ASSURING THE SAFETY, QUALITY AND EFFICACY OF VETERINARY MEDICINES

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MUTUAL RECOGNITION PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

AquaVac ERM Concentrate for Dip Suspension for Rainbow Trout

PuAR correct as of 09/02/2018 when RMS was transferred to IE. Please contact the RMS for future updates.

MODULE 1

PRODUCT SUMMARY

EU Procedure number	UK/V/0210/001
Name, strength and pharmaceutical form	AquaVac ERM Concentrate for Dip Suspension for Rainbow Trout
Applicant	Intervet UK Ltd Walton Manor Walton Milton Keynes Buckinghamshire MK7 7AJ
Active substance(s)	*Yersinia ruckeri*
ATC Vetcode	QI10BB03
Target species	Rainbow Trout
Indication for use	In Rainbow Trout of 2 grams weight or over: Active immunization against Enteric Redmouth disease (ERM) to reduce mortality caused by the Hagerman Type I strain of *Yersinia ruckeri.* 336 degree days are required for the development of full immunity (28 days at a water temperature of 12°C). The time for development of protective immunity will depend on water temperature. A duration of immunity of 78 days has been shown under laboratory conditions. Under field conditions, protection may be expected for at least 6 months. A booster vaccination administered 4 months after primary vaccination may induce a better level of protection.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Medicines Agencies (veterinary) (HMA(v)) website (www.hma.eu).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Mutual Recognition application in accordance with Article 32 (2) of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	27th July 2005
Date product first authorised in the Reference Member State (MRP only)	3rd August 2004
Concerned Member States for original procedure	Austria, Czech republic, Denmark, France, Germany, Greece, Ireland, Italy, Norway, Poland, Portugal, Slovakia, Slovenia, Spain

I. SCIENTIFIC OVERVIEW

AquaVac ERM Concentrate for Dip Suspension for Rainbow Trout is an inactivated bacterial vaccine which is intended for use in rainbow trout to reduce mortality due to enteric redmouth (ERM) disease.

ERM is a sub-acute to acute systemic infection caused by the bacterium *Yersinia ruckeri*. It is principally a disease of rainbow trout but all salmonid species are considered as potential hosts and clinical disease has also been reported in other species. The disease is most severe in fingerlings at temperatures of 15°C - 18°C, whilst in larger fish the disease is less severe and more chronic. Whilst fish usually have to be exposed to large numbers of bacteria for the disease to develop, asymptomatic carrier infection can develop after exposure to low levels of bacteria, and clinical disease may then occur as a result of increased stress. Whilst ERM has been controlled using broad spectrum antibiotics, resistance has developed in many areas. In addition, it has been reported that disease can recur after antibiotic treatment has finished. Thus, vaccines are considered the most economical and effective means of controlling the disease.

AquaVac ERM Concentrate for Dip Suspension for Rainbow Trout consists of formaldehyde inactivated cultures of *Y. ruckeri* serotype I (Hagerman). It is supplied in one litre containers for dilution 1 in 10, prior to administration to fish by immersion.

Vaccination is not recommended for fish intended as broodstock, nor for fish weighing less than 2 grams.

II. QUALITY ASPECTS

A. Composition

The product contains inactivated cells of *Yersinia ruckeri* (Hagelman type I strain, relative percentage of survival in rainbow trout ≥ 75% after vaccination. The excipients are formaldehyde and sodium chloride solution.

The container systems are high density polyethylene bottles, closed with a rubber stopper and sealed with an aluminium cap containing 1 litre of vaccine. The particulars of the containers and controls performed are provided and conform to the regulation. The bottles and stoppers comply with the requirements of the European Pharmacopoeia. There is no pharmacopoeial monograph for the aluminium overseals as these do not come into contact with the product.

The choice of the vaccine strain and inactivating agent are justified. The inactivation process and the detection limit of the control of inactivation are correctly validated. The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

AquaVac ERM Concentrate for Dip Suspension for Rainbow Trout has been developed for the active immunisation of susceptible rainbow trout against ERM. Its main effect is to reduce mortality. The Hagerman Type I strain of *Y. ruckeri* was chosen for inclusion in the vaccine because it is the most common and most virulent serotype, and vaccines based on Type I strains appear to be protective against infection by other serotypes.

The product has a simple formulation, in which the inactivated bacteria are suspended in saline.

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site. Process validation data on the product have been presented in accordance with the relevant European guidelines.

C. Control of Starting Materials

Vaccine Antigen

There is no pharmacopoeial monograph for the active substance, *Y. ruckeri*. It was first obtained from a diseased rainbow trout in the Hagerman Valley region of Idaho in 1976. It was stored on a suitable culture medium and, when needed, the stock was expanded by inoculating some of the bacteria into rainbow trout and collecting the new bacteria from the kidneys of these fish. The stock bacteria are now stored in a freeze-dried state, using a stabilising solution.

When required for manufacture of the product, a bulk supply of bacteria is produced using a well-established microbial culture process in which a sample of stock bacteria is taken and successively cultured under controlled conditions until there are enough bacteria to start a culture in a production-scale fermentation vessel. This culture is monitored to determine the time of optimum bacterial growth. At this stage, the pH of the culture is adjusted and the bacteria are inactivated by the addition of formaldehyde, a process which has been shown to be effective.

Checks are made to ensure that the correct bacteria have been produced, that they are not contaminated with other organisms or agents and that they have been inactivated.

Other Substances

Other ingredients include the formaldehyde solution which is used to inactivate the bacteria, hydrochloric acid, sodium hydroxide, sodium chloride and purified water, all of which comply with the relevant requirements of the European Pharmacopoeia. The stabilising solution in which the bacteria are stored includes some ingredients which comply with the requirements of the European Pharmacopoeia and others for which there are no such requirements. In this latter case, the company has identified the source of each ingredient, explained how its quality is controlled and provided relevant certificates of analysis. Not listed in a pharmacopoeia are the working and master seeds, bovine serum albumin and tryptone broth.

D. Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

All components of the product have been demonstrated to comply with relevant guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicines.

E. Control tests during production

The tests performed during production are described and the results of 3 consecutive runs, conforming to the specifications, are provided.

F. Control Tests on the Finished Product

The product is manufactured in accordance with Good Manufacturing Practice and, where applicable, conditions, equipment and materials are sterile. The volume of active ingredient is calculated and mixed with the required quantity of saline to give the desired concentration of bacteria in the finished product. The pH is adjusted if necessary.

In-process control tests have been described in detail, and include tests for sterility, inactivation of the bacteria, amount of residual formaldehyde, pH, appearance and fill volume.

Finished Product Quality Control

The tests conducted after manufacture include checks on the appearance of the product and its sterility, and the safety and potency of the product for rainbow trout. The results of the tests on three batches of the product have been provided, and these demonstrate that a product which consistently meets the agreed specification can be produced.

G. Stability

Stability data on the active substance have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

Finished Product
The company provided data on three batches of product stored refrigerated for 27 months. At the end of this time, each batch complied with the quality control criteria. On the basis of this information, a shelf-life of 24 months has been agreed.

In-Use
The product should be stored in a refrigerator at between 2°C - 8°C, and should be protected from light. The product is not to be frozen. Product diluted for use should be used within 5 hours.

The in-use shelf-life of the reconstituted vaccine is supported by the data provided.

H. Genetically Modified Organisms

Not applicable.

J. Other Information

The product should be stored in a refrigerator at between 2°C - 8°C, and should be protected from light. The product is not to be frozen. Product diluted for use should be used within 5 hours.

III. SAFETY ASSESSMENT

Introduction

The vaccine is intended for use in fish weighing 2 g or more, and a primary course consists of the immersion of fish for 30 seconds in a 1 in 10 dilution of the product. 1 litre of vaccine will treat 100 kg of fish. A booster vaccination administered 4 months after the primary vaccination may induce a better level of protection.

Safety and efficacy data were generated for an earlier version of the same product, (AquaVac ERM), using one of two formulations, which were essentially

the same apart from diluents. The provision of results from these previous studies was considered acceptable. The use of different diluents was deemed to have no impact on the finished product. Some assays cited below were performed in order to test both the safety and efficacy of the product, (numbered correspondingly within the relevant sections).

Laboratory trials

In the first study (1), the efficacy of AquaVac ERM was tested on fish of approximately 1 g. A suitable number of fish were divided into three groups. One group was immersed in AquaVac ERM vaccine at a 1 in 10 dilution for 30 seconds, a second group were immersed in vaccine at a 1 in 5 dilution for 60 seconds (overdose), and a third batch of fish were sham-vaccinated as negative controls. The fish were left for 28 days after which they were challenged with *Y. ruckeri* by immersion. The fish were then monitored for a further 21 days. All fish vaccinated at a 1 in 10 dilution survived. Fish given the 1 in 5 diluted product produced a 10% mortality rate. The vaccine was demonstrated to be safe for fish of approximately 1 g, when used at the recommended dose.

A further study (2) followed the same format as described above, with five groups of 50 fish between 0.8 0 - 0.9 g treated to a single dose or an overdose of the product. A control group were also analysed. A further series of tests observed seven groups of 50 fish at weights between 1.0 g and 1.25 g. Again, the same protocol was followed. No deaths occurred in tests, demonstrating that the product is safe for use when administered at the recommended dose.

An extinction test (3) was performed in which the safety of a single dose and a repeated dose were analysed. The product was administered at a 1 in 10 dilution to rainbow trout on a commercial farm. A suitable number of fish were immersed according to the recommended dose. No adverse reactions were seen.

A further study investigated a comparison of immersion times (4) in 12 g rainbow trout. The product was diluted 1 in 10 as recommended. Four out of five groups of fish were immersed in vaccine for 10, 30, 60 or 120 seconds respectively, with the fifth group acting as unvaccinated controls. No deaths were seen in any vaccinated groups. A dilution-effect study (5), with product being diluted at half $\log_{10}$ intervals over a range of 10^{-1} to 10^{-4} also had no detrimental effect on tested fish.

A study was performed which analysed the safety of a single dose of AquaVac ERM Oral vaccine in fish previously primed with immersion in AquaVac ERM. No deaths occurred in any vaccinated groups. A further study on the safety of one administration of an overdose did not produce any unforeseen effects. Further studies were performed demonstrating that AquaVac ERM was safe for fish of the smallest recommended size

No studies on reproductive performance were done as the vaccine is not indicated for use in broodstock.

Field studies

A suitable number of fish fry, weighing between 2.7 - 3.1 g were exposed to a primary immersion in AquaVac ERM and then divided into four groups. Six months later, the first group of fish were given a booster dose of AquaVac ERM Oral, the second group AquaVac ERM by immersion, the third group AquaVac ERM by injection and the fourth group were not given a booster. No adverse reactions due to the vaccine were seen in tested fish.

A second trial analysed the safety and efficacy of AquaVac ERM by immersion (6). Two groups of fish, once vaccinate group and one control group were exposed to a natural infection of ERM. No adverse reactions due to the vaccine were seen.

In a third study, a suitable number of fish were immersion vaccinated before being divided into four groups. Booster vaccination was given four months later to two groups, two groups were not boosted. No adverse reactions attributable to the vaccine were seen. A final study performed at a different premises supported findings that the vaccine was safe to use when used as directed (7).

CONCLUSIONS ON SAFETY AND RESIDUES

The company provided data which comply with the legal requirements and provide adequate information to assess the safety of the product.

The laboratory tests and field trials demonstrated the overall safety of the vaccine for rainbow trout weighing 2 g and over, when administered as recommended. Overdose studies showed that the product has a good margin of safety. There has been no evaluation of the effects of the vaccination on reproductive performance as the vaccine is not intended for use in broodstock.
A zero days withdrawal period is acceptable.

Conclusions on User Safety

A user risk assessment indicates that the product does not present a particular hazard for those administering the vaccine to fish. However, because of the likelihood of skin contact with the product, it is recommended that rubber gloves should be worn during the vaccination procedure.

Conclusions on Consumer Safety

None of the ingredients of AquaVac ERM Concentrate for Dip for Suspension for Rainbow Trout are such as would cause unacceptable residues in treated fish. There is no need for a withdrawal period and no consumer safety concerns.

Ecotoxicity

An environmental risk assessment indicates that the risk to the environment from the use of AquaVac ERM Concentrate for Dip Suspension for Rainbow Trout is minimal. The product is formaldehyde inactivated and contains no live organisms. Fish are vaccinated in an enclosed environment with waste water being commonly channeled through settling tanks before release into a

waterway, with spent vaccine being diluted in farm drainage. In addition, the fish oil in the product makes the pellets hydrophobic, which minimizes leaching into the environment. General advice for the disposal of any unused product is provided in the summary of product characteristics:

Any unused veterinary product or waste material derived from such veterinary medicinal products should be disposed of in accordance with local requirements.

The approved product label in different member states may provide further information on how to meet these requirements.

IV CLINICAL ASSESSMENT (EFFICACY)

Clinical Studies

The vaccine is intended for use in fish weighing 2 g or more, and a primary course consists of immersion for 30 seconds in a 1 in 10 dilution of the product.

Laboratory Trials

The efficacy of the vaccine was checked using a challenge test. A suitable number of fish of between 5 - 15 g were immersed in appropriately diluted product for 30 seconds. Vaccinates and controls were challenged with *Y. ruckeri* and monitored for 21 days. The decline in numbers in control fish was as expected.

A test was performed in order to evaluate the efficacy of AquaVac ERM on rainbow trout (1).
Two batches of fish were treated with either AquaVac ERM at the recommended dose or at a 1 in 5 dilution for 60 seconds, which comprised an overdose. A thirds group of fish were sham-vaccinated. No adverse reactions were seen.

A further test (2) observed five groups of 50 fish between 0.8 0 - 0.9 g, treated to a single dose or an overdose of the product. A control group was also analysed. A further series of tests observed seven groups of 50 fish at weights between 1.0 g and 1.25 g. Again, the same protocol was followed. No deaths occurred during tests, demonstrating that the product is safe for use when administered at the recommended dosage. The relevant potency criteria were attained. An extinction test (3) found that the product was efficacious when used as directed.

A comparison of immersion times on the efficacy or AquaVac ERM was performed (4). A suitable number of four groups of fish weighing approximately 12 g were immersed in appropriately diluted AquaVac ERM for 10, 30, 60 or 120 seconds. A control group were not vaccinated. After challenge, no deaths occurred in any fish receiving the vaccine.

A study was performed to examine the effect of dilution on the efficacy of AquaVac ERM using suitable groups of 6 g rainbow trout (5). The vaccine was diluted at half-$\log_{10}$ intervals over a range of 10^{-1} to 10^{-4}. After challenge, no deaths attributable to the vaccine were seen. A further study analysed the efficacy of AquaVac ERM in rainbow trout fry weighing approximately 1 g, no deaths occurred that were attributable to vaccination or sham vaccination, and

after challenge, mortality in the control group was 98% compared to 18% in the vaccinated group.

A study was conducted investigating the safety and efficacy of AquaVac ERM Oral and AquaVac ERM vaccines, administered as primer and booster vaccinations. Three groups of suitable numbers of fish weighing 2 g were immersed in AquaVac ERM and observed over 30 days. A further group of fish were used as controls. A certain number of fish were challenged with *Y.ruckeri* 53 days after vaccination. Other fish were challenged 78 days after vaccination. Fish in one group were given a booster vaccination with AquaVac ERM by immersion 46 days after vaccination and challenged after a further 32 days. Another group of fish were given a booster vaccination with AquaVac ERM Oral 46 days after primary vaccination and subjected to only the second challenge. Other goups of fish were challenged 53 or 78 days after first vaccination. A decline in protection was observed with AquaVac ERM alone between days 30 and 90. Fish which started booster vaccinations at day 30 showed a good level of protection.

A laboratory study was performed in order to determine the time required for the development of immunity in 1 g rainbow trout, following immersion with AquaVac ERM vaccine. A suitable number of 1 g fish were vaccinated, and the same number of fish being sham vaccinated; acting as controls. The fish were challenged at days 7,14 and 21 and observed for 21 days. Mortality rates reflected a rapid onset of immunity.

Field Trials

A series of trials were performed for AquaVac ERM. A number of historical, non-GLP[1] studies from the early 1980s affirmed the efficacy of Aquavac ERM.

A more recent study evaluated the safety and efficacy of AquaVac ERM in 1g rainbow trout by immersion (6). A large number of fish were selected on a fish farm infected with ERM during the period of the study. The fish weighed 1 g on average. A large number of trout were also selected as controls. Both groups of fish were exposed to a natural infection of ERM. Any mortality seen in vaccinated fish was always below that of controls.

A further study was performed to evaluate the safety and efficacy of AquaVac ERM vaccine administered by immersion and injection, and of AquaVac ERM Oral used as a booster injection. A large number of fishing ranging in size from 2.7g to 3.1g were vaccinated. A booster dose was given by immersion 6 months later with either AquaVac ERM Oral or AquaVac ERM, or an injection was given with AquaVac ERM. A control group were not vaccinated. Weekly mortality rates were lower in boosted fish than in non-boosted fish, in the presence of a natural infection of ERM.

A final study analysed AquVac ERM vaccine, AquaVac ERM Oral vaccine, AquaVac Vibrio vaccine and AquaVac Vibrio Oral vaccine on a farm naturally infected with ERM (7). Two groups of rainbow trout, one of which was used as control group, were used in the study. Group one were vaccinated by immersion for 60 seconds in a mixture of AquaVac ERM and AquaVac Vibrio. After 77

[1] Good Laboratory Practise.

days, the fish were boosted with the same mixture. The second groups were used as negative controls. An RPS[2] of 87.63% was established for the vaccinated group.

CONCLUSIONS ON EFFICACY ASPECTS

The laboratory studies showed that AquaVac ERM, when administered by immersion to fish weighing at least 2 g in a 1 in 10 dilution, is effective at reducing or even preventing mortality due to ERM disease. At water temperatures of 12°C, immunity was shown to have fully developed by 28 days after vaccination and to last for 78 days. Under field conditions, protection may be expected to last for at least 6 months.

It is noted that the development of immunity in fish is affected by the water temperature. The data in these studies were collected from fish kept in water at a constant 12°C, and the advice to users of the product therefore explains that the time in field conditions will depend on the temperature. At 12°C it takes 28 days, which is expressed as 12 x 28, i.e. 336 degree days. If the temperature is lower, the actual number of days will increase and if it is higher the actual number of days will decrease.

Field trials on commercial fish farms confirmed the efficacy of the product and indicated that long-term efficacy is enhanced by administering a booster four months after the primary vaccination.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the benefit/risk profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

[2] RPS – Relative Percentage Survival.

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)

The post-authorisation assessment (PAA) contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

The PAA for this product is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)