17.8±4.9 mm
Albendazole-treated segment at 10 mg/kg for 3 days	11.5% (6 of 52 fishes)	6.7±3.4 cysts per fish	9.4±5.3 mm
Albendazole-treated segment at 10 mg/kg for 10 days	10.4% (5 of 48 fishes)	5.0±3.5 cysts per fish	8.6±3.6 mm
Albendazole-treated segment at 40 mg/kg for 3 days	10.4% (5 of 48 fishes)	2.6±2.3 cysts per fish	7.4±4.7 mm
Albendazole-treated segment at 40 mg/kg for 10 days	12.2% (6 of 49 fishes)	6.3±4.9 cysts per fish	8.7±4.7 mm

Example 8

[0064] <Anthelmintic effects of benzimidazole-based drugs against the myxosporidian parasite *Kudoa yasunagai*>

This example investigated the anthelmintic effects of different benzimidazole-based drugs against *Kudoa yasunagai*. Juvenile fishes produced in the same way as in Example 1 were transferred to an offshore sea cage, reared for 10 days, and transferred back to an onshore rearing facility. By means of this rearing in a sea cage, the juvenile yellowtails were naturally infected with *Kudoa yasunagai*. The juvenile yellowtails transferred again to the onshore facility were divided into 10 groups each consisting of 40 fishes (2 groups each in the control segment, the albendazole-treated segment, the febantel-treated segment, the triclabendazole-treated segment, and the flubendazole-treated segment), and the fishes in each group were housed in a 200 L tank. Water injection into the tank was done under the same conditions as in Example 1. On the last day of acclimation, the test fishes were measured for weight and determined to weigh about 10 g. After acclimation, the test fishes

were fed a test diet for 10 consecutive days. The dose of administration of the benzimidazole-based drugs was 40 mg/kg fish body weight/day, given once daily. The benzimidazole-based drug-medicated diets and the control diet were prepared by the same procedure as in Example 1. After administration of a test diet for 10 days, the fishes were fed a commercially available diet in an amount of 2% of the fish body weight. The rearing period was 40 days. The water temperature was held at about 20.5°C during the rearing period.

[0065] Upon completion of the rearing test, all the fishes in all segments were captured, and brain smears were made from the fishes, stained with Diff-Quick stain, and observed at 400-fold magnification at 40 different angles under an optical microscopy to determine the presence and number of spores. Evaluation was done by comparing the control segment with each of the benzimidazole-based drug-treated segments, in terms of detection rate ($\text{number of fishes with detected spores} / \text{number of test fishes} \times 100$) and the number of spores in individual fish with detected spores.

[0066] Results and discussion

The albendazole- and flubendazole-treated segments showed evidently lower values for detection rate and number of spores as compared to the control segment. On the other hand, the febantel-treated segment had a larger number of spores as compared to the control segment. Therefore, these results revealed mainly the following findings: albendazole and flubendazole exerts an anthelmintic effect against *Kudoa yasunagai*; and febantel augments the proliferation of this parasite.

[0067] It was also demonstrated that not all benzimidazole-based drugs have an anthelmintic effect against *Kudoa yasunagai*, and that not all of those drugs which are proved to be anthelmintic against Boko disease necessarily have an anthelmintic effect against *Kudoa yasunagai*.

[0068] Additionally, 7 fishes and 1 fish were reported to die in the control segment and the albendazole-treated segment, respectively. The dead fishes were all thin, and the cause of death of these fishes was presumably due to the same reason as in Example 6.

[0069] [Table 10]

Test segment	Detection rate	Number of spores in individual fish with detected spores
Control segment	52.1% (38 of 73 fishes)	4.0±4.2 spores per fish
Albendazole-treated segment	35.4% (28 of 79 fishes)	2.9±2.1 spores per fish
Febantel-treated segment	50.0% (40 of 80 fishes)	8.1±13.2 spores per fish
Triclabendazole-treated segment	55.0% (44 of 80 fishes)	4.3±6.7 spores per fish
Flubendazole-treated segment	31.3% (25 of 80 fishes)	2.9±2.6 spores per fish

INDUSTRIAL APPLICABILITY

[0070] The present invention can provide antiparasitic agents capable of controlling Beko disease caused by microsporidia parasitic in marine fishes, or myxosporean diseases through oral medication.

CLAIMS

1. A therapeutic agent for Beko disease or a myxosporean disease in a marine fish, the therapeutic agent comprising, as an active ingredient, any of albendazole, febantel, fenbendazole, oxfendazole, mebendazole, and flubendazole.
2. The therapeutic agent for Beko disease according to claim 1, wherein the Beko disease is caused by a parasite which is a microsporidian parasite belonging to the genus *Microsporidium* or the genus *Spraguea*.
3. The therapeutic agent for a myxosporean disease according to claim 1, wherein the myxosporean disease is caused by a parasite which is a myxosporidian parasite belonging to any of the genus *Kudoa*, the genus *Enteromyxum*, and the genus *Leptotheca*.
4. The therapeutic agent according to any of claims 1 to 3, wherein the marine fish is a fish of the order *Perciformes*, the order *Pleuronectiformes* or the order *Tetraodontiformes*.
5. The therapeutic agent according to claim 4, wherein the fish of the order *Perciformes* is a fish belonging to the genus *Seriola*, the family *Sparidae*, or the genus *Thunnus*; wherein the fish of the order *Pleuronectiformes* is a fish belonging to the family *Paralichthyidae*; or wherein the fish belonging to the order *Tetraodontiformes* is a fish belonging to the family *Tetraodontidae*.
6. The therapeutic agent according to claim 5, wherein the fish belonging to the genus *Seriola* is any of yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gold striped amberjack (*Seriola lalandi*), Almaco jack (*Seriola rivoliana*), Guinean amberjack (*Seriola carpenteri*), lesser amberjack (*Seriola fasciata*), samsonfish (*Seriola hippos*), fortune jack (*Seriola peruana*), and banded rudderfish (*Seriola zonata*); wherein the

fish belonging to the family *Sparidae* is any of Okinawa seabream (*Acanthopagrus sivicolus*), king soldier bream (*Argyrops bleekeri* Oshima), yellowback seabream (*Dentex tumifrons*), crimson seabream (*Evynnis tumifrons*), red seabream (*Pagrus major*), blackhead seabream (*Acanthopagrus schlegelii*), and goldlined seabream (*Rhabdosargus sarba*, *Sparus sarba*); wherein the fish belonging to the genus *Thunnus* is any of Pacific bluefin tuna (*Thunnus orientalis*), Atlantic bluefin tuna (*Thunnus thynnus*), southern bluefin tuna (*Thunnus maccoyii*), bigeye tuna (*Thunnus obesus*), albacore tuna (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), longtail tuna (*Thunnus tonggol*), and blackfin tuna (*Thunnus atlanticus*); wherein the fish belonging to the family *Paralichthyidae* is any of olive flounder (*Paralichthys olivaceus*), California flounder (*Paralichthys californicus*), summer flounder (*Paralichthys dentatus*), fivespot flounder (*Pseudorhombus pentophthalmus*), cinnamon flounder (*Pseudorhombus cinnamomeus*), ocellated flounder (*Pseudorhombus dupliciocellatus*),argetooth flounder (*Pseudorhombus arsius*), and pygmy whiff (*Tarphops oligolepis*); or wherein the fish belonging to the family *Tetraodontidae* is tiger puffer (*Takifugu rubripes*) or purple puffer (*Takifugu porphyreus*).

7. The therapeutic agent according to any of claims 1 to 6, wherein the therapeutic agent is intended for orally administering the active ingredient at a dose of 5 to 100 mg/kg fish body weight per day.