

# Koi keeping

# The Virkon® Aquatic story

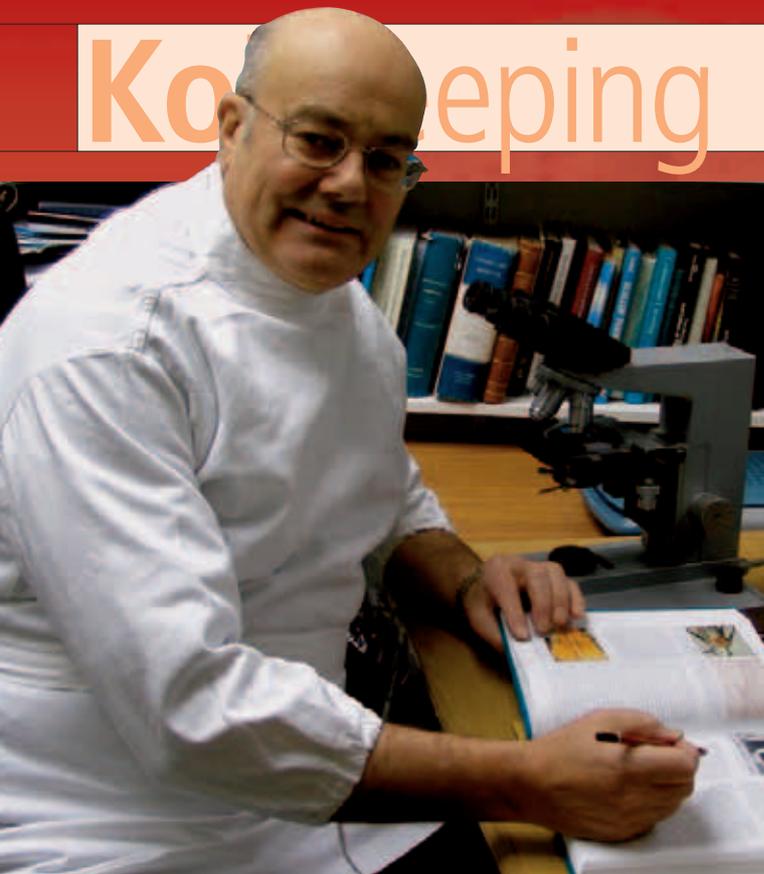
Leading fish vet Professor Ronald Roberts was closely involved with the development of the first in-water pond sanitiser, Virkon® Aquatic – we sent **Keith Holmes** to find out more...



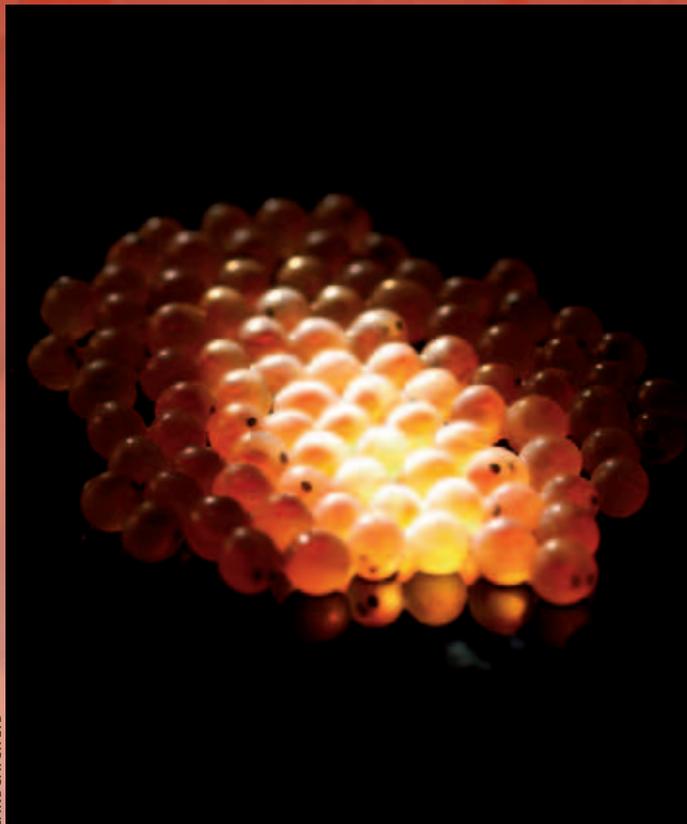
Keith Holmes

Keith Holmes is manager of Koi Water Barn and has recently finished co-writing his second book, *The Interpet Manual of Koi Health*. His Koi and aquatics knowledge is based on 12 years' experience in the trade.

# Koi Keeping



Above: Professor Ronald Roberts has worked throughout the world for the past 40 years, specialising in fish diseases and in-water sanitisation techniques  
 Right: A fine clutch of eyed ova from a biosecure hatchery



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**P**rofessor Ronald Roberts, Fellow of the Royal College of Veterinary Surgeons, the Royal College of Pathologists and the Royal Society of Edinburgh, has been at the leading edge of fish pathology for 40 years. His book, *Fish Pathology*, has been translated into nine languages, including Japanese, and is the standard academic text on the subject. He started his work on fish epidemics in the University of Glasgow Veterinary School in the 1960s and has worked throughout the world as a fish disease specialist. He is best known for his role as leader of the UN international mission investigating **epizootic** virus diseases of carp and other freshwater species in Asia in the 1980s and for his development of techniques for in-water sanitisation against fungal and viral diseases. In his retirement he is Distinguished Visiting Professor at the University of Idaho and a director of Landcatch, one of the world's leading fish breeding companies. He is also Deputy Chairman of the EU's Food Standards Authority Veterinary Panel.



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A box of hand-selected ova that have been disinfected and are ready to be sent to South America

fungal problem in Koi and other carp in Japan while working with my friend and colleague, the very eminent Professor Tokuo Sano, or Sano-sensei as he is known. When some vets closely involved with Koi health in the UK told me about the widespread losses carp viruses were causing, I was immediately interested. I have been working on the control of fish virus epidemics for 40 years so it was really just another fascinating challenge.

One of the big problems with KHV is diagnosis as there are interpretation problems with both the **PCR** and antibody tests and **tissue culture**, which is the gold standard, is both difficult and very expensive.

### Where did the concept of sanitising water while the fish were present come from?

In 1990 a colleague, Dr Guy Willoughby, and I were asked to resolve the problem of salmon egg mortalities caused by the *Saprolegnia* fungus. The traditional treatment was to bathe the eggs in a high dose of malachite green every few days but it was found that if the fungus was already established the treatment was ineffective. After studying the life cycle of the fungus and the effect of the chemical, we showed that the mature fungus on the eggs was highly resistant but that the spores in the water were very vulnerable to even a hundredth of the bath concentration. So, by continuously dripping extremely low concentrations of malachite into the inlet water, the spores were killed within the water column before they could settle on the eggs, and we resolved the problem.

### But *Saprolegnia* is a fungus not a virus – so how can we apply the concept of sanitising water to the treatment of viruses in fish?

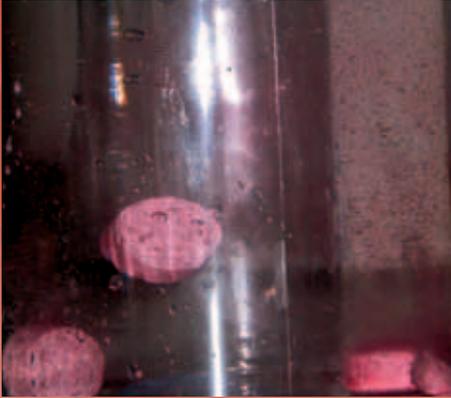
In 2000 there was, for the first time in the UK, a very severe outbreak of the salmon influenza virus Infectious Salmon Anaemia (ISA). At that time government scientists were recommending the use of disinfection regimes which, in the human and animal context, were, I believed, outmoded. At the same time there was the SARS outbreak in Asia and the UK had just suffered the foot-and-mouth epidemic. In both of these

### How did you get involved with Koi viruses?

In many Asian countries, and in Idaho where I now do much of my research, the farming of aquarium species is part of the overall fish culture industry. In Indonesia and Sri Lanka, for example, tropical aquarium fish production is big business. I had also witnessed a

Virkon® Aquatic is now the disinfectant of choice in salmon and shrimp farming





Above: Virkon® is only part of a total biosecurity programme; other measures like hand washes must be considered  
 Above left: Virkon tablets should never be added directly to a pond – they must be dissolved in water first  
 Left: Once the tablets have dissolved the water will be tinged pink – then the solution can be added slowly to a pond

outbreaks the standard viral disinfectant recommended and used was the specially formulated virucide Virkon®. In countries throughout the world this is the specified virus disinfectant in **biosecurity** plans. I started to specify it in all of the farms where I had any veterinary involvement for all biosecurity purposes – foot baths, tank cleaning and so on. However, it had one or two serious drawbacks. It was particularly toxic to fish if they came into contact with it and it was highly scented which meant that it tainted the flesh of fish it came into contact with as well as putting fish off eating. In discussion with the manufacturer, Antec Ltd (subsequently DuPont Animal Health Solutions which, as part of the world's largest speciality chemical company, has unrivalled research chemists), I explained that the ideal would be Virkon's viral properties without the toxicity or taint. They managed to remove the scent and then further work on the new formulation demonstrated an excellent toxicity profile without loss of the high virus-killing activity. The result is Virkon® Aquatic, a new product which is now becoming the disinfectant of choice in salmon and shrimp farming for cleaning tanks and nets, for foot baths, for spraying buildings and decontaminating after outbreaks.

**Do you think the average Koi keeper should use this product for disinfecting their ponds and filters after a viral problem? How would you advise this?**

I think that all responsible Koi keepers have to address this area, which we call 'biosecurity', given the new problems in the industry and the increasing value of their stocks, but it is such a big subject that I think we must save it for another article.

**Virkon® Aquatic is now becoming available in the Koi industry, not only for general hygiene but also for in-water sanitisation. How did that come about?**

In salmon farming there is a very serious disease called Infectious Pancreatic Necrosis (IPN). It is even more acutely lethal to young fish than KHV and is much tougher to destroy than any herpes virus. Like herpes viruses it integrates into the cells of carrier fish to be released later

**In 2000 there was, for the first time in the UK, a very severe outbreak of the salmon influenza virus (ISA)**

under stress. Farms in Europe and South America were suffering major losses from it but, however early the farmer observed it, nothing could be done to stop it. That was when I had the rather rash idea that we might be able to utilise the technique that Guy Willoughby and I had published in 1990 to attack the new problem. At the University of Idaho, Professor Hardy and I established a set of trials in triplicate to test the toxicity of adding Virkon® Aquatic directly to the water of growing trout. We found that up to 10ppm they were perfectly happy. Growth was actually enhanced at 4ppm and the fish had outstandingly good condition and fins, suggesting that this would be an acceptable level for continuous exposure – but we did not know how such a low level would affect viruses.

In fact, the results were better than we dared expect. Provided the farmer got the water treatment going quickly enough, infections were halted in their tracks. Many farmers now apply a continuous low-level dose of Virkon® Aquatic to their header tanks on a routine basis. It is really the first time that control of fish viruses by sanitising the water column has been shown to be effective. It should only be used in conjunction with high standards of biosecurity and great care should be taken with stock origins, which we will discuss in a subsequent article.

Trout farmers had great results when they used Virkon® Aquatic in their growing-on tanks



**Clearly this has been a great benefit for the salmon and shrimp industries.**



Water changes are necessary when using Virkon® Aquatic as one of the side effects is slight foaming of the water

### Where did Koi come into the picture?

The use of the technique in farmed fish has now proven itself and been approved by DuPont Animal Health Solutions for their product support. It has been shown not to require Medicines Act approval since it is the water and not the fish that are being treated. When I was advised of the general problems with viruses in Koi, I had discussions with other specialist fish vets, such as William Wildgoose MRCVS in London and Chris Walster MRCVS in Birmingham, and they confirmed that there was a serious risk from both KHV and SVC, which is a notifiable disease. I had seen the same disease that we now know as KHV in carp in Indonesia in 1984, so it is not a brand-new disease.

The first requirement was to determine the toxicity of Virkon® Aquatic for Koi. I had little doubt about its efficacy since all herpes group viruses are very susceptible to Virkon. They are much more delicate than the very tough IPN virus in salmon. Professor Hardy and I repeated the toxicity work in triplicate in populations of Koi and showed that Koi were even more resistant to any toxic effects than salmon. Virkon® Aquatic had no pathological effects even up to 12ppm and that was with continuous addition at the appropriate rate, in a running water system, on a 24-hour basis. The Koi noticed the change in the water when it was first added, but thereafter resumed feeding and showed no observable effect; when slaughtered at the end of the study they had no microscopic damage internally or externally.

### Jargon buster?

**Epizootic** refers to a disease which suddenly and temporarily affects a large number of animals.

**PCR** stands for Polymerase Chain Reaction and is a test used to detect virus particles, live or dead. It is similar to genetic fingerprinting.

**Tissue culture** is the standard technique used for growing viruses from infected animals using live cells grown in the laboratory from susceptible species.

**Biosecurity** is the series of techniques involving legislative, physical and chemical means, for example UV light and disinfectants, by which human or animal populations are protected from external pathogens such as bacteria or viruses. An example of a biosecurity measure is when lorries were disinfected during the foot-and-mouth outbreak in the UK.

**Lipophilic surfactants** are special detergents that destroy the outer membrane envelope of viruses like the Koi Herpes Virus.

### As you know, Koi are normally kept in complex recirculation systems with biological filters and protein skimmers, so how would Virkon® Aquatic be used in such a system?

This has been the biggest problem when investigating the use of in-water sanitisation techniques for Koi. In salmon or trout culture the water is used once and flows through the system to waste. Any new virucide, added at the header tank, continually flows through the system allowing a constant sanitising level. Tests by DuPont Animal Health Solutions have shown that when Virkon® Aquatic is added to any water system it breaks down quickly and is gone within 24 hours; in the presence of UV light this breakdown is even more rapid. After months of investigation using test ponds, the conclusion is that, instead of continuous on-line dosing at 2–4ppm, which is ideal, alternative recommendations have to be made for Koi. This is made even more difficult by the fact that even DuPont Animal Health Solution's chemists have found it impossible to devise a test kit for such low levels of the product. Fortunately, toxic levels are several times higher than recommended water sanitising levels and herpes viruses are very susceptible to Virkon® Aquatic. Users have found that, as long as the fish appear bright and the water clarity is slightly better than before, the effect is there.

DuPont Animal Health Solutions, in conjunction with Bradan, the fish biosecurity company with whom they work, now recommend the addition of one 5g Virkon® Aquatic tablet a week per 1,000 gallons of water in a Koi pond with the proviso that 5% of the system water is also changed during this time; this is even more important in summer when evaporation is a risk. This dose ensures a high level of viral sanitisation. The water change is necessary because although almost all of the Virkon® Aquatic constituents break down to harmless products, a very small amount of a specialised **lipophilic surfactant** is present, necessary to deal specifically with enveloped viruses like KHV. This does not break down as fast so slight, harmless foaming may occur after a few weeks of use with no water change. If there is a suspicion that KHV is present, or for use in a quarantine system, more frequent use (every three days; two to four tablets per 1,000 gallons) with water changes of up to 10% a week are recommended to avoid foaming. ■

...next month: Biosecurity and quarantine for the Koi owner