Investigating Sanitation of Surface Water for Poultry Using Chlorine - IBDV Models - II

A report for the Australian Egg Corporation Limited

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Foreword

Water quality is particularly important to the productivity of the poultry industry. Significant numbers of Australian poultry farms rely on surface water sources (dams, creeks and rivers) for drinking water or for other production needs, especially cooling during warmer weather periods. Exposure of surface water sources to wild birds, wild waterfowl particularly, can pose disease risks from serious avian viral and bacterial pathogens, to contamination of commercial flocks. These include significant pathogens such as avian influenza virus, Newcastle disease virus, and infectious bursal disease virus (IBDV) as well as 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 treatments of surface waters, so as to ensure effective inactivation of poultry pathogens, are not available worldwide.

If however simple treatment of surface water can inactivate viral contamination reliably and economically on poultry farms, a significant "weak spot" for entry of potentially pathogenic viral infections into flocks can be removed.

Based on its physio-chemical characteristics IBDV is likely to be amongst the most resistant of the avian pathogens to inactivation by disinfectants. It was therefore chosen for the laboratory-scale experimentation using chlorine to attempt to inactivate IBDV in water as is described in this report.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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James Kellaway
Managing Director
Australian Egg Corporation Limited

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The Australian Egg Corporation Limited is thanked for their encouragement of these studies into sanitation of surface waters for poultry use, and the financial support given for the 2000/01 research project and the current 2002/03 investigations. The poultry industry is also acknowledged for their considerable interest in this project area, and particular thanks to the poultry farms who provided water samples for analysis..

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Specific interests
Research and development for improved prevention and control of viral infections of poultry, currently Avian Leukosis Subgroup J; removal of viruses from surface waters, postgraduate veterinary education; international development assistance projects.

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Abbreviations

CEF	Chicken embryo fibroblast cells
Cl	Chlorine
EID_{50}	Egg infectious doses 50%
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
mg/L	milligrams per litre
NDV	Newcastle disease virus
ppm	Parts per million
SS	Suspended solids
$TCID_{50}$	Tissue culture infectious doses 50%
TDS	Total dissolved solids
TKN	Total nitrogen
TSS	Total suspended solids

Executive Summary

Half or more of Australia's poultry production sites, in particular egg-laying farms, are regularly dependant on surface water supplies (dams, reservoirs, creeks or rivers) for their poultry drinking, spraying or washing purposes. Bodies of surface water however are a potential disease interface between wild waterfowl and domestic poultry. Over time Newcastle disease and egg drop syndrome virus intrusions are known to have occurred, but most recently high pathogenicity avian influenza viruses have entered domestic poultry flocks with catastrophic effects in Europe (2003), North America and Asia (2004). Such incursions are likely to increase in frequency in the 21st century with the increasing domestic poultry populations.

Chlorination has been identified as the most appropriate and cost effective technology for treatment of water for poultry sites in Australia. (Bagust, T.J. PIX 2000 Proceedings). Although chlorine treatment is widely used for water sanitation in this country, how to control treatment is in general only poorly understood by poultry farmers especially for major factors which can inhibit effectiveness such as concentration x contact time, pH, temperature and seasonal variation in biological loadings.

During 2000/01 an RIRDC Egg Program-funded preliminary investigation was undertaken to commence developing a scientific basis for quantifying and managing the risk of surface waters in Australian poultry production. Laboratory technology capable of monitoring the effectiveness of simple chlorine as a sanitising treatment was developed, and showed that contamination with either infectious bursal disease virus (IBDV, which is likely the most hardy viral pathogen) or Newcastle disease virus (which appears relatively fragile) are both susceptible to the inactivating effects of 3-5ppm free chlorine in clean water.

For the 2002/03 project, funded by AECL, three key questions addressed were:

#1. For surface waters in Victoria, what are the quality standards for the waters which are being currently used by industry?
#2. Can a simple treatment for surface water e.g. chlorine be used to eliminate the potential site quarantine risk of use of these surface waters?
#3. Could an alternative simple chemical treatment product which is available in Australia, i.e. Citrox™ be used by smaller poultry producers as an emergency measure to reliably sanitise their surface water supplies?

For determining the water quality profiles (Objective #1) samples of surface water were collected from five commercial poultry production sites from various locations in Victoria, covering a geographical distribution of some 25,000 square kilometres. Samples originating from dam water or from creeks were collected for analysis from the supply points beside the poultry houses. The results indicate (1) that the quality of the surface water being used varied greatly and (2) that simple treatment before chlorination such as sand filtration could still leave very high levels of dissolved solids and of total nitrogen present in water. Total nitrogen levels are significant as these exert a major "quenching" effect on the oxidative action (hence the disinfecting capacity) of chlorine in water.

Objective #2, For determining whether chlorine can be used at 5 ppm to eliminate virus-contaminated surface waters as a site quarantine risk, the water sample from each site A through E was tested individually using an infectious bursal disease virus (IBDV) model as a challenge system, as detailed in the RIRDC Egg Program Report 2001. Use of IBDV had been specifically requested by the RIRDC Egg Program Advisory Committee as a representative test virus of a relatively high degree of resistance to inactivation by chemicals in water, i.e. sanitisation treatments.

To field water samples for which the native chlorine demand had been depleted, the diluted virus and an additional amount of chlorine was added so as to yield a final treatment level of 5ppm in this sample at the end of a 2 hour 20^{o}C treatment period. The chlorine level was then monitored during the subsequent 2 hour period, with additional chlorine being added if the chlorine level was found to have dropped below 5ppm.

For each water sample from a field source a virus control, which consisted of distilled water and IBD virus with no chlorine added was used at the same time. The virus content was titrated after one and then 2 hours, along with the sample and the distilled water control. All samples and the distilled water control were tested using 7 replicates, while the virus control was titrated in triplicate.

Results: After 1 hour, each of the samples of field water tested showed virus titres to be reduced but viable virus was still present at low levels. IBDV which had only been diluted in distilled, i.e. non-surface, water was completely inactivated i.e. no detectable infectivity remained. For each of the field water samples after 2 hours exposure to 5 ppm chlorine however, there was no evidence of viable IBDV remaining in any of the samples. Full details are shown in the report.

Note

Extrapolation to viruses of Newcastle disease (NDV) and avian influenza (AIV). Studies in these laboratories during 2001 - 2002 have previously shown that Newcastle disease virus (NDV) could be inactivated in untreated dam water by maintaining a level of 5 ppm chlorine for one hour. As both NDV and AIV viruses have similar physio-chemical properties, it is likely that any treatment that is effective in inactivating NDV will also be effective in inactivating AIV. Hence any treatment that is capable of inactivating IBDV within 2 hours, i.e. maintaining 5ppm chlorine as we have established as an experimental parameter here, should be more than adequate to ensure that both NDV and AIV do not survive. If chlorine is used at 5 ppm for treatment, levels will decline to potability levels for poultry, i.e. $\leq$ 3ppm within a few hours at most.

To examine the effects of suspended solids on chlorine levels, experimentation using Kaolin (hydrated aluminium silicate) was undertaken with suspended solids mirroring the natural range that was found in the field water samples, i.e. 2 – 90 mg/L while a further 3 fold concentration was also included in the testing. Suspended particles were found to have only a minor effect on the level of free chlorine present in a water sample which was undergoing treatment.

To enable direct examination of the potential of nitrogen to interfere with inactivation effects of chlorine, distilled water with levels of nitrogen ranging from 2 to 12 ppm (mg/L) in 2mg/L increments were tested. There was an immediate reduction in the level of free chlorine when concentrations of nitrogen were present at 2ppm or greater. Temperature variation from 4^{o}C to 37^{o}C did not prevent chlorine from inactivating IBD virus when 5ppm was maintained for 2 hours.

Citrox™, a non-rinse biocide derived from orange extract, was also tested at the request of AECL. At twice the manufacturer's recommended concentration for disinfection ie 3.2 mL per litre and using only distilled water as a test substrate, low levels of residual IBDV infectivity continued to be detectable in the test system. On the data obtained here therefore, the use of Citrox™ as a water sanitiser could not be recommended for reliable removal of viral infectivity.

For the most complex surface water samples which we encountered , containing dissolved solids up to 260mg/L, total nitrogen levels up to 11 mg/L and suspended solids to 89 mg/L, treatment with 5ppm chlorine for 2 hours was able to inactivate the challenge dose of IBDV.

As a result of the studies undertaken here, the following system is recommended to be employed for use on Australian poultry production sites which are using surface waters.

(1) A batch treatment system which uses 2 tanks in parallel, one for treatment when the other is being used for supply of water to poultry houses

(2) Ensuring the maintenance of free chlorine levels at 5ppm throughout a 2 hour treatment period, by monitoring through electronic sensing and reinjection of fresh chlorine as required.

These two operational elements should become the basis of standard practice for water biosecurity in treating surface water before use on production sites within the Australian poultry industry in future.

Introduction

Of production sites for the commercial poultry industry in Australia, almost half are dependent to a larger or smaller degree on the use of surface waters as a major source of water whether for poultry drinking or other purposes e.g. cooling sheds.

During 2000/01 an RIRDC Egg Program-funded preliminary investigation was undertaken to commence developing a scientific basis for quantifying and managing the risk of surface water in Australian poultry production. Laboratory technology capable of monitoring the effectiveness of simple chlorine as a sanitising treatment was developed, and showed that contamination with either infectious bursal disease virus (IBDV which is likely the most hardy viral pathogen) or Newcastle disease virus (which appears relatively fragile) are both susceptible, but to varying degrees, to the inactivating effects of 3-5ppm free chlorine in clean water. These studies also confirmed when using simple chlorine treatment, that surface water should undergo pre-treatment processing e.g. filtration to an average particle diameter in the order of 20 microns.

These findings pointed strongly to the need for developing of an industry minimum standard of pre-treatment of surface water, regardless of the system of filtration used. Bench study monitoring of free chlorine levels also indicated the difficulty of being able to maintain chlorine treatment at required levels, over periods longer than 15 minutes, even in clean water (For more details, see Report to RIRDC Egg Program, November 2001.)

Objectives

Objectives were developed towards answering three (3) key questions:

1. For Australian surface waters in Victoria (as a representative major egg-producing state), what are the quality standards for the waters which are currently being used by industry?
2. Can a simple treatment for surface water e.g. chlorine at 3-5ppm, be used to eliminate the potential as a site quarantine risk posed by use of these waters (as in #1 above)?
3. Could a simple chemical treatment product available in Australia, e.g. Citrox™, be used by smaller poultry producers as an emergency measure to reliably sanitise their surface water supplies from IBDV contamination as an alternative to chlorine?

Methodology

Assays of free and total chlorine

A chlorine meter (Hannah Instruments, HI 93734 Free & Total Chlorine HR ISM) was used along with the reagents needed 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.

IBD virus: Propagation in cell culture

A sample of IBDV GT 101, an Australian virus strain of infectious bursal disease virus which has been adapted to grow in chicken embryo fibroblast cells (CEF's) was prepared, using several serial passages using freshly prepared primary CEF cultures. 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.

Assay systems for effects of chlorine treatment on IBDV

Cell culture propagated IBDV

The IBDV virus stock of $10^{5.6}$ $TCID_{50}$/mL was able to be diluted 1/200 for use in the inactivation trials performed using chlorine.

Using the testing protocol developed through a previous project (2001 RIRDC Egg Program report), treatments of virus were undertaken at 5 ppm of chlorine against IBDV suspension which was diluted 1/200 in the water samples under test. Following an exposure period of either 1 hour or 2 hours at room temperature, neutralisation of the residual chlorine 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 in order to assess residual infectivity. After a further 5 days incubation at 37^{o}C in an incubator with 5% CO_2, tissue culture microtitre trays were scored for cytopathic effect of residual IBDV infectivity.

Detailed Results

1. Field Surface Water Samples

Samples of surface water were collected from five commercial poultry production sites from various locations in Victoria, see Figure 1. The geographical distribution of these sites represented an area of some 25,000 square kilometres. Samples originated from dam water or from creeks being used at these locations, and were deliberately obtained from a sampling point located as close as possible to the building where the poultry flock was being housed. At these production sites, the treatments of water before use varied from filtration, softening and chlorination before use, through to the water being fed directly into the header tank and then into the poultry drinking lines with no treatment being undertaken.

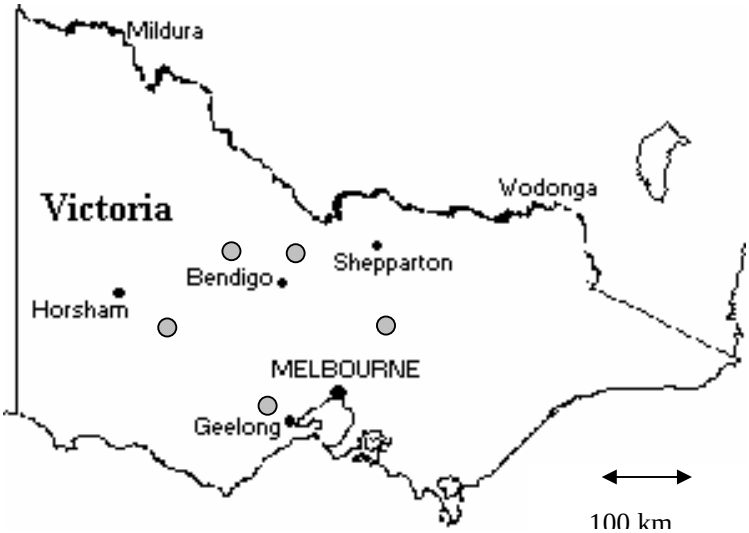

Figure 1: Locations of sites in Victoria where field water samples were collected ○

From each site a 5 litre sample was collected, prior to any sanitising treatments being administered. This sample was transported to the laboratory on wet ice.

Within 24 hours a 500mL sample was delivered to an independent analytical laboratory that is accredited for water quality testing. Profile testing was performed for:

Suspended Solids (SS)
Total Dissolved Solids (TDS)
pH
Total nitrogen (TKN = NH_3 and organic nitrogen)

Results are shown in Table 1

Table 1 Water profile testing for poultry production sites in Victoria

Property	**A**	**B**	**C**	**D**	**E**
pH	6.5	6.7	7.5	7.8	7.2
Total Dissolved Solids mg/L	95	64	260	71	190
Total Nitrogen mg/L	11	1.1	4.7	3.8	4
Suspended Solids mg/L	4	2	2	22	89
Water treatment prior to collection	Gravity settled	Sand filter	Sand filter	Not treated	Not treated

These results show that the quality of water varied greatly between the different sites. The pH of the samples tested ranged from 6.5 to 7.8. It was noted that all were within the range pH 6 – 8 where

chlorine treatment can be most effective (Dychdala 1991). The levels of suspended solids ranged from 2 mg/L to 89 mg/L, reflecting both the starting quality of the water and the level of pretreatment applied to the water samples. The two untreated samples (D and E) had much higher levels of suspended solids than the gravity settled or the sand filtered water samples. The levels of total dissolved solids however ranged from 64 to 260 mg/L, with one of the two sand filtered samples (B & C) showing that very high levels of dissolved solids can be retained. The levels of total nitrogen varied between 1.1 and 11 mg/L likely reflecting the concentrations of organic matter in the water samples. Clearly this will vary depending on the contamination levels of the run-off area for collection (particularly for dams) as well as on water levels. Both these factors will vary seasonally.

2. Determinations of chlorine demand of surface water samples

The source of chlorine used during this project was a commercially available chlorine solution (Milton Anti-bacterial solution, Proctor & Gamble Australia Pty Ltd). For this project work, readings of free (available) chlorine were used.

Each water sample tested had concentrated liquid chlorine solution added to yield initial levels of 5 ppm. Additional chlorine was then added over the 2 hour period as needed in order to maintain the 5ppm level of free chlorine. Free chlorine levels were assayed at 30 minute intervals for a total of 2 hours after mixing chlorine with the water samples in order to establish the native (natural) chlorine demand of the water sample from a production site. This allowed deduction of the level of chlorine input which is required to ensure that 5ppm free chlorine can remain as a residual in the water sample at the conclusion of the 2 hour treatment period. Distilled water was used as a control sample, to indicate the chlorine demand of "clean" water.

For the sample from Property E, it was not possible to zero the chlorine meter using the field water sample as it was too cloudy for the meter to accept as a "zero" sample so the chlorine meter was zeroed using distilled water. The unreacted sample was then read and this reading subtracted from the reading obtained after the sample was reacted and the colour developed. It is noted that this procedure may have caused some minor inconsistency in the readings obtained.

Table 2: Native chlorine demand of water samples

Property	**Distilled water**	**A**	**B**	**C**	**D**	**E**
Chlorine demand (ppm)	5	50	10	20	16	20

Results

As shown in Table 2, the native chlorine demand of the individual water samples varied from twice to 10 times the demand level demonstrated using distilled water. The sample with the lowest native chlorine demand (Property 3) had the lowest levels of suspended solids, dissolved solids and total nitrogen. Also of interest to note, water sample A which had the highest native chlorine demand, was obtained from the producer site whose water contained the highest concentration of total nitrogen. Accordingly this aspect was investigated in more detail during this project (see Section 6).

3. Chlorine demand to inactivate IBDV

For each test sample, the use of infectious bursal disease virus (IBDV) had been requested by the RIRDC Egg Program Advisory Committee as a representative test virus with a relatively high degree of resistance to chemical inactivation (sanitisation). While this virus had been propagated in media

containing minimum protein levels this medium had been found in experimentation by our laboratory to marginally increase the chlorine demand of water samples by some 7.5ppm over 2 hours when the diluted virus was added. This increased demand was allowed for within the calculations here and, as can be seen in Table 3, this amount of chlorine was minor compared with the chlorine demands which the surface water samples required.

To field water samples for which the native chlorine demand had been depleted, the diluted virus and an additional amount (determined previously) of chlorine was added so as to yield a final treatment level of 5ppm in this sample at the end of a 2 hour 20^{o}C treatment period. The chlorine level was then monitored during the subsequent 2 hour period, with additional chlorine being added whenever the chlorine level was found to have dropped below 5ppm.

For each field water sample, a distilled water control with virus and chlorine was run at the same time as the sample. A virus control, which consisted of distilled water and virus with no chlorine added was used at the same time, with the virus being titrated after one and 2 hours, along with the sample and the distilled water control. All samples and the distilled water control were tested using 7 replicates, while the virus control was titrated in triplicate.

Results: First the chlorine demand after addition of diluted IBDV was determined and the results for each test sample of surface water were shown in Table 3.

Table 3: Chlorine demand after addition of diluted IBDV virus

Property	**Distilled water**	**A**	**B**	**C**	**D**	**E**
Chlorine demand (ppm)	7.5	66.25	20	40	47.5	35

Sampling for virus infectivity testing was then undertaken with residual infectivity being determined at 1 and then 2 hours after exposure to chlorine treatment.

Results: After 1 hour, each of the samples of field water tested showed virus titres which were reduced, but viable virus was still present at low levels – refer Figure 2. The same infectivity level of IBDV which had only been diluted in distilled water however was completely inactivated, i.e. no infectivity remained (data not shown). For each of the field water samples after 2 hours exposure to 5 ppm chlorine, there was nil evidence of viable IBDV in any of the samples. The residual levels of IBDV infectivity persisting after 1 and then 2 hours were $10^{2.45}$ and $10^{2.35}$ respectively in the untreated IBD virus control i.e. no detectable inactivation of untreated IBDV had occurred during this experiment.

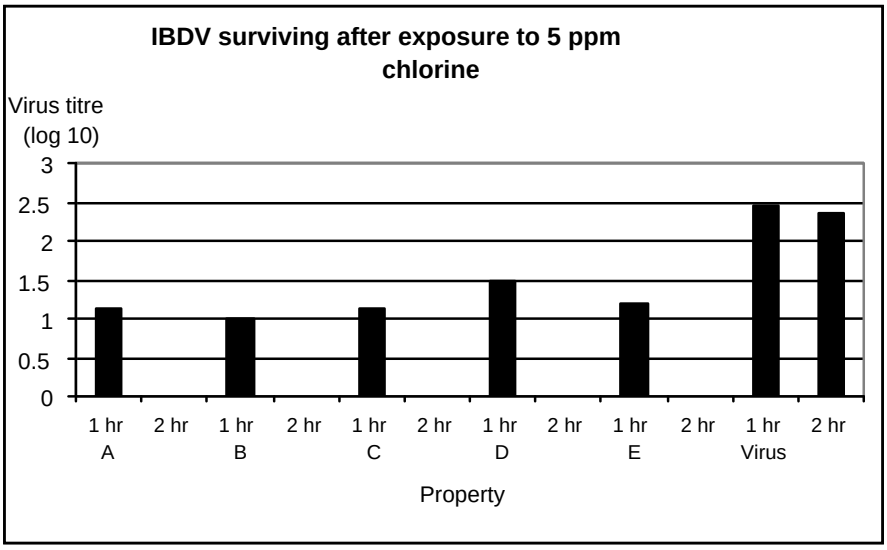

Figure 2. IBDV survival after treatment with 5ppm chlorine treatment for 1 and 2 hours in field surface water samples (Note: All the water samples treated for 2 hours were negative)

Conclusions to this point:

Even for the most complex of the surface water samples which we examined, having dissolved solids up to 260mg/L, total nitrogen levels up to 11 mg/L and suspended solids to 89 mg/L, treatment with levels of 5ppm chlorine maintained for 2 hours, was able to inactivate the challenge dose of IBDV.

Important Note: Extrapolation to Newcastle disease and avian influenza. Studies in these laboratories during 2001 - 2002 have previously shown that Newcastle disease virus (NDV) could be inactivated in untreated dam water by maintaining a level of 5 ppm chlorine for just one hour. As both NDV and avian influenza (AIV) viruses are enveloped RNA viruses which have similar physio-chemical properties, it is almost certain that any treatment that is effective for inactivating NDV will also be effective in inactivating AIV. Hence any treatment that is capable of inactivating IBDV within 2 hours, i.e. maintaining 5ppm chlorine as we have established as an experimental parameter here, will ensure that both NDV and AIV do not survive. It can also safely be assumed from data available on chlorine levels in water (Bagust 2000) that chlorine treatment levels of this order. i.e. 5 ppm, will then decline to be within potability levels for poultry, i.e. $\leq$ 3ppm, within a few hours at room temperature of completion of treatment.

4. Effect of suspended solids on chlorine levels

Suspended solids were found to vary from 2 – 4 mg/L (samples A, B, C) for the surface water samples which had undergone gravity settlement or sand filtration to 7 (D) – 30 (E) times these levels in untreated surface water as it was being used in the poultry sheds.

For purposes of experimentation, suspended solids mirroring the natural range found in the field water samples, i.e. 2 – 90 mg/L while a further 3 fold concentration was examined to reflect the highest concentration found in the field samples. To assess the effects of suspended solids present in water on chlorine levels, varying levels of Kaolin (hydrated aluminium silicate) were added to distilled water. Chlorine was added to a level of 5ppm and the chlorine level of the samples monitored over a 2 hour period. As can be seen here in Figure 3, the presence of these suspended particles had only a minor effect on the level of free chlorine present.

Technical Note: While assessing the effect of suspended solids on chlorine levels, it was discovered that at 50mg/L, it was no longer possible to effectively zero the chlorine meter when using such a sample. In order to overcome this technical problem the chlorine meter was zeroed using distilled water between each of the high reading samples. Each sample was then read and this reading was then subtracted from the reading obtained after the sample was reacted with the chlorine reagent and the colour had developed.

Results: As shown in Figure 3, the amount of chlorine required to maintain a level of 5 ppm chlorine was only marginally increased from a sample with no suspended solids, to the sample which contained more than three times the level of suspended solids found in any of the surface water samples tested. It is concluded therefore that while filtration can improve the aesthetic appeal of water and can contribute to a reduction in the number of microorganisms present in a water sample (Gadgil 1998), it will only marginally reduce the chlorine demand of water.

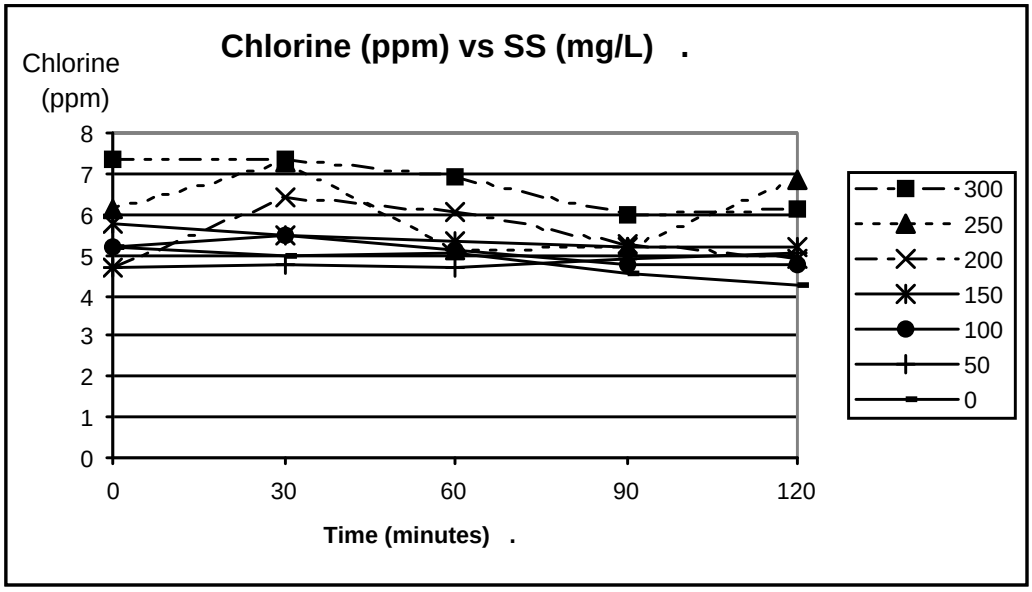

Figure 3: Effect of suspended solids on chlorine levels in water.

5. Dissolved solids

Systematic studies were not able to be conducted in the present work as there is insufficient definition of what dissolved solids comprise and in what ratios.
NB: Dissolved solids = total solids – suspended solids.

6. The effect of nitrogen on chlorine levels

It was found, that total nitrogen levels of the surface water samples varied between 1.1 and 11 mg/L (see Table 1). To enable a direct examination of the potential of nitrogen to interfere with the inactivation effects of chlorine, distilled water with levels of nitrogen which ranged from 2 to 12 ppm (mg/L) in 2mg/L increments were prepared, as well as a distilled water control. Chlorine was added to a level of 5ppm for each sample, the chlorine level monitored immediately after addition and then at 30 minute intervals for a total period of 2 hours. There was an immediate reduction in the level of free chlorine at 2ppm and slightly greater reductions at higher nitrogen levels (see Figure 4). Overall, the reduction in chlorine levels did not vary greatly between the lower and higher concentrations of nitrogen over 2 hours as can be seen from Figure 5.

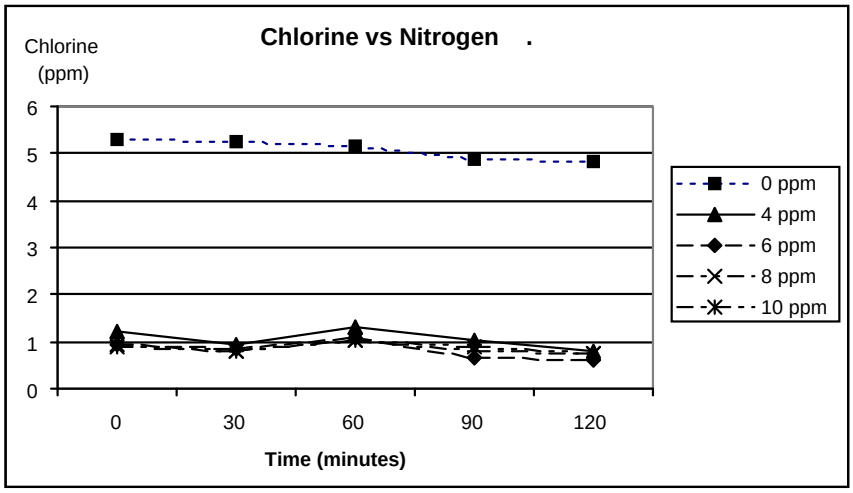

Figure 4: The effect of nitrogen on chlorine levels

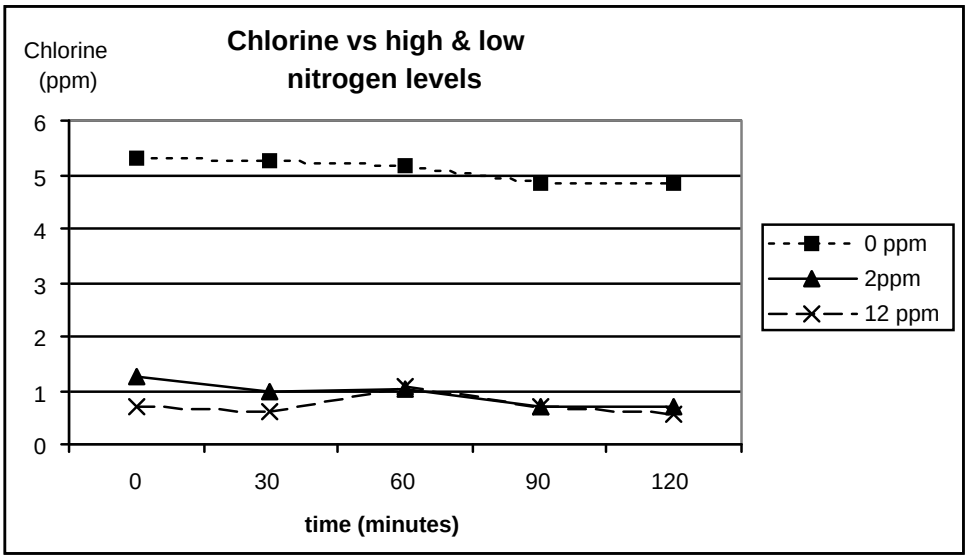

Figure 5: Chlorine levels with high (12ppm) and low (2ppm) levels of nitrogen.

7. Chlorine inactivation of IBDV at extreme temperatures

Temperatures of water supplies can vary from lows of below zero to highs of more than 40°C. To assess the effect of temperature on the ability of chlorine to inactivate IBD virus, samples of distilled water were held at 4°C, 20°C and 37°C. These temperatures were selected as being likely to be close to the extremes found in water in storage tanks. Once the water samples had equilibrated at each temperature, an aliquot of IBDV was added. Chlorine was added to maintain 5ppm for 2 hours. At the end of the 2 hour treatment period, the residual chlorine was inactivated and the water samples assayed for virus infectivity. At all three temperatures, no residual virus infectivity was detected. The amount of chlorine required to maintain 5ppm for 2 hours is shown in Table 4.

Table 4: Chlorine demand after addition of diluted IBDV virus at extreme temperatures

Property	**4°C**	**20°C**	**37°C**
Chlorine demand (ppm)	17.5	7.5	22.5

8. Citrox™

Citrox™ is a non-rinse biocide derived from orange extract. It is recommended for use at 0.8 to 1.6mL per litre of water. There was no recommendation from the manufacturer on the pH level at which Citrox™ should be used, and therefore it can reasonably be assumed that the efficiency of Citrox™ operation is pH independent. Citrox™ was tested for toxicity in distilled water and it was found to be toxic to the cells in our assay system, unless the pH was adjusted to 7 when the cells were able to survive and were used to assay residual IBDV infectivity as previously described for experimentation with chlorine.

Results: At the lowest dilution tested (0.8mL/L), Citrox™ had no detectable effect on the titre of IBDV. At 1.6mL/L, Citrox™ reduced the virus titre only 2-fold from $10^{2.8}$ to $10^{2.5}$, and this level of inactivation of IBDV is not experimentally significant. At twice the manufacturer's recommended dilution, i.e. 3.2mL/L, the titre of the IBDV challenge was reduced from $10^{2.8}$ to $10^{0.34}$, i.e. by some 99%. Residual IBDV infectivity however continued to be detectable even after a full 2 hour treatment period. Higher concentrations of Citrox™ were not able to be tested as they were found to be toxic to cells after the pH was neutralised.

Summary:

Citrox™ appears to have been designed for use on clean surfaces, such as coolroom walls and in dip tanks. The results from the current studies would indicate that Citrox™ showed promise as a virucidal agent in clean water, and when used at twice the strength recommended for use in recirculating spray systems or coolroom wall fogging devices. While likely to be suitable as a surface disinfectant, at this stage it does not appear to be so promising for sanitisation of surface water because when used at twice the recommended strength Citrox™ was still not able to completely inactivate a challenge dose of IBDV infectivity (clean water treatment period of 2 hours). While in the same treatment period, maintaining 5ppm of free chlorine could achieve complete inactivation of the same IBDV challenge in surface water.

Conclusions

1. The quality of surface waters in Australia will vary greatly. The present studies examined water samples from rural Victoria only, but did take in samples across a 25,000 km^2 area. However the results of the present studies were encouraging in that, providing the pH of water samples is 6-8 (chlorine effectiveness is lost at more alkaline pH's) and that the levels of free chlorine can be maintained at 5 ppm for a 2 hour treatment period (by dosimeter type continuing injection and monitoring), then additional hazards to effective disinfection of viruses, e.g. dissolved protein nitrogen can be overcome. Hence effective sanitation of surface water with simple chlorine treatment can be achieved.
2. To reduce variability in some sources of surface waters, it is highly advisable to undertake simple pretreatment before chlorination on site, such as allowing gravity settlement while holding and/or sand filters. These will not however control dissolved solids or nitrogen levels in surface water.
3. For achieving effective treatment of surface water for removal of viruses using chlorine, the major variable factor is likely to be nitrogen, rather than suspended solids or pH (other than when pH > 8, see 1st conclusion). Water with levels in the order of 1 ppm nitrogen or above are going to exert a strong "quenching" effect on chlorine treatments. Consequently, chlorine must be systematically added and monitored by dosimeter until 5 ppm are being achieved and maintained for 2 hours as the treatment period for any batch.

   The alternative of just calculating the amount of chlorine mathematically needed, then adding it to a volume of surface water and not monitoring chlorine levels subsequently, cannot comply with achieving effective biosecurity for the treatment of surface water on poultry production sites in Australia.

4. The ensuring of free chlorine levels of 5 ppm for 2 hours contact time at varying ambient temperatures can provide Australian poultry sites with an effective antiviral disinfectant cover against threat viruses such as NDV, vvIBDV and likely also for high pathogenicity avian influenza ("bird flu") that has emerged during 2003/4.

Discussion of Results

As has been shown here even with sampling from just several production sites in one representative state, the standards of quality of the surface waters which are currently being used on Australian poultry industry production sites will vary considerably. Some sites use surface water directly with no pretreatment, while other sites use systems with varying combinations of flocculation, filtration, softening and disinfection prior to use.

The surface water samples tested during this project all had pH values within the range between pH 6 and pH 8 where chlorination is able to be effective. As a first and simple precaution then, it should be mandatory that poultry farming operations regularly test their surface waters to ensure that pH's are < 8 and preferably < 7.5. Regrettably, at pH > 8, chlorine is ineffective. But solutions should be simple for acidification of such waters, as this can be readily achieved for swimming pools, and pH test papers suitable for swimming pool maintenance will suffice.

Where some pretreatment of water had been undertaken such as gravity settlement or sand filtration, the levels of suspended solids was low (2 - 4 mg/L) while with no pretreatment, levels of up to 89 mg/L were found to occur. Total dissolved solids however varied from 64 to 260 mg/L with or without simple pretreatments as did levels of nitrogen. Simple prefiltration will not necessarily redress this situation but adequate chlorination levels at pH's 6-8 will (see next paragraph). Variations in nitrogen and dissolved solids will likely reflect the load of organic material which

naturally accumulate in surface waters, with higher levels to be found particularly on sites where animals graze in the areas used for run-off of rain water into dams or creeks and seasonal variation with rainfall influxes of soils.

It was therefore able to be determined in the present studies that, using the surface water sampled from across 25,000 km^2 area of rural Victoria, simple chlorine treatment at 5 ppm was able to eliminate IBDV contamination as a site quarantine risk when the free chlorine levels were maintained at 5 ppm, and for 2 hours.

It is critical however to monitor the level of chlorine during this 2 hour treatment period, because chlorine levels were found to readily fall below 5 ppm. While in some cases a gradual decline in the chlorine level was noted, in others where protein/nitrogen concentrations were greater than 1 mg/L, the decline was more precipitous. In both cases however, relatively inexpensive chlorine automatic injection and sensing systems (in the order of $5,000) should enable free chlorine levels to be maintained for treatment at 5 ppm. Further, any protein/nitrogen problem will be relatively simple to detect and economical to manage by (1) use of a simple colour test kit, eg. swimming pool type to check if nitrogen levels are likely to be sufficient to interfere with chlorine and (2) dosimetric addition and chlorine monitoring to ensure 5 ppm.

Citrox™ an alternative chemical treatment product to chlorine sanitation, which is readily available in Australia, was also assessed at the request of AECL for its ability to inactivate IBDV in clean water. After a 2 hour treatment period, using twice the manufacturers recommended strength, residual IBDV infectivity was still detectable in the water sample. While Citrox™ would likely reduce the overall numbers of microorganisms in water, IBDV infectivity was reduced but the challenge dose of this virus was not totally inactivated by this Citrox™ treatment. On this data obtained here using Citrox™ in just distilled water, its effectiveness in water treatment for sanitation must be assessed as questionable.

Implications

Were IBDV, especially the very virulent strains of IBDV which are endemic in Asia but exotic to Australia, transferred mechanically through migratory water fowl to contaminate the surface water being supplied to chickens in a commercial poultry operation in this country, the water treatment regimens currently in place would likely NOT be adequate to inactivate this virus. This is particularly the case where there is no treatment or sanitisation of surface water before being used by poultry. NDV or even high pathogenicity avian influenza, are each more readily inactivated by chlorine than IBDV and should be inactivated in a shorter exposure time to 5 ppm free chlorine.

To be certain of inactivating IBDV however, monitoring of the chlorine levels in order to ensure water exposure to 5 ppm free chlorine will be necessary for a 2 hour exposure time. This can be achieved by using an automated system, whereby chlorine levels are monitored and extra chlorine is added automatically if the levels drop. A working figure to establish this type of system is an initial cost in the order of AUD5,000 for a batch treatment system that can deliver 100,000L of treated water per day.

Recommendations

The following procedure should provide an effective shield against target viral pathogens (NDV, AIV & vvIBDV) as well as endemic EDS adenovirus resident in Australian ducks.

1. Water treatment using 2 batch tanks e.g. 100,000 litre capacity, one for supply to chickens while treatment is undertaken in the other.
2. Chlorine added at a variable rate to each batch by automatic (dosimeter) injection to maintain 5 ppm free chlorine and continual (electronic) monitoring of free chlorine levels
3. Hold for 2 hours with occasional agitation.
4. Monitoring checks should be undertaken by producers using simple swimming pool kits, on key water quality features which will detrimentally affect water sanitation, in order to ensure that the pH is 6-8 (critical!) and to monitor protein nitrogen concentrations.

Note, Chlorine oxidative inactivation effects on virus are quickly diminished at pH's more alkaline than 7.5, and cannot be effective at pH > 8

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