

PHARMAQ

ALPHA JECT micro[®] 1Noda

**THE FIRST COMMERCIAL
VACCINE AGAINST RGNNV
IN EUROPE**



zoetis

ALPHA JECT *micro*® 1Noda

THE FIRST COMMERCIAL V

PHARMAQ's vaccines are manufactured at our well recognized facility in Norway.



A GLOBAL LEADER IN VACCINES FOR AQUACULTURE

PHARMAQ is the global leader in vaccines and innovation for aquaculture and part of Zoetis, the world leader in animal health. The company provides environmentally sound, safe and efficacious health products to the global aquaculture industry through targeted research and the commitment of dedicated people. Production facilities, administration and research and development activities are based in Norway.

Our focus on quality is absolute in all steps of production, from raw materials to finished products. Quality is built in every operation, from development to the final product is approved by the authorities and released.

As a pharmaceutical company, we are subject to very stringent demands, regulated in the principles and guidance of Good Manufacturing Practice (GMP) and are on a regular basis, approved according to GMP by the Norwegian Medicines Agency (NoMA).



L VACCINE AGAINST RGNNV IN EUROPE



DELIVERING SOLUTIONS TO PREDICT, PREVENT AND TREAT DISEASE

We put pride in being flexible and create customer satisfaction through high quality service and products. By preventing or treating against diseases our products contribute to improving animal welfare, environment and food safety. In the end, farmers can grow their business and improve their productivity. By combining our broad competencies, experiences and the commitment of our people with dynamic decision processes and ability to adapt to changes, we will remain in the forefront of new technologies and product launches.

“ALPHA JECT micro® 1 Noda has been developed in response to fish farmers’ needs in the Mediterranean to fight against the most common viral disease affecting farmed sea bass in the Mediterranean. PHARMAQ is committed to the continuous development and supply of innovative health solutions for fish farmers globally.”

Morten Nordstad,
President of PHARMAQ

KEY FACTS ABOUT PHARMAQ

- More than 30 years of dedicated R&D and high tech efforts
- Solid competence within biotechnology
- Invest resources to high-impact projects
- Focus on biologicals, therapeutics, diagnostic and technical support
- Quality is the basis for all our activity and production

WE MAKE AQUACULTURE PROGRESS



Viral Nervous Necrosis (VNN), also called Viral Encephalopathy and Retinopathy (VER), is considered the most important disease in the Mediterranean area; this is linked to the economic impact of the disease, caused both by the mortality rate and the growth reduction in the affected batch.

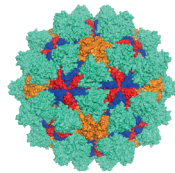


Conclusions of "Fish Health Mediterranean Aquaculture meeting", EAFP Congress of 2015.

VIRAL NERVOUS NECROSIS, A TECHNICAL UPDATE

→ AETIOLOGICAL AGENT

The causative agent of VNN is classified into the genus Betanodavirus within the family Nodaviridae.

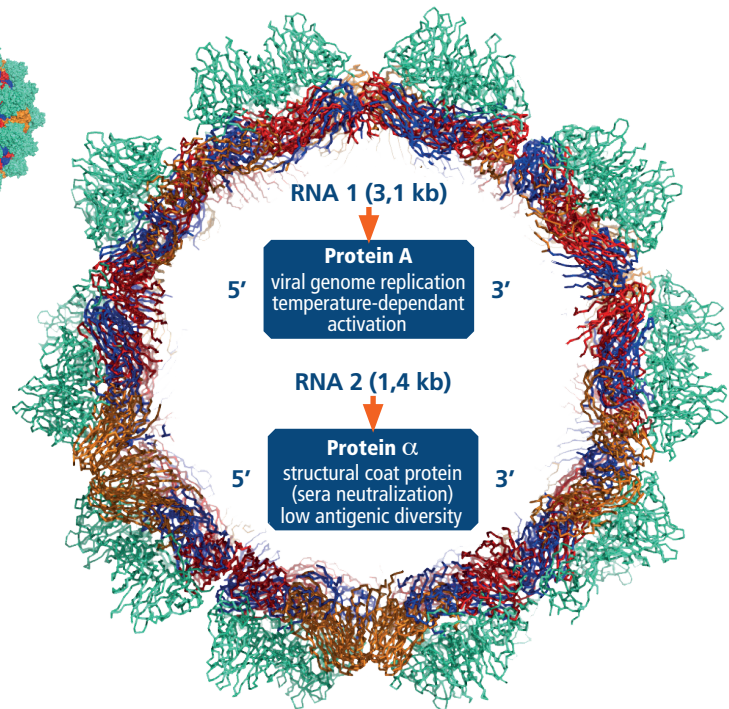


→ VIRUS DESCRIPTION

Nodavirus is a small, non-enveloped virus with an icosahedral-shaped capsid of 25 nm diameter that surrounds the genetic material. This genetic material is made up of two molecules of single-stranded positive-sense RNA¹.

→ NODAVIRUS CLASSIFICATION

Betanodaviruses are clustered in four different genotypes, based on a variable region of RNA1, and in three serotypes according to polyclonal antibodies virus neutralization^{2,3,4}.



<http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1005203>

Every genotype correlates with different host species and in optimal growth temperature^{3,4}:

GENOTYPE	SEROTYPE	TARGET HOST FISH	OPTIMUM GROWTH TEMPERATURE
Striped Jack Nervous Necrosis Virus - SJNNV	A	Striped jack	20-25°C
Tiger Puffer Nervous Necrosis Virus - TPNNV	B	Tiger puffer	20°C
Barfin Flounder Nervous Necrosis Virus - BFNNV	C	Cold-water fish: Atlantic halibut, Atlantic cod, flounders, etc.	15-20°C
Red-spotted Grouper Nervous Necrosis Virus - RGNNV	C	Warm-water fish: Asian sea bass, European sea bass, groupers, etc.	25-30°C

Viral Nervous Necrosis caused by an RNA virus belonging to Betanodavirus genus and may affect different fish species and all production stages of sea bass. Due to its viral nature, no treatments are feasible neither prevention tool was available up to date.

Officially reported from most worldwide regions except South America, linked to intensive production of marine species^{5,6}.

RGNNV genotype is the most common in the Mediterranean area due to its broader host range and to the specific higher water temperature requirements.



➔ **EPIZOOTIOLOGY OF NODAVIRUS INFECTION**

Betanodaviruses are highly resistant in the aquatic environment and the disease has been reported in more than 50 fish species⁷.

INFLUENCING FACTORS IN BETANODAVIRUSES

Age	Mortality rate is age-dependent, so the earlier the disease signs occur, the greater is the rate of mortality, often reaching 100% mortality in larval stages ⁸ .
Water temperature	Higher mortality at temperatures around 25°C, temperatures > 31°C inhibit the proliferation of RGNNV ⁹ .
Stress	Chronic stress may suppress the immune system of the fish and increase the probability of outbreaks

INFECTION SPREADING

- **Horizontal transmission** is the most common way of disease transmission¹⁰. But also in some species **vertical transmission** may play a key role, being the virus detected in brood stock gonads and even in early stage development embryos¹¹.
- Water is the most important abiotic vector spreading the infection during a clinical outbreak⁷.
- Cannibalism on dead fish may contribute to spreading the virus¹²
- **Reservoirs** in the wild are the original source of infection of farmed populations¹³; trading of infected juveniles contributes to spread the virus geographically⁷.



>>> VIRAL NERVOUS NECROSIS, A TECHNICAL UPDATE

➔ CLINICAL SIGNS, LESIONS AND DIAGNOSIS

Quite unspecific **external clinical** signs are observed, barely a progressive change in pigmentation and some cutaneous erosion in cranial areas due to traumatic lesions caused by visual disturbances.

Main **behaviour signs** of the disease are caused by the viral replication in the cells of brain¹⁴ and retina, and include:

- Uncoordinated swimming and nervous signs, including erratic swimming behaviour patterns (spiralling, whirling or belly up at rest)^{15, 16}.
- Swim bladder hyperinflation, diseased fish are often seen at the surface.
- Flexing of the body, muscle tremors.
- Anorexia, lethargy, exophthalmus...

Histopathology reveals extensive vacuolation in nervous tissue, brain, spinal cord and nervous layer of the retina. Malacia and gliosis are also observed often with intracytoplasmic inclusions⁵.

Diagnosis must be made after matching compatible clinical findings with positive results from different analysis⁷:

- Behavioural signs.
- Microscopic findings (vacuolation and necrosis) and virus visualization by electron microscopy.
- Virus isolation from SSN-1 or E-11 cell lines culture (Gold Standard for VNN diagnosis).
- Agent detection tools like direct ELISA antigen detection method, ImmunoHistoChemistry and ImmunoFluoresce technics from brain and retina tissue samples, and finally PCR-based methods (RT-PCR, Nested PCR or RealTime PCR).

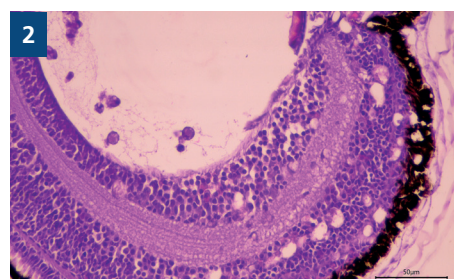
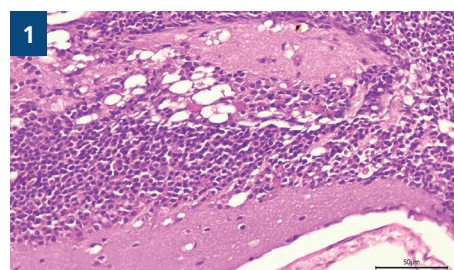


Brain congestion



Hyperinflated swim bladder

Property of Aquavet



Vacuolization in brain cells (1) and retina (2) of sea bass infected by VNN.

Images provided by ICTIOVET

ALPHA JECT *micro*[®] 1Noda

EMULSION FOR INJECTION FOR SEA BASS

ALPHA JECT *micro*[®] 1 Noda is the first vaccine licensed in EU to immunize sea bass against Nodavirus (RGNNV genotype). The first of a new micro-dose (0.05 ml) PHARMAQ generation vaccines at Mediterranean market, allowing vaccination of fish down to 12 g.

MAIN PRODUCT CHARACTERISTICS

Composition



Inactivated Red-spotted Grouper Nervous Necrosis Virus (RGNNV) ≥ 0.07 antigenicity units per dose (quantity of antigen measured in vaccine) in **emulsion for injection**.
Adjuvant: Liquid paraffin (mineral oil)

Indication



For active immunization of **European sea bass (*Dicentrarchus labrax*)** to reduce mortality caused by Red-spotted Grouper Nervous Necrosis Virus (RGNNV).

No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.

Posology



The recommended dose is **0.05 ml per fish of a minimum weight of 12 g**.
The vaccine should be administered by **intraperitoneal (IP) injection**.

Technical features



Multiple efficacy and safety studies have been performed:

- Safety has been evaluated by scoring of local reactions (adhesions and pigmentation) in the abdominal cavity, and efficacy has been documented by challenges up to 12 months post vaccination.
- The vaccine reduces mortality caused by Nodavirus (RGNNV genotype) in European sea bass up to 12 months post vaccination.
- Onset of immunity is documented at 466 degree-days after vaccination.



ALPHA JECT *micro*® 1Noda



- ➔ **Reduces mortality** caused by RGNNV in sea bass.
- ➔ A new generation of PHARMAQ's **micro dose** (0.05 ml per fish) vaccines.
- ➔ **Reduces fish size** at vaccination to **12 g.**
- ➔ **Onset of immunity:** 466 degree days.
- ➔ **Duration of immunity:** 1 year.

PACKAGING: Flexible and robust injection bags easy to handle and store.

REFERENCES:

1. Mori K et al. "Properties of a new virus belonging to nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis". *Virology* 187:368–371 (1992). 2. Nishizawa T et al. "Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene". *Appl Environ Microbiol* 63:1633–1636 (1997). 3. Iwamoto T et al. "Cloning of the fish cell line SSN-1 for piscine nodaviruses". *Dis. Aquat. Org.*, 43, 81–89 (2000). 4. Mori K et al. "Serological relationships among genotypic variants of betanodavirus". *Dis. Aquat. Org.*, 57, 19–26 (2003). 5. Shetty et al. "Betanodavirus of Marine and Freshwater Fish: Distribution, Genomic Organization, Diagnosis and Control Measures". *Indian J. Virol.* (July–September 2012) 23(2):114–123. 6. Panzarin et al. "In vitro study of the replication capacity of the RGNNV and the SJNNV betanodavirus genotypes and their natural reassortants in response to temperature". *Veterinary Research* 2014, 45:56. 7. 2017 © OIE. "Viral encephalopathy and retinopathy. Manual of Diagnostic Tests for Aquatic Animals - 13/07/2017". Chapter 2.3.12. 8. Breuil G et al. "Viral infection (picorna-like virus) associated with mass mortalities in hatchery reared sea-bass (*Dicentrarchus labrax*) larvae and juveniles". *Aquaculture*. 1991;97:109–16. 9. Yuasa K et al. "Effect of high water temperature on betanodavirus infection of fingerling humpback grouper *Cromileptes altivelis*". *Fish Pathol.*, 42, 219–221 (2007). 10. Castric J et al. "Sea bass *Sparus aurata*, an asymptomatic contagious fish host for nodavirus". *Dis Aquat Organ.* 2001;47:33–8. 11. Kuo et al. "Nervous necrosis virus replicates following the embryo development and dual infection with Iridovirus at juvenile stage in grouper". *PLoS ONE* (4): e36183 (2012). 12. Manin BO et al. "Experimental evidence of horizontal transmission of betanodavirus in hatchery-produced Asian seabass, *Lates calcarifer* and brown-marbled grouper, *Epinephelus fuscoguttatus* fingerling". *Aquaculture*. 2011;321:157–65. 13. Ciulli S et al. "Detection and characterization of Betanodavirus in wild fish from Sicily, Italy". *Ittiopatologia*, 3, 101–112 (2006). 14. Munday et al. "Betanodavirus infections of teleost fish: a review". *J Fish Dis* (25): 127-42 (2002). 15. Mori et al. "Properties of a new virus belonging to nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis". *Virology* (1): 368-71 (1992). 16. Comps et al. "Purification and characterization of two fish encephalitis viruses (FEV) infecting *Lates calcarifer* and *Dicentrarchus labrax*". *Aquaculture* (1-2): 1-10 (1994).

ALPHA JECT *micro*® 1 Noda, emulsion for injection for sea bass. Composition per dose (0,05 ml): Inactivated Red-spotted Grouper Nervous Necrosis Virus (RGNNV) strain ALV1107 ≥ 0.07 antigenicity units¹. ¹ quantity of antigen measured in vaccine (short version AgU). **Indications for use:** For active immunisation of sea bass to reduce mortality caused by Red-spotted Grouper Nervous Necrosis Virus (RGNNV). **Contraindications:** None. **Amounts to be administered and administration route:** The recommended dose is 0.05 ml per fish of a minimum weight of 12 g. Should be administered by intraperitoneal (IP) injection. Should be left to slowly reach 15 – 20 °C by keeping it at room temperature and should be well shaken prior to use. **Special warnings:** Vaccinate healthy animals only. **Withdrawal period:** Zero degree days. **Onset of immunity:** 466 degree days. **Duration of immunity:** 1 year. **Special storage precautions:** Store and transport refrigerated (2 °C – 8 °C). Do not freeze. Protect from light. **Package sizes:** 250 ml bag (5 000 doses) and 500 ml bag (10 000 doses). **Spanish MA no.:** 3597 ESP; **Italian MA no.:** Sacca da 250 ml A.I.C. n.105071017 and Sacca da 500 ml A.I.C. n.105071029; **Croatian MA no.:** UP71-322-05/17-01/405; **Greek MA no.:** 108576/1-12-2017. **Marketing Authorisation Holder:** PHARMAQ AS, 7863 Overhalla, Norway. **Under veterinary prescription.**

PHARMAQ Spain Aqua

Avda. de Europa 20B
Parque Empresarial La Moraleja
28108 Alcobendas, Spain
Tel: +34 91 419 19 35
Mob: +34 606 114 291
customer.service@pharmaq.no

PHARMAQ AS

Harbitzalléen 2A, 0275 Oslo, Norway
P.O.Box 267 Skøyen, 0213 Oslo, Norway
Tel: +47 23 29 85 00
Fax: +47 23 29 85 01
customer.service@pharmaq.no

AQUAVET SA

48, Antiochias Str.
14341 Athens, Greece
Tel: +30 210 2517807
Mob: +30 6932 494354
aquavet@otenet.gr

www.pharmaq.com

