A Report on the Use of Intravenous Hydrogen Peroxide (H_2O_2) in the Treatment of Aspergillosis in Ostriches

Stacey Gelis BVSc(Hons) MACVSc (Avian Health)[1]

CASE 1

An adult 2 year old male ostrich was examined on 24th February 1995. This bird had been diagnosed with aspergillosis some 4 months earlier via serology (AGID- 4 bands positive for *Aspergillus fumigatus)* and at this time was treated via fogging in an enclosed shed with enilconazole smoke (Clinafarm Smoke Generators). The treatment consisted of lighting the smoke generator & inhaling the enilconazole smoke produced and left in the shed for at least 4 hours & in some cases overnight. This was repeated twice weekly for 2 weeks. However, there was no resolution of clinical signs following treatment.

At the time of re-examination the bird appeared bright & active but exhibited marked prolonged expiratory effort accompanied by dropping of the wings, open-beaked breathing & had mild weight loss. Auscultation revealed audible squeaks at the end of each expiration with the left side slightly worse affected.

Treatment of choice at this stage included providing oral itraconazole, preferably in conjunction with enilconazole smoke. The owner did not consider it feasible to treat the bird twice daily with itraconazole for the required 2-6 weeks, and had declined further enilconazole smoke therapy. Instead the owner requested using an intravenous infusion of hydrogen peroxide which had been suggested to him by his physician. After discussing potential risks & my own misgivings with the owner, I reluctantly agreed to institute intravenous H_2O_2 therapy. Due to a lack of published protocol for this treatment, I was advised to use a 0.175 % solution of H_2O_2. This was achieved by diluting 5 ml of 30% "medical grade" H_2O_2 with 1 litre of 0.9% NaC1. The bird was initially restrained in an "ostrich crush", hooded & an indwelling 20 g butterfly catheter was placed in the ulnar vein. The bird was initially given 300 mls of this infusion over 20 minutes. The bird struggled a little but accepted the treatment well. Another 700 mls was given on 27 February 1995, and on 1 March 1995 1 litre of the H_2O_2 solution was given IV at an unimpeded flow rate. This was repeated on 3 March 1995 and on 6 March 1995.

What was noticed was that within minutes of each infusion commencing, the bird would begin coughing, often expectorating mucus containing necrotic material. This coughing would cease shortly after the treatment was finished.

The bird had clinically shown no improvement following this treatment regime, with rales & squeaks still audible upon thoracic auscultation & continued increased expiratory effort. At this stage it was agreed to trial doubling of the H_2O_2 concentration infused to 0.35%. After 700 mls of this infusion the bird showed weakness & ataxia. The infusion was immediately stopped, the hood removed and 100 mg Dexamethasone and plain 0.9% NaC1 given IV. During this period the bird struggled & hit its head against the restraint rail & died. A necropsy was immediately performed. This revealed a generalised thickening of abdominal & thoracic air sacs which were milky in colour. Fibrin tags traversed the air sac lumina, and several areas of thickening with necrotic centres were evident. However, no fungal fruiting bodies were seen. The liver exhibited alternating areas of pallor and congestion along the lobe margins but was otherwise grossly unremarkable. The owner declined to have either histopathology or haematology/biochemistry performed.

CASE 2

[1] Kingston Veterinary Clinic, 32 Lower Dandenong Road, Mentone VIC 3194

On 28 February 1996 1 was called to examine an adult male ostrich which had been exhibiting "open-beaked breathing" for 2 days. Clinical examination revealed the bird to be in good body condition, though there was a noticeable paling of the red areas of the beak & legs. The bird was open-beak breathing at rest, was coughing up mucus & showed increased respiratory effort. Examination of the oropharynx revealed the mucus to be of tracheal origin, rather than from the choana or oesophagus. Rales were auscultated bilaterally in the thorax. No squeaks were audible through the respiratory cycle. The bird was treated with lincomycin/spectinomycin (Lincospectin Injection- Upjohn) at 1000 mg Lincomycin, 2000mg spectinomycin given subcutaneously once daily for 5 days & multivitamin injection (Multi- Heriot Agvet), 8 mls once daily by intramuscular injection for 5 days. Blood samples were taken for FBE/VMBA & Aspergillus serology. Haematology revealed a marginally elevated PCV & basophilia, with no WBC or RBC morpholgy changes. Biochemistry revealed elevated protein, albumen & globulins, and decreased chloride, creatinine, phosphorus & cholesterol. The pathologist did not consider these diagnostically significant, and attributed the globulin increase to haemolysis. The Aspergillus serology, however, yielded a significantly positive result. By AGID, this bird exhibited a 2 band positive result to *A. fumigatus* and *A. niger* and a 1 band positive result to A. *flavus*.

The bird was re-examined on 4 March 1996. Auscultation revealed the rales to be still present. The bird was caught up & placed into a horse float. A 19g x 19 mm butterfly catheter was inserted into the right ulnar vein and the bird was given 400 ml of 0.175% solution of hydrogen peroxide intravenously before it appeared agitated so the infusion was stopped. The owner agreed to also treat the bird with enilconazole smoke (Clinifarm Smoke Generator) and this was also performed whilst the bird was in the horse float.

This procedure was repeated on the 8 March 1996 and this time the bird did cough initially, but did not show any signs of distress following the intravenous H_2O_2 infusion & concurrent smoke therapy.

Similar treatments were repeated on the 13, 20 & 26 March & on 1 & 8 April. By the end of the treatment regime the respiratory rales had improved considerably but were still audible. However, the open-beak breathing & coughing had ceased & the pink colour on the bird's beak & legs had re-intensified.

On 12 September 1996 the owner called & informed me that the bird in question remained free of clinical signs & had in fact produced its first batch of fertile eggs.

The bird was re- examined as recently as the 12 July 1998. It remained free of clinical signs, auscultation failed to reveal any abnormal respiratory sounds & was showing aggression & early reproductive activity such as cantling, intensification of the red areas of the beak & legs & chasing of the hen around the paddock.

Discussion

Aspergillosis is an important disease of ostriches both within Australia & overseas. It can effect birds of any age, but the commonest form seen is chronic airsacculitis in adolescent & adult birds. It is believed that these birds were probably infected when young and go on to develop the clinical signs of the disease following an episode of immunosuppression eg stress (eg transport, onset of reproduction), poor nutrition, concurrent disease and environmental conditions. Of course, exposure to large numbers of *Aspergillus* spores and concurrent poor ventilation, dustiness & dampness all add to the risk of contracting & exhibiting signs of this disease.

The species considered most pathogenic is *A. fumigatus*. There is however believed to be different pathogenicity in different isolates of *A. fumigatus (Richard*, 1991). *A. niger & A. nidulans* have also been associated with aspergillosis in ratites (Love & Gill, 1995).

Treating aspergillosis in ostriches has traditionally been difficult. The two problems have been, firstly, finding medications which are effective against the *Aspergillus* organism in vivo and secondly, delivering the medication at therapeutic levels to the site of infection. Initially, ketoconazole was trialed with poor success (Munro, 1993). More recently, the treatment regime of choice has been as follows:

1) "Clinafarm Smoke" (enilconazole)- aerosol therapy twice weekly for 2-6 weeks.
2. "Sporanox" capsules (itraconazole -100 mg capsules)' 1 capsule per 20 Kg bodyweight once-twice daily for 14 days.
3. Antibiotics eg lincomycin/spectinomycin ("Lincospectin"- Upjohn) at 10-20 mls subcutaneously for

5- 10 days.

This regime was reported to effect a complete cure in 20-25% of cases, and remission in 60 % of cases, with total failure in 15 % of cases (Love & Gill, 1995).

However, there have been several problems encountered with this regime. Firstly, procuring the "Clinafarm Smoke" generators has often been difficult. This item is a shed disinfectant & not registered for use on live animals. Hence, the relevant authorities were not keen on its use on ostriches, unless it was to be registered for such use. This was not feasible. To my knowledge this product is no longer available to veterinarians in Australia.

With the "Sporanox Capsules", the problem lies in being able to administer 4-8 capsules to an adult ostrich daily for an extended period. For birds that must be caught up daily, the stress involved may negate any positive effect gained by the subsequent treatment. It is also very expensive.

The antibiotics are used to treat secondary bacterial infections which are often found to concurrently exist with the *Aspergillus* organisms and have been found to be the end cause of death. (Love & Gill, 1995).

The lack of response & difficulty in procuring some treatments led many to seek alternative therapies. Such was the case with the farmer whose birds are discussed in the above case reports. The use of hydrogen peroxide (H_2O_2) was suggested by him in response to his physician's suggestions.

Hydrogen peroxide has been used for treating various ailments for many years but objective scientific data on its therapeutic benefits are more limited. The theory behind its therapeutic effectiveness is that being an unstable molecule, H_2O_2 when in contact with water breaks down to produce hydroxyl radicals which are cytotoxic. The cytotoxicity does not appear to be selective, so damage of healthy tissue also occurs.

In vitro, hydrogen peroxide was found to be 99.9% sporicidal to the conidia of *Aspergillus flavus* and *A. terreus* at ph 3.79 (Buchen & Marth, 1977a), and at concentrations varying from 2-6 % but the time required for this varied from minutes to one hour depending on the strains *of Aspergillus (* Buchen & Marth, 1977b). Geissler *et al* (1986) reported that inactivation of *A. fumigatus* in suspension was achieved with exposure to 4.5% hydrogen peroxide for 3 hours. These concentrations are much higher than those used in the treatment protocol described in the 2 case reports above.

The antibacterial properties of H_2O_2 have also been reported, eg Elzanowski et al (1995). Andryunin (1983) found that in vitro, *Escherichia, Brucella, Salmonella, Listeria & Staphylococcus* could all be inactivated by exposure to 4 % H_2O_2 alone or by 2 % solution plus 0.5 - 1% lactic acid (ph 3.75) at a concentration of 0.5 litres for 30 minutes. However, Shenep *et al* (1985) found a lack of antibacterial activity after intravenous hydrogen peroxide infusion in experimental *Escherichia coli* sepsis.

Much has been written on the potential toxicity of hydrogen peroxide, however. Hata *et al* (1997) found that hydrogen peroxide was toxic to cultured colonic epithelial cells. When H_2O_2 enters the intracellular space, it is converted to the more reactive and harmful hydroxyl radical leading to cellular injury. Gas emboli have been reported secondary to the intraoperative use of H_2O_2 (Garcia-Velasco, 1997; Konrad 1997). Lubec (1996) reported brain lipid peroxidation and hydroxyl radical attack after an intravenous infusion of hydrogen peroxide in an infant. Its ability to cause in vitro erythrocyte haemolysis in common marmosets was investigated by Ghebremeskel *et al* (1990). Their findings showed that marmoset erythrocytes were abnormally susceptible to oxidative damage by H_2O_2 compared to other primate species. This was exacerbated when a vitamin E deficiency was experiment, ally induced. Vitamin E therapy was reported to be beneficial in the prevention of haemolysis in tamarins (Baskin *et al*, 1983; Gutteridge *et al*, 1986) and in owl monkeys (Sehgal *et al*, 1980).

Fidler (1976) found that hydrogen peroxide reduced the rate of growth, water consumption, and usually reduced glutathione peroxidase activity in the liver, plasma and lower small intestine in chicks. He found that the effect of H_2O_2 was primarily attributable to decreased selenium retention and that neither ethoxyquin nor excess supplemental selenium were effective in reversing toxicity.

Even from these limited studies it would appear that the therapeutic value of intravenous hydrogen peroxide

therapy is questionable. The concentrations required for in vitro fungicidal activity were much higher than those used in intravenous infusions and the potential side effects are many. Certainly no conclusions can be derived from the two case reports described here. One can only comment on the observations made, these being that 2 different birds survived a series of 1 litre infusions of 0.175% H_2O_2; both birds were seen to cough shortly after the infusions commenced; 1 bird exhibited ataxia & collapse following infusion of a 0.35 % solution of H_2O_2; one bird showed resolution of clinical signs after being given 7 infusions of H_2O_2 at intervals of 4- 7 days as part of the treatment regime.

These observations merely open the door for further discussion on the role of hydrogen peroxide in veterinary therapy, and highlight the need for controlled clinical studies in this field. This is particularly the case at this time when many untested "alternative" therapies are popular with the general public, particularly for those ailments where traditional medicine has failed to provide an effective cure. On the other hand, it does, I believe, behove us as clinicians to keep an open mind to different ways of treating such conditions, whilst still maintaining our scientific objectivity.

References;

Andryunin, Yu, I. (1983). Dezinfektsiya zhivotnovodcheskikh pomeshchenii i veterinarnaya sanitariya na transporte.(Disinfection of animal buildings and the veterinary hygiene aspects of transport). 10-13.

Bachev, K., Kolev, G., Kamburov, P. (1973). Experimental effects of aqueous solutions of bromine and hydrogen peroxide on the development of *Ascaris lumbricoides* and *A. suum* ova. *Nauchmi Trudove Visshiya Veterinarno Meditsmski Institut.* 23:599-602.

Black, **D. (1994).** Aspergillosis in ostriches in Australia. *Australian Ostrich Association Journal.* 24: 39-42.

Buchen, S.Y., Marth, E. H. (1977a) Sporicidal action of hydrogen peroxide on conidia from toxigenic strains *of Aspergillusfiavus* and *Aspergillus parasiticus.* 1. Effects of growth and recovery medium, temperature of treatment, concentration of peroxide and age of conidia. *Journal of Food Protection.* 40: 9, 617-621.

Buchen, S.Y., Marth, E.H. (1977b). Sporicidal action of hydrogen peroxide on conidia from toxigenic strains *of A,pergillus flavus* and *A,pergillus parasiticus.* II. Effects of pH, sucrose, glucose and sodium chloride. *Journal of Food Protection.* 40: 10, 698-703.

Elzanowski, H. et al. (1995). Bactericidal properties of hydrogen peroxide and copper or iron-containing complex ions in relation to leucocyte function. *Free Radic Biol Med.* Mar; 18: (3), 437-449.

Fidler, J.W. (1976). Selenium metabolism in the chick. I. Effects of peroxide administration on selenium utilization, growth deficiency symptoms, and glutathione peroxidase activity in chicks fed controlled selenium diets. II. Induction of glutathione peroxidase activity by selenium in the chick. *Dissertation- Abstracts International.* 36 B:11, 5517.

Garcia-Velasco, P. (1997). Gas embolism secondary to intraoperative use of hydrogen peroxide. *Rev Esp Anestesiol Reanim.* Mar; 44(3): 124-6.

Geissler, H., Weingarten, M. and Hafez, H.M. (1986). Inactivation of *Aapergillus fumigatus. Deutsche Tierarztliche Wochenschrifi.* 93: 1, 23-24.

Ghebremeskel, K., Williams, G., Harbige, L., Spadetta,M., Summers, P.(1990). Plasma vitamins A and E and hydrogen peroxide-induced in vitro erythrocyte haemolysis in common marmosets (*Callithrix jacchus*). *Veterinary Record.* 126: 429-431.

Hata, Y., et al. (1997). Hydrogen peroxide-mediated cytotoxicity to cultured colonic epithelial cells. *Life Sciences.* 60 (24): 2221-30.

Konrad, C., et al. (1997). Pulmonary embolism and hydrogen peroxide [letter]. *Can. J. Anaesth.* Mar; 44 (3):338-9.

Love, S.C.J. and Gill, H.S. (1995). Aspergillosis in ostriches: epidemiology, treatment and diagnosis. *Proceedings of the Fifth Australian Ostrich Association Conference.* 4-6 August. Gold Coast QLD. 53-59.

Lubec, B., et al. (1996). Brain lipid peroxidation and hydroxy radical attack following the intravenous infusion of hydrogen peroxide in an infant. *Free Radic Biol Med.* 21 (2): 21923.

Munro, B. (1993). Infectious diseases of ostrich. *Ostrich Odyssey. Proceedings of the Meeting of the Australian Ostrich Association (Vic).* 23 July 1993, 79-82.

Richard, J.L. (1991) Aspergillosis, in B.W. Calnek, H.J. Barnes, C.W. Beard et al (Eds), *Diseases of Poultry*, 9th edn. Ames, Iowa: Iowa State University Press, pp 326-334.

Shenep, J.L. et al (1985) Lack of antibacterial activity after intravenous hydrogen peroxide infusion on experimental *Escherichia coli* sepsis, *Infect Immun* Jun; 48 (3): 607-610.