RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Investigating Sanitation of Surface Water for Poultry using Chlorine-IBDV Models.

A report for the Rural Industries Research and Development Corporation

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Foreword

Improvements in productivity of the poultry industry relate to all elements of production, but water quality is particularly critical. A significant proportion of Australia's poultry farms rely on surface water sources (dams, creeks and rivers) for drinking water and other production needs. This can pose disease risks from serious avian viral and bacterial pathogens, through exposure to wild waterfowl allowing contamination by infectious agents. These include significant pathogens such as Avian Influenza, Newcastle Disease Virus, Egg Drop Syndrome and Infectious Bursal Disease Virus (IBDV) as well as a number of other microorganisms including enteric bacteria and viruses.

A review of the main methodologies used for water sanitation in Australia indicates that chlorination is the most appropriate technology for poultry sites if effective treatment can be assured. Objective parameters, particularly for chlorination water sanitation treatments, so as to ensure effective inactivation of poultry pathogens are not available worldwide.

If effective viral decontamination of surface water, using simple treatment, can be undertaken reliably and economically on poultry farms, this would remove a significant "weak spot" for entry of potentially pathogenic viral infections into flocks.

Based on its physio-chemical characteristics IBDV is likely to be amongst the most resistant of the avian pathogens to inactivation by disinfectants. Sighter laboratory-scale experimentation using chlorine to attempt to inactivate IBDV in water is described in this report. The outcome of a preliminary investigation to test the susceptibility of Newcastle Disease virus (NDV) to chlorine treatment is also described.

This project was funded by the RIRDC.

This report, a new addition to RIRDC's diverse range of over 700 research publications, forms part of our Egg R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian Egg Industry.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

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Managing Director
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Contents

Abbreviations

CEF	Chicken embryo fibroblast cells
Cl	Chlorine
ClO_2	Chlorine dioxide
EID_{50}	Egg infectious doses 50%
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
NDV	Newcastle disease virus
ppm	Parts per million
$TCID_{50}$	Tissue culture infectious doses 50%
TSS	total suspended solids

Executive Summary

Some half of the commercial poultry production sites in Australia draw on surfacewaters (dams, creeks, rivers) as their major source of drinking water. In some cases this is used untreated save for settling procedures and crude filtration, and in the majority of others, simple addition of chlorine is the only treatment. While a relatively economical treatment process, no data (worldwide) is available as to the likely efficiency of chlorine for the removal of infectivity (= inactivation or "killing") of viral poultry pathogens. At the request of the RIRDC Egg Industry Program, IBDV, (likely to be the avian virus most resistant to inactivation) was tested for sensitivity to inactivation by simple chlorine treatment of water. The inactivation of Newcastle Disease virus was also tested.

The aim of this study was to develop a laboratory-based assay to enable quantification of inactivation of infectious bursal disease virus (IBDV) by chlorine treatment of drinking-quality water. Then to apply this assay for assessing the likely effectiveness using the levels of treatment routinely used in the field for the production of potable water supplies for poultry, i.e. 1 – 10 ppm. Chlorine dioxide, a more complex compound, was also tested.

Using an Australian isolate of IBDV, which had been adapted to growth in chicken embryos by serial subculture, a stock of virus with a relatively high titre and a low concentration of protein was prepared. The protein level was important as protein can neutralise the effects of chlorine.

Treatments of virus were undertaken at 1, 3, 5 or 10 ppm of chlorine against IBDV suspension diluted 1/100 in water. To maintain the concentration of chlorine, measurements were taken immediately after addition of virus, at 30 minutes and again at the end of the 60 minute incubation. It was found that additional chlorine was needed to maintain the target chlorine levels. Following an exposure period of 1 hour at room temperature residual chlorine (or chlorine dioxide) was neutralised. These solutions were then inoculated into cell cultures and incubated for 5 days before the cells were examined for the presence of cytopathic effect caused by surviving virus.

In clean water exposure to 1 ppm chlorine for 1 hour reduced levels of IBDV infectivity some 3 fold (33%), while maintaining a level of 3 ppm reduced the infectivity 10-fold, i.e. 90% of infectivity was removed.

Repeating this investigation, but including chlorine dioxide (ClO_2) for comparison at similar levels confirmed this initial result but also indicated that ClO_2 also was not fully effective for the inactivation of IBDV at 0.35 ppm (Figure 1). When a treatment level of 0.7 ppm was used however, IBDV infectivity was effectively reduced to below a threshold of detection of this test system, i.e. $\geq$33 fold or $\geq$ 97% reduction.

Water from a production water-source dam was obtained from a representative commercial poultry farm in Victoria. This was visibly turbid, and was used without any form of filtration. Chlorine at 1, 3, 5, and 10 ppm was added and measurements were taken throughout the 1 hour incubation period. Additional chlorine was added as needed to maintain the target chlorine levels. ClO_2 was also tested at target concentrations of <0.05, 0.05, 1.3 and 4.4 ppm, using the same test conditions of 20.4^0C and a pH of 7.30 and a 1 hour incubation period. No additional ClO_2 was added.

At the maximum concentration tested, i.e. 10 ppm for one hour chlorine did not inactivate IBDV in unfiltered dam water. At concentration of 4.4 ppm (available ClO_2), ClO_2 was able to inactivate IBDV under the same conditions.

Concerns about the ability of NDV to spread via water supplies led to our including testing chlorine and ClO_2 in both distilled and unfiltered dam water for ability to inactivate NDV. In distilled water, chlorine was able to inactivate more than $10^{3.5}$ (7,000) egg infectious doses of NDV at a concentration of 1 ppm as was ClO_2 at 0.35 ppm after 1 hour.

When suspended in the dam water, chlorine was less effective, requiring 5 ppm (available chlorine, maintained by monitoring available chlorine levels and adding additional chlorine) to inactivate NDV in this test system. Chlorine dioxide was effective in inactivating NDV $\leq$ 0.05 ppm available chlorine dioxide after 1 hour incubation.

While ClO_2 appears to be effective at inactivating virus at a lower concentration than Cl, ClO_2 is (currently) in the order of 10 times more expensive than simple chlorine to use in water sanitation.

While IBDV and NDV each appear sensitive to inactivation by simple chlorine treatments at 5 ppm in clean water, a minimum requirement is for pre-treatment by adequate (sand) filtration of surfacewater supplies, prior to chlorine treatment. Hence we have recommended that the key findings of this laboratory pilot study, i.e. that. contaminations with IBDV (likely the most hardy poultry viral pathogen) and NDV (relatively fragile) in poultry drinking water appear to be susceptible to the inactivating effects of 3 ppm free chlorine in clean water, need to be confirmed and extended in their practical relevance to effectiveness of Cl-IBDV inactivation parameters. Specifically by quantification of the water quality (especially total suspended solids, pH and protein levels) being produced and used on several, typical industry production sites after their practical treatments, e.g. sand filtration, and re-testing the efficiency of simple chlorine treatment using these field water samples as substrates.

Introduction

Some half of the commercial poultry production sites in Australia draw on surfacewaters (dams, creeks, rivers) as their major source of drinking water. In some cases this is used untreated save for settling procedures and crude filtration, and in the majority of others, simple addition of chlorine is the only treatment. While a relatively economical treatment process, no data (worldwide) is available as to the likely efficiency of chlorine for the removal of infectivity (= inactivation or "killing") of viral poultry pathogens.

Such information is an important step in risk assessment for biosecurity of poultry production sites using surfacewater for drinking or other purposes, e.g. cooling sprays, as surfacewaters are known to provide an interface between wild waterfowl and domestic poultry. Significant viral pathogens such as **Newcastle disease, avian influenza** and **egg drop syndrome (haemagglutinating avian adenovirus)** are all potentially contaminants from wildbird populations in Australia. Of further concern is **infectious bursal disease virus (IBDV)**, for which strains of moderate pathogenicity are already present in Australia and for which the threat of introduction of very virulent strains already endemic in Asia (and other overseas) poultry is increasing. To enable more objective assessment of the risks inherent in the Australian poultry industry's current use of surfacewaters, data on inactivation of the potential viral contaminants in water was required to be developed.

At the request of the Egg Program of the RIRDC, a preliminary study was commissioned for 12 months during 2000 – 01. Their research committee requested that the virus inactivation in water studies be conducted using the (likely) most resistant concern virus IBDV be used as the research model virus system. Also that chlorine-based treatment be used to initiate development of laboratory capacity for quantifying the effectiveness of treatments to sanitise their water supplies. Within this scoping study, the effectiveness of inactivation of Newcastle disease in surfacewater was subsequently included because of the concerns re biosecurity with NDV outbreaks being encountered by Australian industry around this time.

Objectives

1. To develop a laboratory-based assay to enable the quantification of inactivation of IBDV by chlorine treatment of IBDV suspended in drinking-quality water.
2. To then undertake sighter experimentation using representative surfacewater to assess the likely effectiveness of simple chlorine inactivation within the levels of treatments, i.e. 1 – 10 ppm, routinely used in the field for the production of potable water supplies for poultry.

Methodology

Assays of free and total chlorine

A chlorine meter (Hannah Instruments, HI 93734 Free & Total Chlorine HR ISM) was purchased along with the reagents need for measuring free and total chlorine. The meter was tested by diluting a stabilised chlorine solution (Milton Anti-bacterial solution, Proctor & Gamble Australia Pty Ltd) in distilled water to give 0, 0.1, 1, 3 and 5ppm. These solutions were repeatedly tested and the readings compared. Over the range tested, readings varied by less than 0.1ppm for both free and total chlorine. This was considered to be sufficiently reproducible to use throughout this project. For this project work, readings of free (available) chlorine were used.

Propagation of IBDV for assays

IBD virus: Propagation in eggs

IBD virus was inoculated into the allantoic sac of eggs and embryos harvested 5 days post inoculation. The embryos were homogenised with a solution of SPA (sucrose, phosphate and albumen) and filtered through multiple layers of cheesecloth to remove clumps of tissue. This was titrated in eggs and found to have a titre of 10^5 EID_{50}/mL.

IBD virus: Propagation in cell culture

When egg propagation of IBDV was discontinued as an alternative, a sample of IBDV GT 101, an Australian virus strain adapted to grow in chicken embryo fibroblast cells (CEF's) was grown, with the aim of attaining a high titre stock of virus with an especially low concentration of protein. Repeated attempts were required to achieve this outcome in that several serial passages using freshly prepared primary CEF cultures were found to be needed to support the growth of IBDV to a titre sufficiently high to be used for this project. A stock preparation of IBDV with a titre of $10^{5.6}$ $TCID_{50}$/mL was grown in media containing the minimum possible level of protein required to support the growth of cell cultures (1% foetal calf serum).

Preparation of Newcastle Disease virus stocks

Newcastle disease virus (V4) was inoculated into the allantoic sac of embryonated SPF eggs at a rate of 1 haemagglutinating unit per egg. The eggs were incubated for 4 days, then chilled and the allantoic fluid harvested. A titre of $10^{9.5}$ EID_{50}/mL was obtained. This readily enabled use in testing for effects of chlorine treatments, as this virus was able to be diluted 1/100,000 (10^{-5}) in water, so as to ensure that any residual egg protein levels would not be significant in reducing chlorine effectiveness.

Assay systems for effects of chlorine treatment on IBDV

Egg propagated IBDV

Using an Australian isolate of IBDV, which had been adapted to growth in chicken embryos by serial subculture, a stock of virus was obtained and infectivity was titrated to be 10^5 EID_{50}/mL (100,000). This virus was diluted to a total of 1/100 for the chlorine inactivation testing, giving a titre of 10^3 EID_{50}/mL. At a dose rate of 0.2mL/egg able to be inoculated after treatment, this gave a working concentration of 200EID_{50}/egg if the solution was used with no further dilution. To determine if there had been a reduction in virus infectivity, 10 fold dilution steps were used. Dilutions of 10^{-1} and 10^{-2} yielded 20 and 2 EID_{50}/egg respectively.

Cell culture propagated IBDV

A virus stock of $10^{5.6}$ $TCID_{50}$/mL was able to be diluted 1/200 for inactivation trials with chlorine. The natural chlorine demand of this substrate at this dilution was found to be much less than the egg cultured IBDV, but was still measurable.

Treatments of virus were undertaken at 1, 3, 5 or 10 ppm of chlorine against IBDV suspension diluted 1/100 in water. Following an exposure period of 1 hour at room temperature, neutralisation of the residual chlorine (or chlorine dioxide) was undertaken by addition of 0.08mL of a 10% solution of skim milk powder per 2mL of sample. This was allowed to remain at room temperature for 20 minutes. A further 10-fold step was then required for the samples not to be toxic for cell cultures. Test solutions were then titrated in serial 2-fold steps from 1/10 through to a maximum of 1/320 to assess residual infectivity. Tissue culture microtitre trays were scored for cytopathic effect after a further 5 days incubation at 37^{o}C in an incubator with 5% CO_2.

To ensure that free chlorine levels were maintained at close to the desired treatment levels, i.e. 1 ppm and 3 ppm, measurements were taken immediately after combining the virus with the chlorine solution virus, at 30 minutes and again at the end of the 60 minute incubation. It was observed that additional chlorine was needed to maintain the chlorine levels, specifically to maintain 1 ppm, a total of 2.5 ppm was added, whilst to maintain 3 ppm a total of 7.5 ppm was required, i.e. in the order of double the requisite.

Detailed Results

1. Stability of chlorine in distilled water

To check the stability of chlorine dilutions over time samples of chlorine were diluted in distilled water to a target concentration of 5 ppm and held at room temperature (~20^{o}C). Chlorine levels were monitored immediately on addition of chlorine, after 1 hour and after 24 hours. For the 5 ppm target, the initial reading showed 5.05 ppm, with 4.20 ppm after 1 hour and 2.97 ppm after 24 hours.

Conclusion

Residual chlorine activity decreased over a 1 hour test period, but not significantly at the target concentration, i.e. 4 – 5 ppm.
NB: Negligible inactivation of virus was found to occur after this contact time so this was selected as adequate for the simple, i.e. non-monitoring, reinjection test system used.

2. Treatment of IBDV with chlorine

Virus propagated in SPF embryos.

Virus IBDV propagated in eggs of infectivity $10^{5.0}EID_{50}$/mL stock was diluted in distilled water and chlorine added to give a final concentration of 0, 1, 3 and 5 ppm of chlorine and a final dilution of 1/100 of virus. The chlorine concentration was monitored over a one hour incubation period. At the end of the hour, the virus/water/chlorine solutions were inoculated into eggs by the chorioallantoic membrane and examined for evidence of IBDV after 5 days incubation. Results obtained showed that within 5 minutes of mixing the free chlorine levels had been heavily reduced (Table 1).

Table 1. Free Chlorine concentration over a 1 hour incubation with egg grown IBDV diluted to 1/100 in distilled water

Chlorine – target concentration (ppm)	0	1	3	5
Actual amount of chlorine added (ppm)	0	1.13	3.08	5.12
Chlorine reading 5 minutes after addition of virus (ppm)	0.05	0.36	0.43	0.28
Chlorine reading after 1 hour incubation (ppm)	0.03	0.06	0.77	0.48

Higher concentrations of chlorine, 5 and 50ppm chlorine, were then used in repeat assays using the same procedures. Immediately on addition of the virus, the free chlorine levels were found to have dropped to from 5 ppm to 0.16 ppm in the 5 ppm sample and from 50 ppm to 4.77 ppm in the 50 ppm sample. After 1 hour incubation this had reduced further to 0.06 ppm and 2.42 ppm for the 5 and 50 ppm samples, respectively. Subsequent virus infectivity titrations of the samples showed no inactivation at either starting concentration after 1 hour.

Conclusion

It was not possible, with this virus propagation system, to control the levels of chlorine making contact with the target virus. Egg propagated IBDV was then discontinued.

b. IBD virus propagated in cell culture

A starting virus preparation of $10^{5.6}$ $TCID_{50}$/mL was used, and the protocol followed as detailed in Methodology. To maintain the concentration of chlorine, measurements were taken immediately after addition of virus, at 30 minutes and again at the end of the 60 minute incubation. It was found that, even with this (low protein) medium, additional chlorine was needed to maintain the target chlorine levels. To maintain 1 ppm, a total of 2.5 ppm was added, to maintain 3 ppm a total of 7.5 ppm was required. Data obtained was as in Figure 1.

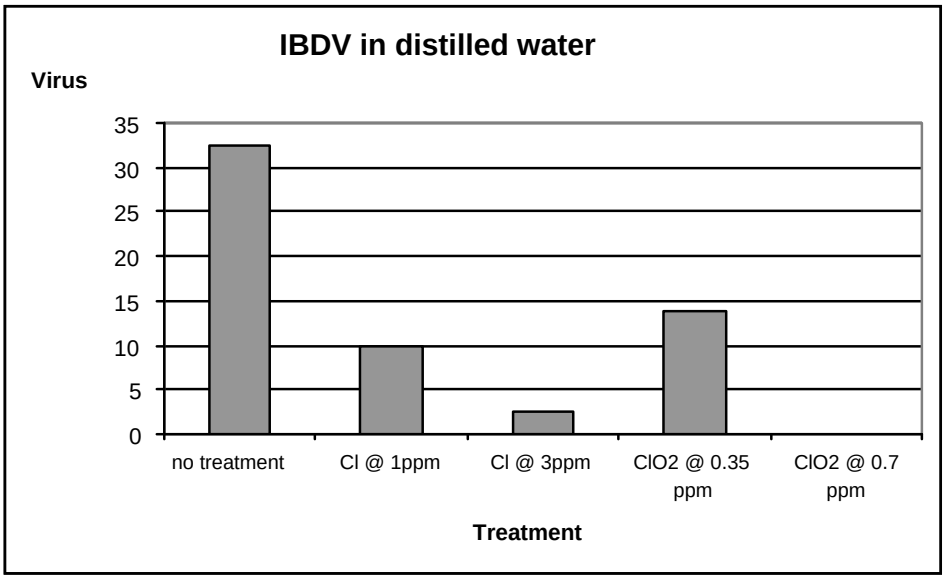

Figure 1. IBD virus titre after 1 hour treatment at 21.6^0C, pH 6.84

Relative to the untreated IBDV suspended in an equivalent volume of chlorine-free water for 1 hour at room temperature (21.6^oC) and near neutral pH (6.84), infectivity in this virus suspension could still be detected to a level shown in Figure 1. Exposure to 1 ppm chlorine for 1 hour reduced levels of infectivity some 3 fold (33%), while maintaining a level of 3 ppm reduced the infectivity 10-fold, i.e. 90% of infectivity was able to be removed.
NB: Drinking water for chickens is generally reduced in attractiveness (C1 odour & taste) at concentration $\geq$ 3ppm at the shed drinkers.

Repeating this investigation, but including chlorine dioxide (ClO_2) for comparison at similar levels confirmed this initial result but also indicated that ClO_2 also was not fully effective for the inactivation of IBDV at 0.35 ppm (Figure 1). When a treatment level of 0.7 ppm was used however, IBDV infectivity was effectively reduced to below a threshold of detection of this test system, i.e. $\geq$33 fold or $\geq$ 97%.

NB: Potability (attractiveness) of drinking water for chickens is generally unaffected by residual levels of ClO_2 in shed drinkers of 0.3 - 0.5ppm.

Conclusions:

1. IBDV is susceptible to the inactivating effects of simple chlorine treatment with exposure to 3ppm reducing infectivity by 90% in 1 hour. It is surmised therefore that treatment levels of free chlorine in the order of 5 ppm would need to be able to be maintained in clean water for at least 1 hour to achieve a $\geq$ 97% reduction in infectivity.
2. It is extremely unlikely that reliable levels of chlorine treatment of water can be achieved other than with systems for automatic monitoring and re-injection. (These systems are available commercially). Further, to ensure effective treatment will also likely require that water be held and treated in batches, rather than being continually run off into sheds as is sometimes the case at present.
3. Chlorine dioxide (ClO_2) proved considerably more effective at inactivating IBDV than using simple chlorine treatments. In this investigation, 0.7 ppm of ClO_2 was sufficient to inactivate IBDV to below the threshold detection levels of this test system ($\geq$ 97% removal of infectivity)

3. Effect of using untreated dam water on efficacy of chlorine treatments (Cl, ClO_2)

Water from a representative production water-source dam (complete with wading ibis!) was obtained from a commercial poultry farm. This was visibly turbid, and was used without any form of filtration. Chlorine at 1, 3, 5, and 10 ppm was added and measurements were taken throughout the 1 hour incubation period. Additional chlorine was added as needed to maintain the target chlorine levels.

Table 2. Chlorine required to maintain the target level of chlorination over a 1 hour incubation period in unfiltered dam water with IBDV

Target chlorine level	1 ppm	3 ppm	5 ppm	10 ppm
Chlorine required	4 ppm	10.5 ppm	12.5 ppm	20 ppm

ClO_2 was also tested at target concentrations of <0.05, 0.05, 1.3 and 4.4 ppm, using the same test conditions of 20.4^{0}C and a pH of 7.30 and a 1 hour incubation period. No additional ClO_2 was added.

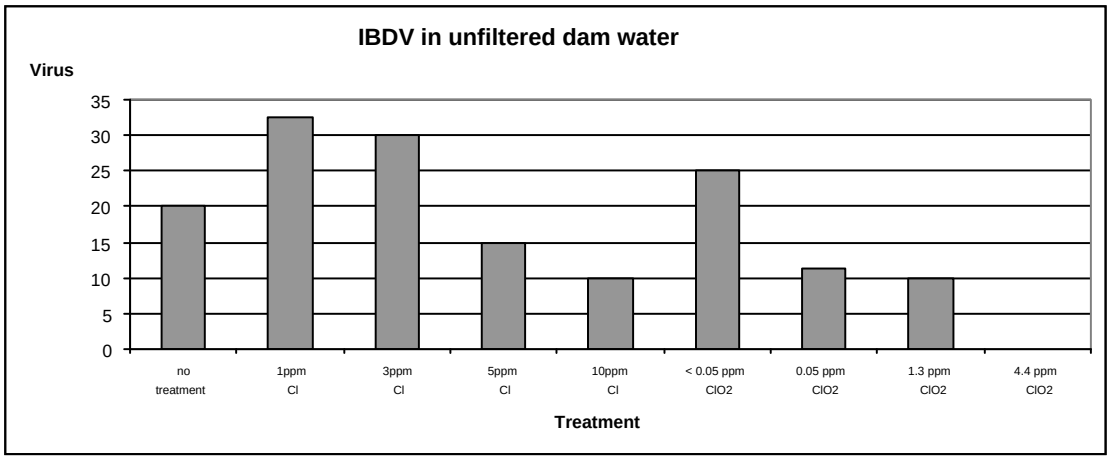

Figure 2. IBD virus titre after1 hour treatment at 20.4^{0}C, pH 7.30

At the maximum concentration tested, i.e. 10 ppm for one hour chlorine did not inactivate IBDV in unfiltered dam water. At an actual concentration of 4.4 ppm (available ClO_2), ClO_2 was able to strongly inactivate IBDV i.e. $\geq$ 97% removal of infectivity under the same conditions.

Conclusions:

1. Pretreatment of water e.g. coarse filtration, is a critical step prior to sanitation using chlorine treatments. Very significant losses in free chlorine can occur otherwise as shown in table 2.
2. Only slight inactivation of (3-fold) could be achieved by 10 ppm chlorine, but was able to be obtained using chlorine dioxide in excess of 3 ppm.

4. Effectiveness of chlorine treatment on NDV suspended in water

Concerns about the ability of NDV to spread via water supplies led to our including testing chlorine and ClO_2 in both distilled and unfiltered dam water for ability to inactivate NDV. In distilled water, chlorine was able to inactivate more than $10^{3.5}$ (7,000) egg infectious doses of NDV at a concentration of 1 ppm and ClO_2 at 0.35 ppm after 1 hour.

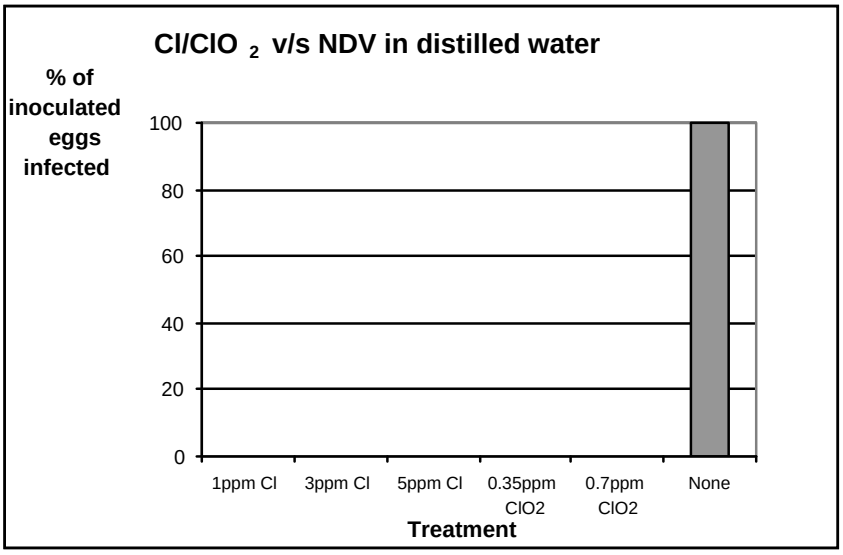

Figure 3. Percentage of eggs infected following inoculation with NDV in distilled water after treatment with chlorine or ClO_2 for 1 hour at 21^0C and a pH of 6.8

When suspended in the dam water referred to in the previous section, chlorine was less effective, requiring 5 ppm (available chlorine, maintained by monitoring available chlorine levels and adding additional chlorine) to inactivate NDV in this test system. Chlorine dioxide was effective in inactivating NDV at a concentration of $\leq$ 0.05 ppm available chlorine dioxide after 1 hour incubation (Figure 4).

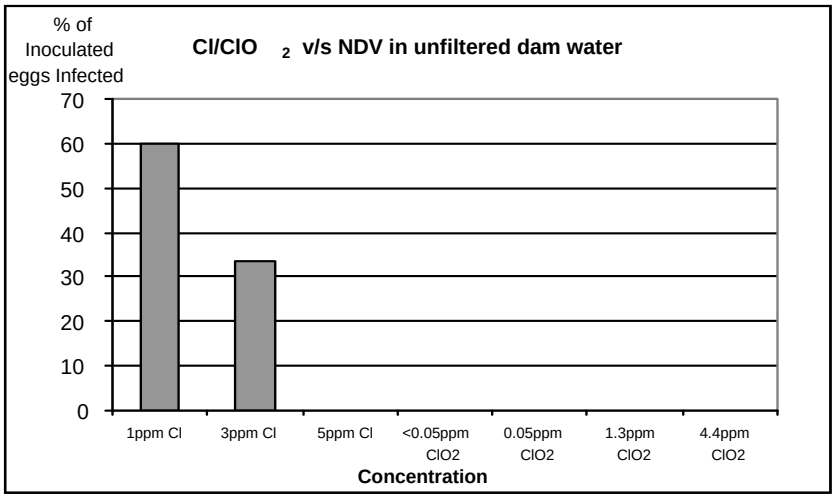

Figure 4. Percentage of eggs infected following inoculation with NDV in unfiltered dam water after treatment with chlorine or ClO_2 for 1 hour at 20.9^0C and a pH of 7.35

5. Industry interactions

Interest has been high throughout the period of this project 2000-01. Enquiries, requests for advice and offers of collaboration in assessing the efficiency of water sanitation treatments have been received from 6 major pharmaceuticals and industry companies, as well as from several producers. Also, personnel in the NSW and Queensland Departments of Agriculture and Victoria's Department of Natural Resources and Environment have made contact to discuss aspects of water sanitation treatments particularly biosecurity and environmental impact aspects.

Discussion of Results

Objectives

#1. To develop a laboratory-based assay to enable the quantification of inactivation of IBDV by chlorine treatment of IBDV suspended in drinking-quality water.

#2. To then undertake sighter experimentation using representative surfacewater to assess the likely effectiveness of simple chlorine inactivation within the levels of treatments, i.e. 1 – 10 ppm, routinely used in the field for the production of potable water supplies for poultry.

1. Objective #1. Development of assays system technology for IBDV-Cl. Achieved.
   Objective #2. Sighter experimentation on the effectiveness of simple chlorine treatment for water sanitation. Achieved to the preliminary stage findings here. It must be stressed however that this scaled study was undertaken with quite limited resources during 12 months, and therefore could not be expected to produce comprehensive answers to an Australia wide problem. For technical reasons, the use of IBDV in the assay system has proved difficult to work with in only being propagatable to low titres and its high level of resistance to chlorine. However the industry's reasoning (on risk-management) for requesting that IBDV be used as a test model virus is understood. It is noted also that any further work undertaken with chlorine should be based around commercial-type chlorine injection-electronic monitoring of dosage to maintain chlorine levels closely during treatments
2. Infectious bursal disease virus (IBDV) infectivity was able to be reduced 90% by exposure to 3 ppm free chlorine for 1 hour in good quality water. This level of treatment in water of a similar pre-filtered quality would also ensure the effective removal of Newcastle disease virus. However if surface water is being drawn (e.g. from dams) and used by treatment with chlorine

after incomplete removal of suspended solids, these results prove that, as would be expected, the effectiveness of chlorine sanitation will be greatly reduced.

3. The results appear to be the first objective data which had been obtained using low levels of Cl against IBDV and NDV in drinking water. While encouraging, this line of work needs to be continued and extended by using water from commercial sites, which has already undergone their practical standard of prefiltration (e.g. sand) and which can be tested as a substrate for testing of chlorine treatment on IBDV infectivity using our laboratory assay systems

Implications for industry

1. While IBDV and NDV each appear sensitive to inactivation by simple chlorine treatments at 5 ppm in clean water, a minimum requirement is for pre-treatment by adequate (sand) filtration of surfacewater supplies. It will therefore likely prove necessary to be able to specify the maximum limits for pH, protein content and total suspended solids (TSS) required to be produced before treating surfacewater using simple chlorine treatment.
2. Should introduction of very virulent IBDV occur into Australia's poultry industry, water supplies will be difficult to sanitise using the simple chlorine treatments now widespread in industry practices, i.e. dositron delivery but non-monitoring of chlorine treatments. In fact presently in some cases water can be experiencing its first contact with chlorine as it moves in the pipes towards the poultry houses. Also the concept of sanitising treatment of drinking water in batches, e.g. 100,000 litres for a specific time for each batch dosed will need careful consideration for adoption as an industry standard.

3. A more complex source of Cl which was found to show considerable promise in the inactivation of IBDV was chlorine dioxide (ClO_2). However the cost-benefit of this type of compound, needs to be carefully evaluated as ClO_2 is (currently) some 10 times more expensive than simple chlorine to use in water sanitation. Several newer types of chemical-based water treatments, e.g. 'Aquasept' treatment concepts and PAP (peracetic peroxidic acid) compounds have also recently emerged. The only information on effectiveness at present are the claims of manufacturers and their suppliers.

4. Industry benefit can flow directly on from having an independent source of water sanitation-biosecurity expertise, both to assist itself in developing reliable practical water treatment standards, and through these to suppliers of water treatments.

Recommendations

The key findings of this laboratory pilot study, i.e. that. contaminations with IBDV (likely the most hardy poultry viral pathogen) and NDV (infectivity relatively fragile) in poultry drinking water appear to be susceptible to the inactivating effects of 3 ppm free chlorine in clean water, need to be confirmed and extended in their practical relevance to effectiveness of Cl-IBDV inactivation parameters. Specifically by quantification of the quality (especially total suspended solids, pH and protein levels) being produced and used on several, typical industry production sites after their practical treatments, e.g. sand filtration, and re-testing the efficiency of simple chlorine treatment using these water samples as substrates.

Because of the importance of maximising the knowledge base in this key biosecurity area of disease prevention, this investigation should be undertaken as quickly as possible, e.g. during 2002.

A short-term experimental proposal for such a focused extension of this study will be submitted late in 2001 for consideration by the RIRDC Egg Program. Such an extension of the present project work will prove a cost-effective investment in site biosecurity in Australia.

Plain English Compendium Summary

Project Title:	Investigating Sanitation of Surface water for Poultry using Chlorine-IBDV Models
RIRDC Project No.:	UM51A
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Objectives	• To develop a laboratory-based assay to enable quantification of inactivation ("killing") of infectious bursal disease virus (IBDV) by chlorine treatment of drinking-quality water and apply this assay to assessing the likely effectiveness of treatments using 1 – 10ppm (parts per million)
Background	Some half of the commercial poultry production sites in Australia draw on surfacewaters (dams, creeks, rivers) as their major source of drinking water. In some cases this is used untreated save for settling procedures and crude filtration, and in the majority of others, simple addition of chlorine is the only treatment. While a relatively economical treatment process, no data (worldwide) is available as to the likely efficiency of chlorine for the removal of infectivity (= inactivation or "killing") of poultry viruses. At the request of the RIRDC Egg Industry Program, sighter studies utilising IBDV, a viral pathogen (cause of disease) of major concern, was tested for sensitivity to inactivation by simple chlorine treatment of water.
Research	IBDV strain GT 101 propagated in freshly prepared chicken embryo fibroblast cell cultures was used to produce virus stocks of sufficiently high virus concentration. Dilutions of this virus in distilled water and in untreated surfacewaters (dam source) were tested against 1, 3, 5 & 10ppm of free chlorine. Chlorine dioxide, a more complex compound, was also tested. The inactivation of Newcastle Disease virus was also tested.
Outcomes	IBDV is susceptible to the inactivating effects of simple chlorine treatment, and it appears that levels of free chlorine in the order of 3 ppm if maintained in clean water for at least 1 hour, can achieve a $\geq$ 90% reduction in infectivity. Higher Cl concentrations reduce infectivity further. It is extremely unlikely that reliable levels of chlorine treatment of water can be achieved however other than with systems for automatic monitoring and re-injection. (These systems are available commercially, To ensure effective treatment will also require that water be held and treated in batches, rather than being continually run off into sheds as is sometimes the case at present. Chlorine dioxide (ClO_2) proved considerably more effective at inactivating IBDV than using simple chlorine treatments. In this investigation, 0.7 ppm of ClO_2 in a clean water substrate was sufficient to inactivate IBDV to below the threshold detection levels of this test system i.e. $\geq$ 97%. NDV was much more susceptible to inactivation in clean water than IBDV. Untreated dam-source water proved highly unsuitable as a substrate for chlorine inactivation of avian viruses. Industry interest in the project is high with many requests for information and two contract research projects are currently under negotiation
Implications	While IBDV and NDV each appear sensitive to inactivation by simple chlorine treatments at 5 ppm in clean water, a minimum requirement is for pre-treatment by adequate (e.g. sand) filtration of surfacewater supplies. It will therefore likely also prove necessary to be able to specify the maximum limits for pH, protein content and total suspended solids (TSS) required to be produced before treating surfacewater using simple chlorine treatment.
Publications	T.J. Bagust, 2000. Assuring effective water sanitation. Proceedings, Poultry Information Exchange. Surfers Paradise, 9-11 April 2000 pp23-35