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F10 PRODUCTS

CONTROL OF CIRCOVIRUS INFECTION IN PSITTACINE BIRDS USING F10SC DISINFECTANT AND AVIAN GAMMA INTERFERON

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Summary

This study investigates the use of F10SC disinfectant in the control of a circovirus outbreak in a collection of aviaries containing a mixed collection of parrots (macaws, grey parrots and cockatoos). The disinfectant was used via nebulisation in combination with avian gamma interferon injections to treat a group of juvenile grey parrots found to be infected with circovirus from the aviaries. 70% of the birds survived using this combination. It was concluded that F10SC was a useful adjunct to the treatment of circovirus infection by reducing the possibility of secondary infections such as aspergillosis.

F10SC disinfectant was subsequently used to decontaminate the aviaries that housed the infected grey parrots. A 1:100 dilution was sprayed onto all aviaries surfaces twice daily for 3 weeks. Wall swabs submitted for PCR examination for circovirus were subsequently found to be negative compared with an infected control aviary simply sprayed with water. The aviaries have since been repopulated and there have been no further cases of circovirus infection in the last 4 years from the facility. It was concluded that F10SC could be used to eliminate circovirus infection from aviaries at the concentrations indicated but care should be taken to ensure the buildings are tested negative for the virus prior to repopulation.

Introduction

Psittacine Beak and Feather Disease (PBFD) is caused by a circovirus and is known to infect most species of psittacine birds. The virus affects rapidly growing cells in young birds. The disease can present in either acute or chronic forms. In the chronic form birds are seen with both feather loss and malformed feathers. Birds produce feathers with unusual colouring ***(Figures 1 & 2)***.

Figure 1. Abnormal colouring in a grey parrot with circovirus infection.

Figure 2. Classical appearance of a grey parrot with circovirus infection. There is loss of the normal architecture of the feathers and the bird has started to feather pick.

Damage to the horn producing cells of the beak leads to chronic deformities and the birds find it painful to eat ***(Figure 3)***. The disease appears to be fatal but some birds will survive up to 10 years if nursed carefully and managed with analgesics.

Figure 3. A Senegal parrot with chronic circovirus infection. The normal architecture of the beak has been lost making it painful for the bird to eat without adequate analgesia. The normal development of the feathers has also been lost.

In all birds the bone marrow is infected leading to immunosuppressive actions. This is seen more dramatically in young Grey parrots that frequently present with the more acute forms of the disease rather than the chronic ***(Figure 4)***.

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Figure 4. Young grey parrots are extremely susceptible to acute circovirus infection. The increase in the popularity of captive breeding and hand rearing has led to a dramatic increase in the spread of circovirus infection throughout the UK breeding population.

A classical haematology profile will reveal severe leucopaenia (especially heteropenia) with signs of anaemia. These birds rapidly succumb to secondary invaders such as *Aspergillus fumigatus* ***(Figure 5)***. The disease is invariably rapidly fatal with few Grey parrots appearing to develop the chronic form of the disease.

Figure 5. Aspergillus in the lung of a young grey parrot secondary to circovirus infection. The fungal hyphae rapidly spread through the immunosuppressed birds leading to death from an overwhelming infection.

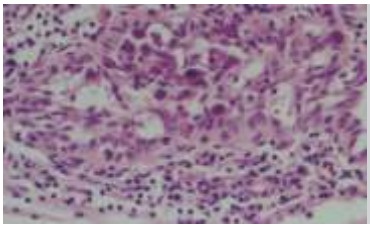

Figure 6. Biopsy of the bursa in a young Grey parrot infected with circovirus. The bursa has numerous inclusion bodies caused by the virus.

It is felt that birds are only able to contract the virus whilst they have a cloacal bursa present (normally the bursa is present up to 16 weeks of age, ***Figure 6)***. PBFD virus is best diagnosed by a PCR test normally using either blood and feather pulp samples in the live bird. Recently is has been noticed that the PCR test is potentially unreliable and positive birds can be missed. Whether this is due to a problem with the test protocol or that there are two forms of the virus is debated and a breeder could certainly not depend on the test to produce a disease free unit when buying in young birds unless repeat testing is performed. The author finds that bone marrow from the tibiotarsus in addition to blood and feather samples give the operator the best chance of finding the virus but avoiding false negatives.

The virus is spread mainly by contact with highly infective feather dust in young birds. Grey parrots tend to produce large quantities of powder down and if the bird is infected there is a high risk of dust being passed between birds. This means that young birds in breeding establishments are particularly at risk ***(Figure 7***). The virus is highly infectious and stable in the environment so once premises are infected it is difficult to ensure that the virus has been eliminated from the environment. This is a big concern in nurseries and hand rearing rooms. It is vital to practice excellent biosecurity in aviaries and to use an "all in-all out" system in the nurseries to avoid introducing the virus to buildings.

The interferons are a group of small protein and glycoprotein cytokines naturally produced by the avian immune system following natural infection or vaccination. Interferons protect the bird from biological

Figure 7. Grey parrots are frequently handreared in the UK due to their popularity as pets. Unfortunately they produce vast amounts of feather down which can become infected with circovirus and subsequently rapidly spread through hand rearing establishments.

attack by suppressing cell proliferation, inhibiting viral replication and augmenting the activity of macrophages and T lymphocytes. There are type 1 and type 2 interferons. Type 1 interferons are essentially antiviral and antiproliferative in their effects. Recently an interferon omega (type 1 interferon) has been produced commercially for the treatment of canine parvovirus and feline leukaemia virus in Europe (Virbagen Omega, Virbac Animal Health Animal Health, Carros, France). Type 2 interferons have a more immunomodulatory effect. Initially the use of interferon was limited due to the difficulty in manufacturing the protein in large enough quantities but the recent development of recombinant DNA technologies has made interferon economic and easy to produce. The use of interferons in humans for the treatment of chronic hepatitis B and various malignancies has transformed treatment success rates with these difficult diseases. The use of interferons and other cytokines has been well researched in the poultry industry in a bid to reduce the use of vaccines and in feed antibiotics with significant success. Interferons are regarded as being species specific and interferon from one species would not normally function in another. Despite this interferon of feline origin has been found to be affective for the treatment of canine diseases. The omega interferon has been found to bind with cells from different species in vitro although there is no data available for avian cells. The commercially available omega alpha 2 interferon exerts its effects by preventing viral replication.

The use of F10 Super Concentrate (F10SC, Health and Hygiene Ltd, South Africa) administered by fogging systems as a preventative treatment for respiratory diseases in poultry in particular aspergillosis is documented. The disinfectant has been shown to dramatically reduce *Aspergillus fumigatus* spore counts in hatcheries so it is felt that it would potentially be the best method of preventing secondary infection in these immune suppressed circovirus positive birds. Most of the young circovirus positive chicks appear to die from secondary *Aspergillus fumigatus* infections. It has the advantage of appearing to be safe for use on both eggs and chicks whilst being effective against many pathogens. Recently F10 has been used by many Veterinary surgeons for the treatment of exotics in particular aspergillosis in avian cases with good results ***(Figure 8)***. The 1:125 dilution of F10 has also been shown to be effective against Chicken Anaemia Virus a common circovirus affecting poultry.

Figure 8. F10SC disinfectant has become a popular non-specific treatment for respiratory disease amongst avian veterinary surgeons. It is normal applied by nebulisation or fogging sytems. It was used in this study in an attempt to decontaminate a commercial breeding aviary from circovirus in the environment.

The disinfectant has also been used successfully for the treatment of respiratory conditions in psittacines and many other pet species.

Use of F10 disinfectant and gamma interferons to treat acute circovirus infection in grey parrots

Once the outbreak had been detected in the aviary facility large numbers of hand reared grey parrot chicks succumbed to the virus. They presented with anorexia, depression and rapid death usually from the secondary infection *Aspergillosis fumigatus*. It was decided to use interferon as a potential treatment for the young greys due to its theoretical immune stimulatory activity. A small amount of avian gamma interferon was obtained from Dr Peter Kaiser (Compton Laboratories UK) in addition to the mammalian interferon of feline origin available commercially in the UK (Virbagen Omega Interferon Virbac, UK). A group of 22 juvenile grey parrots testing positively for circovirus by PCR test were randomly split into two groups.

All 22 birds exhibited profound leucopaenia with total leukocyte counts below 1 × 10^9 (normal range 3-15 × 10^9) in all cases. Each bird was injected daily with one million international units of an alpha type 1 interferon of feline origin intramuscularly for 90 days. Additional measures involved fogging the birds for 15 minutes twice daily with F10SC at a dilution of 1:125. The fogger produced a droplet size of 6um. The birds were monitored by determination of serial total leukocyte cell counts at intervals. Only 2 birds were surviving at week 30. Both surviving birds were still leucopenic and found to be PCR positive for circovirus on samples obtained in week 30. The birds were euthanased ***(Figure 9)***.

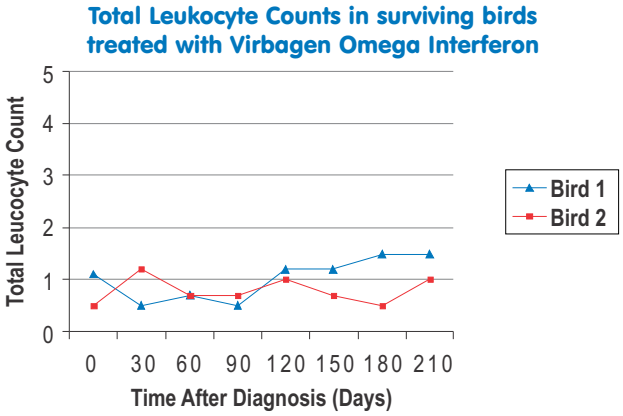

Figure 9. Demonstration of leukocyte counts in Grey parrots treated with mammalian interferon. There was no improvement in the condition of the birds and a decision was made to euthanase them.

A second group consisted of 10 grey parrots that had been presented with clinical signs of circovirus, which were confirmed by PCR test. All 10 birds were found to be severely leucopaenic. These birds were treated using an avian gamma interferon derived from poultry cell culture. The birds were injected once daily using one million units of avian interferon gamma intramuscularly for 90 days. The birds were fogged with F10SC as with the initial group. Seven of the 10 birds were alive at week 30. The increase in total leukocyte counts in these birds over the time period is shown in ***figure 10***. By day 180 all 7 birds were exhibiting normal total white blood cell counts. Using a **t2** comparisons of means test there was a statistical difference between the total leukocyte count in the same birds between day 210 and day 1 (with 95% confidence limits). Samples of blood and feather pulp taken in week 30 were found to be negative for circovirus by PCR test in all 7 birds. The birds were still alive and not exhibiting any clinical signs of circovirus infection, either chronic or acute, 9 months post diagnosis.

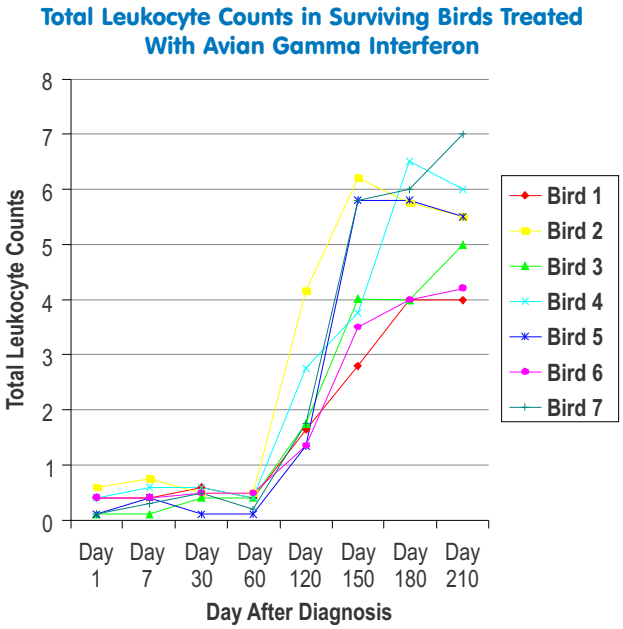

Figure 10. Graph demonstrating the improvement in leucocyte counts in the birds treated with avian interferon. After 60 days there was a steady improvement in the total white blood cell count. After 6 months the birds was negative by PCR for circovirus.

On the basis of this study interferon gamma of poultry origin would appear to have a potential use in the treatment of circovirus infection in young grey parrots. Despite the costs involved in daily injections and quarantining the birds this treatment was considered cost effective due to the high cost of baby psittacines. Mammalian interferon was not satisfactory. Interferons are considered species specific and a mammalian interferon would not be expected to have a significant action in avian patients but cross-species reactivity has been reported in birds. No side effects were seen from the repeated interferon injections in any bird. The results are encouraging and a double blind placebo trial would be indicated in the future.

By this stage it was felt that although the protocol appeared potentially effective for a previously untreatable disease the cost was potentially prohiberative for most clients given the low success rate. It would be expected that interferon would have to be administered until the PBFD virus had been eliminated which in theory meant 90 days treatment in the average case. It was; however, felt that interferon would be potentially far more useful in combination with F10 preventing initial infection. In poultry the administration of interferon has been found to improve growth rates and prevent common infections without the need to vaccinate. The interferon is applied by a fogging system and has been found to be more economic than the use of antibiotics and vaccines in commercial poultry systems.

Use of F10 disinfectant to decontaminate the aviaries

Due to the resistant nature of circo viruses in outbreaks of PBFD identification of infected birds and subsequent treatment or euthanasia of birds testing positive is only the starting point. The environment that

housed the infected birds should be considered infected with circovirus for a long period of time (years rather than months) unless completely decontaminated. In reality this leads to any new stock purchased by the owner having the risk of being exposed to the virus for many years from the date of the initial infection unless a thorough clean up operation has been performed and the buildings have been demonstrated to be free from the virus by PCR testing. This explains why many pet shops, breeders and hand rearing facilities act as reservoirs for the disease.

In this case the owners had 7 aviaries containing a variety of psittacine birds ***(Figure 11)***. They were all of traditional wood and galvanised wire construction making disinfection difficult. Unfortunately the aviaries were also all linked together by covered walkways so any infection could spread through the premises relatively easily. Once the infection had been detected in the grey parrots all the birds at the premises were tested by PCR test. All positive adult birds were euthanased after 2 positive test results. In reality this was limited to the cockatoos. All the parrots that tested negative for PBFDV were placed in quarantine. In addition swabs were taken from the environment and tested for PBFDV from all 7 aviaries. Each aviary was found to be positive on 2 or more wall swabs.

Figure 11. Typical construction of the aviaries involved in the study. The wood and galvanised wire construction made disinfectant application difficult.

The aviaries were thoroughly sprayed twice daily with F10SC (1:100 dilution) for one week by the same individual. Care was taken to ensure that the spray covered all surfaces including throughout the covered walkways (***Figure 12***). One aviary was kept as a control and sprayed twice daily with water. After 7 days the aviaries were swabbed for circovirus infection a second time (3 swabs per aviary). The control aviary was still positive for PBFD virus in addition to three of the other 6 aviaries on one or more wall swabs. Three aviaries were negative to PBFD virus on all three swabs.

Figure 12. Fogger used to spray contaminated aviaries.

The results were encouraging but demonstrated the resistant nature of the virus but it was decided to continue with the trial. After three weeks of spraying twice daily with F10SC (1:100 dilution) further swabs taken from the aviaries were all found to be negative for PBFDV by PCR test. The control aviary was still positive at this time. The control aviary was sprayed for 3 weeks with F10SC and demonstrated to be negative for PBFDV on 3 wall swabs after this time. Four months later all the aviaries were re swabbed for PBFDV and all were found to be negative. At this stage the aviaries were repopulated with psittacine birds. There have been no further cases of Psittacine Beak and Feather Disease at the premises since the initial outbreak in 2001. All young stock released from the aviary is tested for circovirus at point of sale with no positive results found.

Conclusions

1) Avian gamma interferon in combination with F10SC nebulisation was found to be a potential treatment for Psittacine Beak and Feather Disease. No apparent side effect was seen with either the interferon or F10SC use at the concentrations described. Avian Gamma Interferon is now being used in Europe in many outbreaks of PBFD including more recently chronic cases and the research into its effectiveness continues.

2) The use of interferon as an immune enhancer prior to PBFD virus infection would also appear to be a useful future use of this new technology. Combining this treatment with the constant application of F10 by nebulisation to reduce circovirus contamination and prevent secondary infections would be a useful protocol in most bird establishments involved in buying birds in or allowing members of the public to handle babies.

3) F10SC was demonstrated to assist in the decontamination of aviaries infected with PBFD virus at the dilution 1:100. Despite the encouraging results it should be noted that aggressive spraying was required to eliminate the disease from the aviaries demonstrating the residence of the virus. Prior to introducing new stock to the aviary best practice would indicate that all surfaces should be swabbed for the virus and submitted for a PCR test. Preventing potential owners handling young chicks would be useful (***Figure 13***)

Figure 13. In an ideal world young birds should be kept behind glass to prevent the spread of circovirus from feather dust.

4) F10SC should also prevent other avian pathogens when used in this way such as polyoma virus and papilloma virus. As it appears safe in contact with birds at the recommended doses F10SC has an important role to play in the bio security of any aviary facility.

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