



IRISH MEDICINES BOARD

Control of starting materials used in vaccine production – Extraneous agents

Location: Dublin, Date: 21st September 2005

Dr Una Moore,
Senior Immunological Assessor,
Department of Veterinary Medicine,
Irish Medicines Board

Control of starting materials – Why?

- Safety
 - Serious consequences
 - Administered via unnatural route
 - Intraperitoneal route
 - Low numbers of infectious particles may be sufficient to cause disease.



IRISH MEDICINES BOARD

Vaccines Contaminated with Extraneous Agents

- Veterinary vaccines:
 - 2002: An inactivated feline and a number of canine vaccines contaminated with a live feline parvovirus
 - 1999 - Bovine respiratory vaccine contaminated with bovine viral diarrhoea virus
 - 1994: Canine vaccine contaminated with bluetongue virus



IRISH MEDICINES BOARD

Biologicals Contaminated with Extraneous Agents

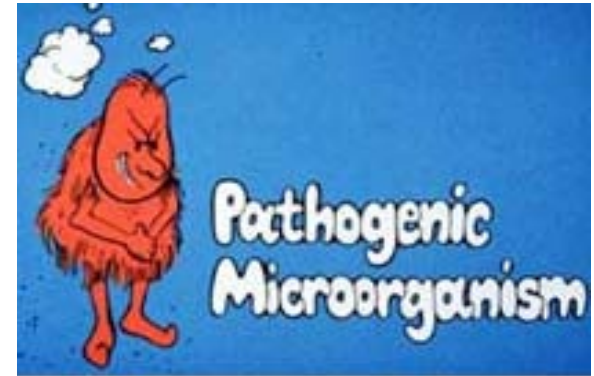
- Human Biologicals:
 - 2000 – polio vaccine recalled due to possible contamination with BSE
 - 1980 – 1990's blood products contaminated with HIV, Hepatitis A, B & C.
 - Gulf war syndrome - caused by mycoplasma contamination????



IRISH MEDICINES BOARD

Sources of Extraneous Agent Contamination

- Where does it come from?
 - Main source
 - biological materials used in manufacture
 - Breach of GMP,
 - Operator,
 - Cross contamination.



IRISH MEDICINES BOARD

Extraneous Agent Contamination

- Biological starting materials:
 - Viral or bacterial seeds,
 - Cell seeds,
 - Bovine serum, trypsin, other cell culture/bacteria supplements,
 - Gelatin/oil adjuvants



IRISH MEDICINES BOARD

Minimising the Risk of Extraneous Agent Contamination of Bacterial Seed

- Bacterial seed:
 - Characterisation of strain:
 - Purity
 - Gram strain
 - Biochemical profile: API sticks, e.g. growth rates
 - Morphology: shape/size, margin, elevation, colour & texture.
 - Identity by serological methods e.g. by western blot
 - No need to screen for viral contamination.



IRISH MEDICINES BOARD

Minimising the Risk of Contamination of Viral Seeds

- Viral seed lot:
 - Propagation: Only expand in cells and use biological materials shown to be free from extraneous agents.
 - Identification
 - Free from bacteria, fungi (Ph. Eur. 2.6.1) mycoplasma (Ph. Eur. 2.6.7) and extraneous viruses – Ph. Eur. 01/2005:0062
 - Screen for specific extraneous agents.
- Must not contain any other live organism.



IRISH MEDICINES BOARD

Minimising the Risks of Cell Bank Contamination

- Master and Working Cell Seeds:
 - Use cell lines where possible
 - Primary cells only where essential
 - Characterisation & Identification
 - Free from bacteria, fungal (Ph. Eur. 2.6.1), mycoplasma (Ph. Eur. 2.6.7) & extraneous viruses – Ph. Eur. 01/2005:50204
 - Screen for specific extraneous agents
- Must not contain any other live organism.



IRISH MEDICINES BOARD

Minimising the Risks of Viral Contamination

- Must screen the viral and cell seeds for:
 - Viral Haemorrhagic Septicaemia virus (VHSV) type I, II and III
 - Infectious Haematopoietic Necrosis Virus (IHNV)
 - Spring Viraemia of Carp Virus (SVCV)
 - Infectious Pancreatic Necrosis Virus (IPNV)
 - Bovine viral diarrhoea virus*

NFG: Specific requirements for the production and control of live and inactivated vaccines intended for fish.

*Ph. Eur. 01/2005.0062: Vaccines for veterinary use

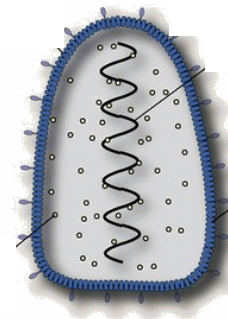
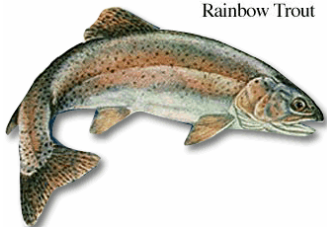


IRISH MEDICINES BOARD

Viral Haemorrhagic Septicaemia virus

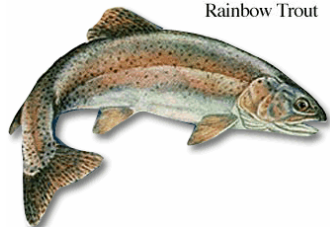
- VHSV:

- Affects marine and freshwater fish
- Four genotypes have been isolated – I, II, III and IV
- Three serotypes have been recognised
- Pathogenicity depends on host species.
- Continental Europe – Rainbow trout farming.
- *Rhabdovirus* family:
sRNA virus, enveloped.
(*Novirhabdovirus*)



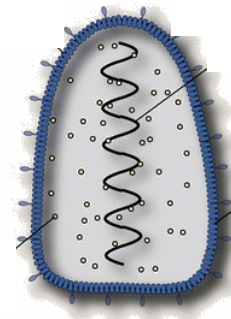
Infectious Haematopoietic Necrosis Virus

- IHNV:



Rainbow Trout

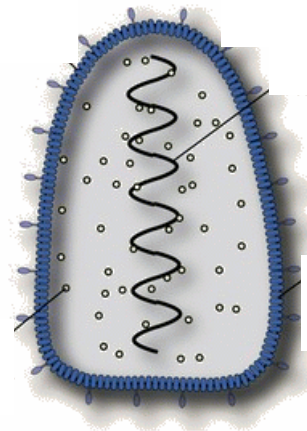
- Important disease of trout and salmon (80%)
- Geographical distribution: Western US; has spread to EU continent and Far East.
- Survivors demonstrate protective immunity and high levels of circulating antibodies.
- Antigen studies indicate one serotype.
- *Rhabdovirus* family
- sRNA virus, enveloped. (*Novirhabdovirus*)



IRISH MEDICINES BOARD

Spring Viraemia of Carp Virus

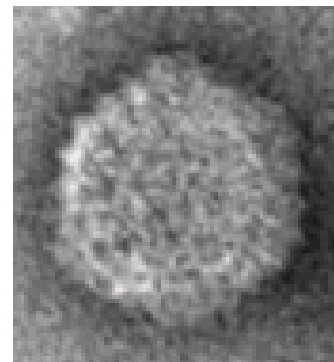
- SVCV
 - Important disease of carp – all ages, seasonal
 - Geographical distribution – European continent with low water temperatures in winter.
 - Survivors demonstrate protective immunity and high levels of circulating antibodies
 - Antigen studies indicate one serotype
 - Rhabdovirus family
 - sRNA virus, enveloped
 - (Vesiculovirus)*



Infectious Pancreatic Necrosis Virus

- IPN

- Important disease of salmonid species under intensive rearing conditions (10 – 90%)
- Geographical: Canada, Europe, North & South US, Asia.
- Survivors demonstrate protective immunity and high levels of circulating antibodies
- Wide antigenic diversity, two serotypes. Majority in serogroup A.
- *Birnavirus* family:
dsRNA, non-enveloped
(*Aquabirnavirus*)



IRISH MEDICINES BOARD

VHSV, IHNV, SVCV and IPNV Screening

	Sensitive cell lines	Visual effect	Detection of virus	Serological responses
VHSV	RTG-2 or BF-2 @15°C	CPE	IFAT, ELISA RT-PCR	Not acceptable
IHNV	BF-2 or EPC @ 15°C	CPE	IFAT, ELISA PCR, DNA probe	Not acceptable
SVCV	EPC or FHM @ 20°C	CPE	IFAT, ELISA	Not acceptable
IPNV	BF-2, CHSE-214 or RTG-2 @ 15°C	CPE	IFAT, ELISA	Not acceptable

Ref: OIE manual of diagnostic tests for Aquatic Animals

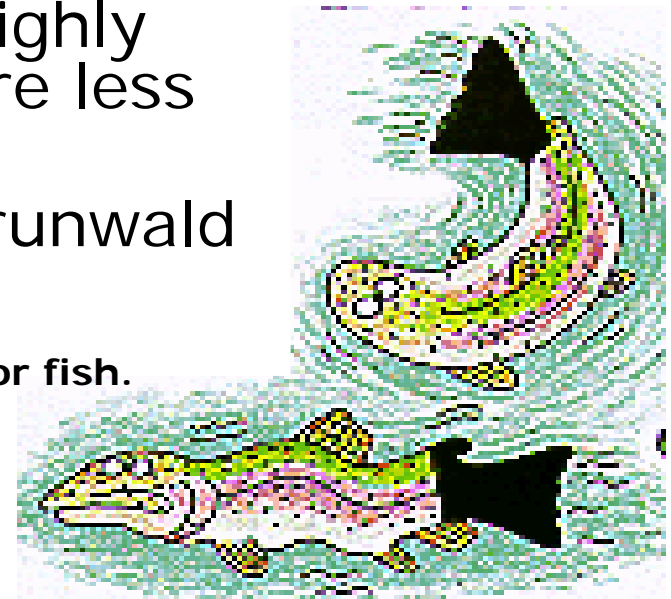


IRISH MEDICINES BOARD

Myxosoma cerebralis

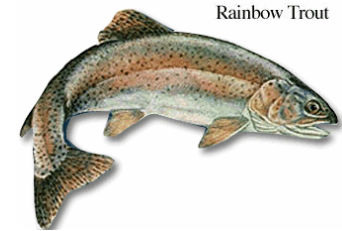
- Parasite of salmonids causing Whirling disease – up to 90% mortality in young fish.
- Geographical distribution: EU, US, South Africa & New Zealand.
- Two host life-cycle involving fish and aquatic worm Tubifex.
- Spores released from fish are highly resistant, spores from worms are less resistant.
- Screening: Staining with May-Grunwald

NFG: Specific requirements for the production and control of live and inactivated vaccines intended for fish.

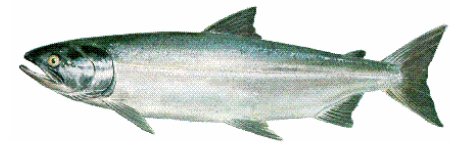


Minimising the Risks of Bacteria Contamination

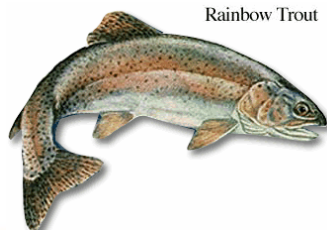
- Bacteria:
 - *Yersinia ruckeri*,
 - *Vibrio anguillarum*,
 - *Aeromonas salmonicida*



Rainbow Trout



NFG: Specific requirements for the production and control of live and inactivated vaccines intended for fish.



Rainbow Trout



IRISH MEDICINES BOARD

Bacterial Screening

	Gram stain Motility	Culture	Morphology
<i>Yersinia Ruckeri</i>	Neg, Variable motility, Rod	TSA @ 18 –22°C	White raised colonies, regular margin, no diffusible pigment
<i>Vibrio Anguillarum</i>	Neg, Motile, Rod	TSA + NaCl @ 13–17°C	Cream circular colonies, no diffusible pigment
<i>Aeromonas salmonicida</i>	Neg, Non-motile, Rod	TSA + yeast @ 18–23°C	Produces brown diffusible pigment, rough/smooth

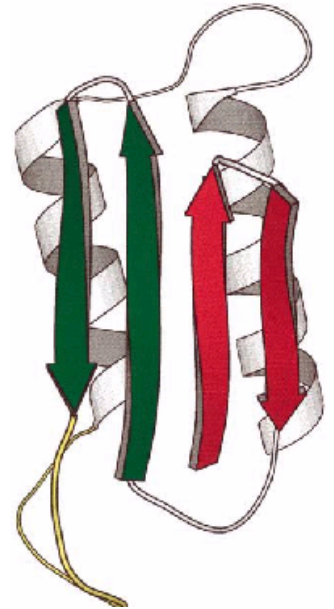
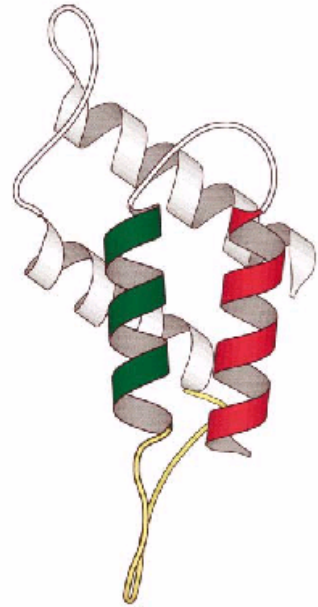


IRISH MEDICINES BOARD

Transmissible Spongiform Encephalopathy (TSE)

- TSE

- Materials of piscine origin are excluded from the scope of the TSE Note for Guidance.
- Conduct a risk assessment to determine if the seed materials could be contaminated with TSE during preparation.
- Relevance of TSE in fish?



IRISH MEDICINES BOARD

Minimising the Risks of Extraneous Agent Contamination

- Materials of Animal Origin for Production:
 - Only use animal materials when necessary
 - When used – ‘Strictest possible selection criteria must be applied (Ph. Eur. 01/2005/50205)’ e.g.
 - Healthy animals
 - Countries free from exotic diseases
 - Countries with a category I or II (or III with justification) rating with respect to TSE.



IRISH MEDICINES BOARD

Minimising the Risks of Extraneous Agent Contamination

- Materials of Animal Origin for Production:
 - Must be subjected to a validated sterilisation or inactivation procedure,
or
 - The substance is tested for absence of extraneous agents (Ph. Eur. 01/2005:50205)
 - Specific requirements for the production and control of live and inactivated vaccines intended for fish.
 - Table of extraneous agents (Vol. 7B of NTA)
 - Guideline on requirements and controls applied to bovine serum used in the production of IVMPs



IRISH MEDICINES BOARD

Summary

- Why?
 - Safety
- How?
 - Extensive screening of biological starting materials to ensure extraneous agents are not present.
 - 'Strictest possible selection criteria'
 - All manufacturing process conducted using GMP.
- Result:
 - Safe vaccine and



IRISH MEDICINES BOARD

Lots of Happy Fish



Wide Smile



Laughing



IRISH MEDICINES BOARD