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Avian Influenza Aerosol Sampling Campaign in the British Columbia Fraser Valley, 9–19 April 2004

Sampling of rare biological events

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Defence R&D Canada – Suffield

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Technical Memorandum
DRDC Suffield TM 2005-032
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Abstract

An outbreak of infectious Avian Influenza (AI) occurred during March-April 2004 in the Abbotsford area of British Columbia. There was a need to determine if infectious viral particles were being spread via an aerosol route. It was anticipated that such particles, should they be found, would occur as rare events. By coincidence, DRDC Suffield has been developing aerosol samplers that can be used to detect such rare events. Two sampling systems, consisting of a slit sampler array and a large column air sampler (XMX virtual impactor) were deployed. The sampling scheme was designed to capture four specific events requested by the CFIA. These were (1) up and down wind sampling of an acutely infected barn prior to euthanasia, (2) up and down wind sampling of a barn being euthanized to capture the displacement effect of carbon dioxide pushing air and possibly virus out the barn, (3) the effect of clearing the barn of carbon dioxide by starting up the ventilation fans after euthanasia, (4) the possible re-aerosolization of virus from a barn being actively composted or depopulated by sampling the up and down wind positions. In addition to these specific events, XMX samples were taken in 10 random positions in and around Abbotsford. The first two were in acutely infected barns with high morbidity/mortality while the remaining eight were randomly taken in the outbreak area. Liquid samples from an XMX device were analysed using PCR as the initial method of identification followed by virus culture to demonstrate live virus. Quantification was to be carried out using plaque assay. Results showed that all slit samples were PCR negative while three of the XMX samples were unmistakable positives. One sample collected from 250 m southeast of the CFIA emergency operation centre (EOC) in Abbotsford was also positive. Estimated viral load yielded a value of 292 viral doses/m^3 of barn air. Results from this exercise will be used for subsequent modelling experiments.

Résumé

Une épidémie de grippe aviaire infectieuse a sévi de mars à avril 2004, dans la région d'Abbotsford, en Colombie-Britannique. Il fallait déterminer si des particules virales infectieuses se disséminaient en aérosol. On avait anticipé que de telles particules, si on en confirmait l'existence, seraient rares. RDDC Suffield ayant mis au point, par coïncidence, des échantillons d'aérosols permettant de détecter de tels rares épisodes, on a déployé deux systèmes d'échantillonnage qui consistaient en un réseau d'échantillonneurs à fentes et d'un échantillonneur pour grande colonne d'air (impacteur virtuel XMX). Le schéma d'échantillonnage a été conçu pour capturer quatre évènements spécifiques requis par l'ACIA. Il s'agissait 1) de l'échantillonnage avec vent en amont et vent arrière d'un poulailler infecté de manière aiguë préalablement à l'euthanasie, 2) de l'échantillonnage avec vent en amont et vent arrière d'un élevage étant euthanasié pour capturer l'effet de déplacement du gaz carbonique poussant l'air, et éventuellement le virus, hors de la grange, 3) de l'effet de purifier la grange du gaz carbonique en démarrant les ventilateurs après l'euthanasie, 4) de la re-pulvérisation en aérosol possible d'un virus provenant d'un poulailler, étant à compost ou dépeuplé, en échantillonnant en positions en amont et en vent arrière. En plus de ces évènements spécifiques, des échantillons XMX ont été recueillis à 10 endroits choisis par hasard dans Abbotsford et aux alentours. Les deux premiers se situaient dans des poulaillers infectés de manière aiguë ayant une haute morbidité / mortalité alors que les huit autres ont été choisis au hasard dans la région de l'épidémie. Des échantillons liquides provenant d'appareils XMX ont été analysés par la méthode PCR comme méthode initiale d'identification, suivie d'une culture de virus visant à prouver l'existence du virus vivant. La quantification devait être effectuée en utilisant la méthode des plages de lyse. Les résultats ont indiqué que tous les échantillonneurs à fentes étaient PCR négatifs alors que trois des échantillons XMX étaient incontestablement positifs. Un échantillon recueilli à 250 m au sud-est du centre des opérations d'urgence de l'ACIA, à Abbotsford, était lui aussi positif. La charge virale estimée correspondait à une valeur de 292 doses virales/m^3 de l'air de la grange. Les résultats de cet exercice seront utilisés pour des essais ultérieurs de modélisation.

Executive summary

This report chronicles an aerosol field sampling investigation requested by the Canadian Food Inspection Agency in April 2004. The purpose of the trial was to rule-in or out the presence of airborne dispersion of the Avian Influenza (AI) virus H7N3 infecting many domestic poultry operations around the Abbotsford British Columbia area. The fulminant development of the epizootic in the lower Frazer Valley had raised the question: was this virus spreading by means other than those cited in the current literature? DRDC Suffield was contacted on April 5, 2004 by the CFIA for help in establishing a means of environmental sampling in the hot zone areas where the virus was considered to be most prevalent and active. The aim was to examine the premise that the virus was being transported on small airborne dust particles emitted from barn floor litter. A team of specialists was fielded and sent to the Abottsford area where air sampling for the virus was carried out using modified slit samplers and a XMX high volume air sampling unit. Ten days of sampling were required to capture four specific events requested by the CFIA. These were: (1) up and down wind sampling of an acutely infected barn prior to euthanasia, (2) up and down wind sampling of a barn being euthanized to capture the displacement effect of carbon dioxide pushing air and possibly virus out the barn, (3) the effect of clearing the barn of carbon dioxide by starting up the ventilation fans after euthanasia (4) the possible re-aerosolization of virus from a barn being actively composted or depopulated by sampling the up and down wind positions. In addition to these specific events, 16 XMX high volume air samples were taken in 10 random positions in and around Abbotsford. The first two were in an acutely affected barn with high morbidity/mortality while the remaining eight was randomly taken in the outbreak area. Two hundred and forty (240) samples were captured using the slit units. Samples were to be analysed using PCR as the initial method of identification followed by virus culture to demonstrate live virus. Once identified, quantification was carried out using plaque assay and a quantified PCR. All slit samples were PCR negative while three of the XMX samples were clear positives. Sample 10 was unexpected in that it was captured 250 m southeast of the CFIA emergency operation centre (EOC) in Abbotsford. Sampling was completed on 17th April 2004.

What is evident from this study is the virus was circulating in the barn sampled and in the surrounding environment. The estimated viral load yielded a value of 292 viral doses/m^3 of barn air. The quantitative, spatial and temporal nature of the dispersion however remains undefined. It would be speculative to say more about the possibility of airborne dispersion without conducting a better-designed study to define viral presence in and around contiguously infected barns. Geomorphic mapping detailing viral plumes and their dynamics based on a meteorological continuum would be especially useful.

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Sommaire

Ce rapport fait la chronique d'une investigation d'échantillonnage d'aérosols sur le terrain qui avait été requise par l'ACIA, en avril 2004. Le but de cet essai était de prouver ou non la présence d'une dispersion en aérosol de la grippe aviaire H7N3 qui infectait beaucoup d'élevages domestiques de volailles aux alentours d'Abbotsford, en Colombie-Britannique. Le développement fulminant de l'épizootie dans les régions basses de la vallée Frazer avait soulevé la question : le virus se répandait-il par des moyens autres que ceux cités dans la documentation ? RDDC Suffield a été contacté le 5 avril, 2004 par l'ACIA pour que le centre aide à établir des moyens d'échantillonnage environnementaux dans les régions des zones les plus critiques où le virus était considéré comme le plus prévalent et le plus actif. Le but était d'examiner l'hypothèse que le virus était transporté par des petites particules de poussière en aérosol qui étaient émises par les déchets du sol d'un poulailler. Une équipe de spécialistes a été envoyée sur le terrain dans la région d'Abottsford où l'échantillonnage d'air contenant le virus a été effectué en utilisant des échantillonneurs à fentes modifiés et une unité d'échantillonnage XMX traitant un grand volume d'air. L'échantillonnage a duré 10 jours pour capturer quatre évènements spécifiques requis par l'ACIA. Il s'agissait 1) de l'échantillonnage avec vent en amont et vent arrière d'un poulailler infecté de manière aiguë préalablement à l'euthanasie, 2) de l'échantillonnage avec vent en amont et vent arrière d'un élevage étant euthanasiée pour capturer l'effet du déplacement du gaz carbonique poussant l'air, et éventuellement le virus, hors du poulailler, 3) de l'effet de purifier le poulailler du gaz carbonique en démarrant les ventilateurs après l'euthanasie, 4) de la re pulvérisation en aérosol possible d'un virus provenant d'un poulailler étant activement à compost ou dépeuplé en échantillonnant les vents en position en amont et arrière. En plus de ces évènements spécifiques, 16 échantillons XMX, traitant un grand volume d'air, ont été recueillis à 10 endroits choisis par hasard dans Abbotsford et aux alentours. Les deux premiers se situaient dans des poulaillers infectés de manière aiguë ayant une haute morbidité / mortalité alors que les huit autres ont été choisis au hasard dans la région de l'épidémie. Deux cent quarante (240) échantillons ont été capturés au moyen de ces unités à fentes. Des échantillons devaient être analysés au moyen de la PCR comme méthode initiale d'identification, suivie d'une culture de virus visant à prouver l'existence du virus vivant. Une fois les échantillons identifiés, la quantification a été effectuée par la méthode des plages de lyse et une PCR quantifiée. Tous les échantillonneurs à fentes étaient PCR négatifs alors que trois des échantillons XMX étaient incontestablement positifs. Nous n'avions pas prévu que l'échantillon 10 recueilli à 250 m au sud-est du centre des opérations d'urgence de l'ACIA, à Abbotsford, serait lui aussi positif. L'échantillonnage a été complété le 17 avril 2004.

Cette étude prouve que le virus circulait dans les poulaillers échantillonnés et dans le milieu environnant. La charge virale estimée correspondait à une valeur de 292 doses virales/ m^3 de l'air de la grange. La nature quantitative, spatiale et temporelle de la dispersion demeure cependant indéfinie. Il est impossible d'en dire plus sans spéculer sur la possibilité d'une dispersion aérosol ; une étude mieux conçue visant à définir la présence virale à l'intérieur et aux alentours des poulaillers infectés par contiguïté devrait être conduite. La représentation géomorphologique détaillant les panaches viraux et leur dynamique en se basant sur un continuum météorologique serait particulièrement utile.

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Preamble

In the spring of 2004, the avian flu problem of spreading infections in Abbotsford, BC was becoming a national issue. It was apparent the infection parameters were complex and no real solution to reducing the incidences of infection was in sight. The CFIA had proceeded to carry out destruction of infected and potentially infected birds. Initial analysis of the infection dynamics produced suggestions that the spread of the disease was much quicker than traditional models could predict. Speculation was that the infecting particles might be airborne. If this were true, assistance from aerosol experts at DRDC Suffield was needed to help CFIA collect evidence to explain the epidemiological observations.

On April 5, 2004 a message was sent to Dr. L. Schofield by Maj. F. Martins, CFB Suffield to contact Dr. Christine Power CFIA. Dr. Schofield made the contact by e-mail and was quickly contacted by Dr. Power. She explained that the epidemiological team tracking the epizootic had some concern that the virus might be spreading on the wind by small particles of exhausted barn litter consisting of sawdust. The virus had never been seen to spread by wind but this possibility needed to be ruled in or out to better understand the current unexplained dynamic and very rapid spread in spite of the established control and surveillance zones. She asked if DRDC had the capability to sample particles of 10 microns or less. Dr. Schofield suggested to her that DRDC Suffield had a capability and that Drs. Ho and Kournikakis were the individuals with the experience and expertise as they had attended and sampled during the SARS epidemic in Toronto in the summer of 2003. Dr. Schofield said he would approach Dr. J. Lavigne, the Chemical Biological Defence Section (CBDS) Head, with Power's request for help and reply as soon as possible. Dr. Lavigne approved the request rapidly while a team was assembled and mobilized by April 8 with all equipment on the road by 1600h to arrive at Abbotsford by noon of April 9. The remainder of the team was on site at the Canada Food Inspection Agency (CFIA) Emergency Operations Centre in Abbotsford by 1500h Good Friday April 9 2004. All expenses for this study were covered by the CFIA during the travel and sampling periods. This report details the proceedings.

Introduction

Avian influenza (AI) is a highly contagious viral disease of many species of birds. The clinical signs range from sub clinical to severe illness with high levels of morbidity/mortality in domestic poultry to unapparent infection in wild and domestic waterfowl. Passerines such as pigeons appear not to be infected, although the possibility of them acting as mechanical vectors exists. Wild birds such as waterfowl appear to be a primary nidus for the disease but as referenced, seldom exhibit signs. AI has been recovered from 20 of the 42 indigenous species of wild ducks, geese and swans in North America. Various strains have been identified around the globe including 10 countries in Southeast Asia [1].

There are 15 subtypes designated H1to H15. Within each subtype are many strains. The strains that can cause high levels of morbidity and mortality within domestic poultry have been termed highly pathogenic avian influenza (HPAI) [2]. Those strains that cause transient and ineffectual respiratory disease are termed the low pathogenic forms of avian influenza.

(LPAI). Since December 2003, HPAI (H5N1) has been responsible for the destruction of approximately 100M birds in southeast Asia. Thirty-three humans who contacted sick or dead birds and associated faecal matter were infected with the virus [3].

The strain associated with the lower Fraser Valley epizootic is H7N3. As of April 27, 2004 the virus has infected a total of 37 flocks. This and H5 are the only subtypes to have ever been highly pathogenic [4]. H3-4-6 are the predominant strains in North American ducks with H5 and H7 being rare. Continued recovery of H3-4-6 suggests these subtypes are host adapted. Although genetically stable in their natural hosts, once an AI virus escapes its niche and crosses a species barrier, there is a real possibility of accelerated mutation leading to gene recombination and the fear of a new and highly pathogenic virus capable of moving into a new host such as people [5]. Therein lies the underlying fear in the current epizootic.

Transmission is generally accomplished through direct contact with body secretions. Secondary transmission occurs by contact with faecal matter, contaminated feed, water or other fomites on which the virus has survived such as vehicle tires, footwear and clothing. The virus survives well outside the host in flesh, water and faecal matter [6]. The cited incubation period is 3-5 days but in this particular outbreak new clusters appeared to be about every 10 days.

Viral aerosols have been measured from urban sources such as sewage treatment plants [7, 8]. While Webster et al. [9] were attempting to study the possible air transmission of H5N1 influenza viruses between different bird species, their experimental set up did not truly represent aerosol dissemination as illustrated by this quote: "Aerosol transmission of virus was studied by placing quail or chickens in cages that were beside the cages of infected geese at a distance of approximately 0.3 m." At this short distance, contact with infected feathers or droplet transmission could not be ruled out. As such there has been no previous published record of avian influenza aerosol measurement. The methodologies employed in this study can be considered new and untested.

Study Purpose

The aim of the study was to advance the theory that the virus was being transported on small airborne dust particles emitted from the floor litter of infected poultry barns. This hypothesis, if demonstrated, would not only change the way in which these epizootics would be managed but would also carry significant veterinary and human public health implications. The reality of avian influenza A are the possibilities of genetic shift due to recombination and accelerated mutation if the virus were to jump species. The sum of these fears would be the transmission to humans in a highly pathogenic form.

Methods and materials

Serial Slit Sampler

Serial slit sampling, a DRDC Suffield innovation [10], is a simple concept that describes a set of electronically networked instruments that can be activated in sequence or any combination thereof. The system permits continuous sampling from short to long time spans. For example, when sampling rare events, the system can be programmed to run for 7.5 hours (as used in this study for avian influenza virus). Conceptually, capturing viral particles was achieved by drawing the aerosol stream at a moderate flow rate, resulting in particles impacting gently on to a liquid surface. Particles with sufficient inertia would be trapped in the liquid, permitting the capture of the rare event that may occur infrequently.

In the first iteration, stock New Brunswick slit samplers [11] were modified by a replacement digital motor used with the appropriate driver board interface, serial switching and timing circuitry. This work was done by a contractor (Dycor Technologies Ltd. Edmonton, Alberta, Canada) and the instrument was designated the Dycor modified New Brunswick (figure 1). As the original slit sampler platform went out of production. There was a requirement for a more efficient replacement sampler with radical design changes. Key components such as the original slit cup (slit dimension 0.15 x 48 mm) and the plastic chamber bowl were obtained from New Brunswick (New Brunswick Scientific Co., Inc. Edison, New Jersey 08818-4005) as spare parts. These items constructed a sampler that performed like the original. While working on the design of the original sampler, it was decided that by putting two slit and bowl units on one base, enhanced operational efficiencies could be realized. The integration and electronics for this dual slit platform was performed by another contractor (HF Research, Medicine Hat, Alberta) and the instrument was designated the HF modified slit system (figure 1). The major specification deviation from the Dycor modified slit sampler was that the flow rate was changed to 30 lpm to accomodate the solenoid valve flow restriction. This instrument had been used to sample rare events like anthrax aerosols in the Brentwood postal station [12] and SARS virus particles in a Toronto hospital [13]. Aerosol particles were captured on standard 15 cm plates with 20 ml of viral capture medium on an agar base.

Viral Capture Medium

Gillespie et al. [14] have described the use of a viral preparation medium that permitted isolation of viable viruses. Brenner et al. [15] used it for capturing viral particles from the air. We have modified this medium for capturing SARS and avian influenza virus in air by using the following modifications. In phosphate buffered saline (Sigma Aldrich, St. Louis, MO) there was 10% antibiotic – antimycotic (Life Technologies, Grand Island N.Y) and 0.75% fetal bovine serum Gibco Invitrogen Corporation, Burlington ON).

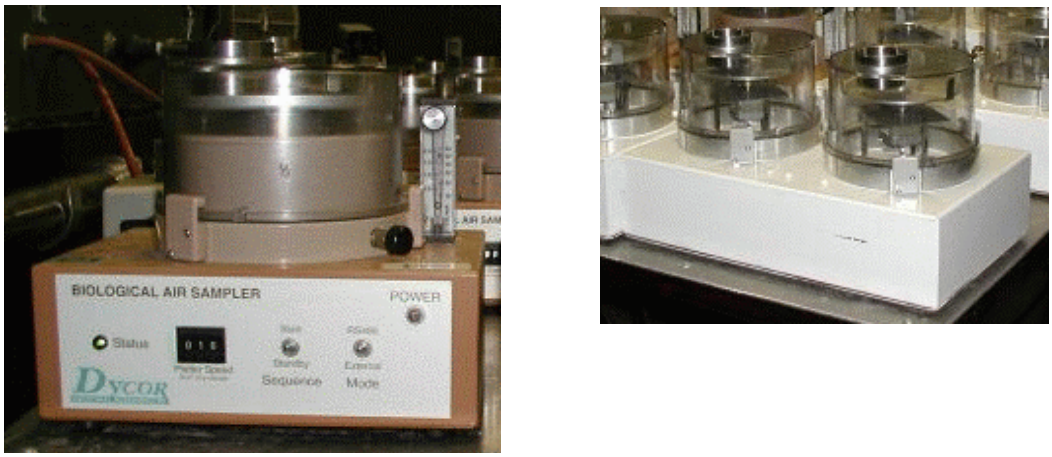

Figure 1. *Slit sampler types*

Dycor modified New Brunswick (left) and HF modified (right)

Large Volume Air Sampler

A virtual impactor [16] was used for concentrating 800 litres of aerosol into 1 lpm. The instrument is a miniaturized version (Model XMX, Dycor Technologies Ltd., Edmonton, Alberta, Canada) of the XM2 sampler first described by Brenner et al. [15] who used it to capture bacterial and viral particles from the air. We altered the XM2 to make it smaller, portable and requiring less power. This instrument was configured to collect particles in 5 to 10 ml of viral capture fluid over 10 to 15 minutes. In some samples, the fetal bovine serum was eliminated to decrease foaming.

PCR and Cell Culture

Samples were packaged and sent to the National Microbiology Laboratory in Winnipeg where these were first analysed by PCR. Samples that tested positive were then inoculated into cell culture to determine if viable virus was present. Positive samples were re-tested using a semi-quantitative PCR technique to estimate the relative amounts of virus present.

Virus Culture

Virus was cultured on monolayers of MDCK cells grown in T75 flasks. Cultures were pre-washed 2 times with 5ml influenza virus growth medium (MEM, hepes, pen-strep-l-glut., sodium pyruvate, nonessential amino acids and TPCK trypsin-2 µg /ml). Medium was then aspirated, and the cultures inoculated with 0.1 ml of specimen mixed with 2.0 ml of virus growth medium. The inoculum was adsorbed for one hour at room temperature during which time the flask was rotated three times. A further 20 ml of virus growth medium was then added, the flask was incubated at 37° C and the contents observed daily for cytopathic effect (CPE).

RNA Extraction, RT-PCR, Real-Time RT-PCR, DNA Purification and Sequencing

Environmental sample RNA was extracted on the QIAGEN BioRobot MDx Workstation (Cat #900600) using the QIAamp Virus BioRobot MDx Kit (Cat# 965652). RNA was amplified in a one-step RT-PCR reaction (QIAGEN) following the manufacturer's recommendations. Briefly, 5 µl of RNA was added to the RT-PCR mixture containing 2 µl QIAGEN OneStep RT-PCR enzyme mix, 10 µl of 5X QIAGEN OneStep RT-PCR buffer, 400 mM dNTP, 0.6 µM of each primer and 10 µl of Q-solution for a final volume of 50 µl. The thermocycler conditions used were: 50° C for 30 min for reverse transcription, 95° C for 15 min for the activation of the HotStart DNA polymerase; then 50 cycles of 94° C for 30 sec, 50° C for 1 min, 72° C for 1 min, followed by an extension of 7 min at 72° C. The PCR products were purified using QIAquick PCR purification kit (QIAGEN) and sequenced on an ABI 377 Sequencer using a fluorescent dye-terminator kit (Applied Biosystems). Samples that tested positive for H7 by conventional PCR were tested by Real-Time RT-PCR using the iCycler IQ system (Bio-Rad Cat #170-8720 and #170-8740) and the QuantiTect Probe RT-PCR Kit (QIAGEN Cat #204443). 5 µl of RNA was added to the RT-PCR mixture containing 0.25 µl QuantiTect RT enzyme mix, 12.5 µl of 2X QuantiTect Probe RT-PCR master mix, 0.4 µM of each primer and 0.2 µM of probe in a final volume of 25 µl. The thermocycler conditions used were: 48° C for 30 min followed by 95° C for 10 min; then 40 cycles of 94° C for 1 sec and 58° C for 20 sec with data collection and real-time analysis enabled for the 58° C step.

Results and Discussion

Samples were collected at three different chicken farms (A, B, C) as well as some random locations in Abbotsford. A total of 240 samples were collected from the slit samplers and an additional 16 from the XMX high volume sampler. None of the 240 samples from the slit sampler (all three farms) were positive by PCR and were not tested further. Of the 16 XMX samples, numbers 1-6 and 10 were PCR positive. Samples 1 and 2 which were collected inside the barn at farm B were PCR positive (figure 2). Samples 3-6 which were collected away from the barn near the command post were also PCR positive. Samples 1 and 2 both yielded live virus by cell culture while all others were cell culture negative. Using a semi-quantitative PCR technique, which is less sensitive than the original PCR, samples 1 and 2 demonstrated a 3500 Tissue Culture Infective Dose fifty ($TCID_{50}$). A single Tissue Culture Infective Dose 50% means a concentration of 1 $TCID_{50}$ will infect 50% of the cell cultures inoculated with the material. Samples 3 and 4 showed 150 $TCID_{50}$, 2 orders of magnitude lower than the higher concentration seen in the barn. All other samples that were positive by the original PCR were negative by the semi-quantitative PCR. These figures were used to estimate viral load (appendix A) which yielded a value of 292 viral doses / m^3 barn air.

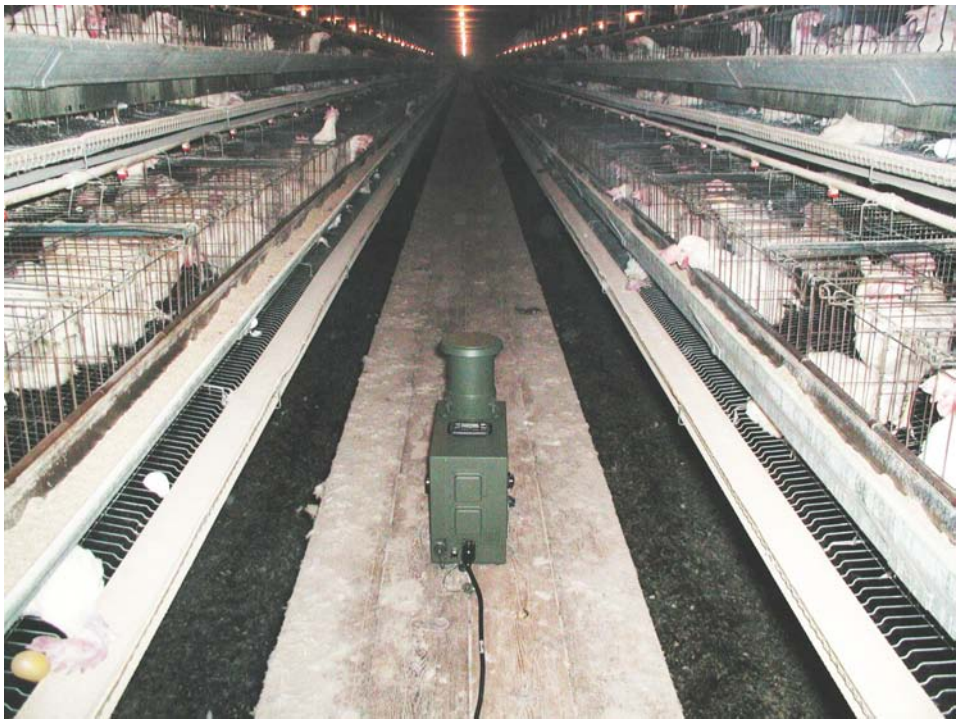

Figure 2. *High volume air sampling with the XMX instrument*

Aerosol is concentrated from 800 lpm into a liquid at 1 lpm

The negative results from the slit samplers may have resulted from a number of factors. As shown in table 1, there were no live viruses sampled from farm A. At this farm, the animals were not yet dying from infection and CFIA swab samples from these birds were positive only for PCR tests. Thus it is conceivable that no viable airborne infectious particles were produced at the time the slit samples were taken.

At farms B and C there were dead infected animals. The slit samplers should have captured particles from the exhaust fans. However, the slit samplers were all placed approximately 75-100m on opposite sides of the barn along the line of the forecast winds for the area. As the winds were variable it was not practical to shift the sampling array once set up. Because of the shifting winds it is possible that viral aerosols might not have reached the sampling sites. In addition, the sampling rate of the slit samplers was 30 lpm (as compared to the high volume XMX sampler which collected 800 lpm) which may have been insufficient to capture adequate material above a detectable limit. This speculation was partially confirmed by tracer smoke (electric smoke generator and smoke grenades provided to CFIA by the RCMP) that was used to determine the volume and direction of airflow from the barn exhaust fans. (figure 3). In both locations the exhaust fans were directed into the wind, resulting in smoke being forced back towards the barns and up over the roof. This meant that the exhausted air particles were well above ground level and would not be low enough to be sampled by the slit samplers positioned nearby (figure 4).

Table 1. *Summary of viral sampling regime*

	DATE (APRIL 2004)							
	Sat 10	***Sun 11***	***Mon 12***	***Tues 13***	***Wed 14***	***Thurs 15***	***Fri 16***	***Sat 17***
Location	Farm A	Farm A	Farm A	Farm B	Farm B	Farm B	Farm C	Farm C
Viral state*	PCR +ve			H7:N3 live virus			H7:N3 live virus	
	No live viruses			Dead chickens			Dead chickens	
Treat-ment	CO_2 applied		Ventilation	CO_2 applied	Ventilation		CO_2 applied	Ventilation
XMX samples				Samples 1 & 2		Sample 10		
				+ve in barn		+ve downwind		

* Viral state was determined by immunological methods on swabs taken from chickens by CFIA agents

Samples 1 and 2 from the XMX sampler clearly demonstrated high levels of live virus within the infected chicken barn (farm B) where infected birds were still alive but dying at a high rate. This suggested that the virus was hardy enough to withstand the physical impact imposed by this high volume sampling system. Samples 3-6, which also were positive on the original PCR were not believed to be true positives, but rather resulted from the "flushing" of residual material remaining in the sampler from samples one and two. This was supported by the rapid decrease in the concentration as determined by the semi-quantitative PCR, with samples 3 and 4 being two logs lower and with samples 5 and 6 being negative with the less sensitive PCR. The sampler was decontaminated after sample 7. The positive sample 10 was notable in that it was not collected at an infected premise but as part of a random collection of samples in different parts of Abbotsford. It was collected in an open field approximately 800 m away from farm B. We felt this was a true positive since samples 8-9 and 11-16 were all negative showing that there was no possibility of any cross contamination between samples. Although positive by the original PCR it was shown to be negative by culture and by the less sensitive semi-quantitative PCR. This indicates that the concentration was very low. Since the sample was collected during daylight hours it was unlikely to find live virus in the sample. Any viable virus would probably have been inactivated by sunlight.

Figure 3. *Demonstration of airflow characteristics using a smoke model*

As shown in this illustration, smoke particles were elevated above the roof level and were carried away beyond the reach of ground level samplers that might have been situated at some distance

Conclusions

Although slit sampling technology has been successfully used for the collection of SARS virus indoors, it was not effective in collecting Avian influenza virus outdoors, as shown in this situation. The concentration of any circulating virus 75-100m from the barns was probably too low to be collected by the relatively low flow rate (30 lpm) of the slit samplers. In addition, the concept of using a fixed sampling position based on the forecast prevailing winds was not optimal as the local micrometeorology (interaction of wind with buildings and trees around the sampling area) resulted in shifting winds and dispersal of air from the barns (figure 3) at least 5-10m above ground level and far above the samplers. One of the reasons the slit samplers were used was to minimize the physical stress on the virus during the sampling process. Only limited use was made of the XMX high volume sampler (800 lpm) because it was unclear whether live virus could survive the physical stress of a high volume sampler. The positive XMX results were not available until the end of the investigation. This was unfortunate as had we known this earlier, we would have made greater use of this sampler. We now know that live virus can be successfully collected using the XMX sampler and that it is possible to collect the virus at some distance from a barn.

Figure 4. *Slit sampler location adjacent to barn*

Due to logistical reasons, the distance of the site to the barn was about 30 m in this illustration. As a result, aerosol particles exhausted from the barn were carried high above this site, resulting in a failure to capture by the sampler

Based on our experience in Abbotsford we can make a number of recommendations regarding both the sampling strategy as well as logistical issues. Outdoor aerosol sampling of this nature would be better served with the more easily portable high volume samplers that can quickly be moved as weather conditions change. By using a smoke generator or smoke bombs, sampling positions can be quickly identified and set up to suit the changing weather conditions. This process can be configured in a downwind geomorphic sampling grid to detail viral plume dynamics based on the current meteorological continuum. Repeated variations would provide a more robust statistical expression of plume and dispersion dynamics. This approach would have had a far greater chance of success than the pre-positioning of samplers at a single point.

In terms of logistics it is clear that such deployments have always occurred on short notice requiring the interruption of R&D activities and the acquisition and packing of needed supplies. Pre-packing of equipment dedicated to urgent requests for aerosol sampling would allow for rapid deployment as it would only be necessary to arrange for the shipping of the equipment. The equipment and supplies to be included in such a rapid deployment will need

careful review to ensure that the deployed team is as self-sufficient as possible. This self-sufficiency must also include the ability to carry out preliminary on-site analysis of collected samples. The time delay, resulting from shipping samples off-site and awaiting results, hampered the mission and should be avoided if at all possible. The inclusion of a driver/mechanical specialist proved to be a very valuable addition to the team on this mission. Dealing with mechanical problems, assisting with sample shipment and re-stocking supplies which, otherwise would have distracted the scientific staff, proved to be invaluable.

In summary, with the results obtain from this work, it may be possible to address the original queries:

> (1) up and down wind sampling of an acutely infected barn prior to euthanasia,

As can be seen, the slit samplers located up and down wind did not capture any viral particles. It can be interpreted that the particles, if present, were carried high above the sampling sites and thus beyond detection.

> (2) up and down wind sampling of a barn being euthanized to capture the displacement effect of carbon dioxide pushing air and possibly virus out the barn,

Again, negative samples were observed. Similar explanation could be used as above.

> (3) the effect of clearing the barn of carbon dioxide by starting up the ventilation fans after euthanasia

Same as for item 2.

> (4) the possible re-aerosolization of virus from a barn being actively composted or depopulated by sampling the up and down wind positions

Same as for item 2. Alternatively and problematic, failure to capture viral particles may also be interpreted that none were in the air at the time of sampling. Such is the difficulty with measuring rare events.

One aspect of the work not previously anticipated was the requirement to decontaminate all the "exposed" equipment including personnel before moving to another site (figure 5). This was made necessary by strict regulations set by the CFIA to prevent cross contaminations of uninfected farms. Although decontamination has been standard practice for microbiologists in laboratories, having to do this in open air required a different skill sets. The lessons learned from this exercise may serve as guidelines for future environmental decontamination exercises.

Figure 5. *Decontamination of equipment*

Removing equipment from a contaminated area to a clean area required meticulous decontamination using a commercial liquid agent known to be effective for viruses. This was a labor-intensive process and required careful planning. The vehicle was used for transporting heavy equipment within the contaminate areas as defined by CFIA. It had to be decontaminated before coming out of the restricted zone at the end of the trial

References

1. Trampuz, A., Prabhu, R.M., Smith, T.F., and Baddour, L.M. (2004). Avian influenza: a new pandemic threat? *Mayo Clin. Proc.*, 79, 523–530.

2. Alexander, D. J. (2000). A review of avian influenza in different bird species. *Vet. Microbiol.,* 74 (1-2), 3–13.

3. Webby, R.J. and Webster, R.G. (2003). Are we ready for pandemic influenza? *Science*, 302, 1519–1522.

4. Suarez, D.L., Senne, D.A., Banks, J., Brown, I.H., Essen, S.C., Lee, C.W., Manvell, R.J., Mathieu-Benson, C., Moreno, V., Pedersen, J.C., Panigrahy, B., Rojas, H., Spackman, E., and Alexander, D.J. (2004). Recombination resulting in virulence shift in avian influenza outbreak, Chile. *Emerg. Infect. Dis.*, 10, 693–699.

5. Dieter, R.A., Jr, Dieter, R.S., Dieter, R.A., 3rd, and Gulliver, G. (2001). Zoonotic diseases: health aspects of Canadian geese. *Int. J. Circumpolar Health*, 60, 676–684.

6. Baigent, S. J. and McCauley, J. W. (2003). Influenza type A in humans, mammals and birds: determinants of virus virulence, host-range and interspecies transmission. *Bioessays,* 25 (7), 657–671.

7. Carducci, A., Gemelli, C., Cantiani, L., Casini, B., and Rovini, E. (1999). Assessment of microbial parameters as indicators of viral contamination of aerosol from urban sewage treatment plants. *Lett. Appl. Microbiol.*, 28, 207–210.

8. Pfirrmann, A. and vanden Bossche, G. (1994). [Occurrence and isolation of human enteroviruses from the air of waste removal and disposal plants]. *Zentralbl. Hyg. Umweltmed.*, 196, 38–51.

9. Webster, R.G., Guan, Y., Peiris, M., Walker, D., Krauss, S., Zhou, N.N., Govorkova, E.A., Ellis, T.M., Dyrting, K.C., Sit, T., Perez, D.R., and Shortridge, K.F. (2002). Characterization of H5N1 influenza viruses that continue to circulate in geese in southeastern China. *J. Virol.*, 76, 118–126.

10. Ho, J., Spence, M., and Duncan, S. (2001). Live Biological Particle Measurement: Comparison of slit sampler performance in a biological aerosol chamber. (DRES TR 2001-139). Defence Research Establishment Suffield.

11. Kedjarune U, Kukiattrakoon B, Yapong B, Chowanadisai S, and Leggat P. (2000). Bacterial aerosols in the dental clinic: effect of time, position and type of treatment. *Int. Dent. J.*, 50, 103–107.

12. Dull, P.M., Wilson, K.E., Kournikakis, B., Whitney, E.A., Boulet, C.A., Ho, J.Y., Ogston, J., Spence, M.R., McKenzie, M.M., Phelan, M.A., Popovic, T., and Ashford, D. (2002). *Emerg. Infect. Dis.*, 8, 1044–1047.

13. Booth, T.F., Kournikakis, B., Bastien, N., Ho, J., Kobasa, D., Stadnyk, L., Li, Y., Spence, M., Paton, S., Henry, B., Mederski, B., White, D., Low, D.E., McGeer, A., Simor, A., Vearncombe, M., Downey, J., Jamieson, F.B., Tang, P., and Plummer. F. (2004). Detection of Airborne Severe Acute Respiratory Syndrome (SARS) Coronavirus and Environmental Contamination in SARS Outbreak Units. *J. Infect. Dis*., 191, 1472–1477.

14. Gillespie, J., Kalica, A., Conner, M., Schiff, E., Barr, M., Holmes, D., and Frey, M. (1984). The isolation, propagation and characterization of tissue-cultured equine rotaviruses. *Vet. Microbiol*., 9, 1–14.

15. Brenner, K.P., Scarpino, P.V., and Clark, C.S. (1988). Animal viruses, coliphages, and bacteria in aerosols and wastewater at a spray irrigation site. *Appl. Environ. Microbiol*., 54, 409–415.

16. Marple, V.A. 2004. History of impactors the first 110 years. *Aerosol Sci. Technol.*, 38, 247–292.

Appendix A – Estimated Viral Load

Assumptions made for calculation of viral load per cubic meter of air in barn. Unit of measure is in tissue culture infective dose fifty ($TCID_{50}$)

1. Flow rate of XMX sample r= 800 lpm
2. Sampling time = 15 min.
3. Total volume of air processed = 12000 liter or 12 m^3
4. Total number of $TCID_{50}$ = 3500
5. Estimated viral load = 3500/12 = 292 $TCID_{50}$/ m^3

Appendix B – Team Members

Dr. L. Schofield.....…Veterinarian and team lead

Dr. J. Ho.....Microbiologist and aerosol sampling specialist

Dr. B.Kournikakis .....Microbiologist and aerosol sampling specialist

Ms N. Mazuryk.....…Microbiology technologist

Ms R. Hilsen.....…Microbiology technologist

Mr. J. Ogsten.....Microbiology technologist

Mr. D. Fode.....Driver/Mechanical specialist

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An outbreak of infectious Avian Influenza (AI) occurred during March-April 2004 in the Abbotsford area of British Columbia. There was a need to determine if infectious viral particles were being spread via an aerosol route. It was anticipated that such particles, should they be found, would occur as rare events. By coincidence, DRDC Suffield has been developing aerosol samplers that can be used to detect such rare events. Two sampling systems, consisting of a slit sampler array and a large column air sampler (XMX virtual impactor) were deployed. The sampling scheme was designed to capture four specific events requested by the CFIA. These were (1) up and down wind sampling of an acutely infected barn prior to euthanasia, (2) up and down wind sampling of a barn being euthanized to capture the displacement effect of carbon dioxide pushing air and possibly virus out the barn, (3) the effect of clearing the barn of carbon dioxide by starting up the ventilation fans after euthanasia, (4) the possible re-aerosolization of virus from a barn being actively composted or depopulated by sampling the up and down wind positions. In addition to these specific events, XMX samples were taken in 10 random positions in and around Abbotsford. The first two were in acutely infected barns with high morbidity/mortality while the remaining eight were randomly taken in the outbreak area. Liquid samples from an XMX device were analysed using PCR as the initial method of identification followed by virus culture to demonstrate live virus. Quantification was to be carried out using plaque assay. Results showed that all slit samples were PCR negative while three of the XMX samples were unmistakable positives. One sample collected from 250 m southeast of the CFIA emergency operation centre (EOC) in Abbotsford was also positive. Estimated viral load yielded a value of 292 viral doses/m^3 of barn air. Results from this exercise will be used for subsequent modelling experiments.

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Avian flu, aerosol sampling, biological sampling, XMX sampler, slit array sampler, PCR, chickens, British Columbia