The Sensitivity of Some Avian Viruses to Formaldehyde Fumigation

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ABSTRACT

Various avian viruses (infectious bursal agent, reovirus, adenovirus, infectious bronchitis, Newcastle disease, poxvirus, avian encephalomyelitis and infectious laryngotracheitis virus) as suspensions in buffer or in a litter slurry were exposed to aerosolized formalin in an attempt to determine the efficacy of this fumigation method for decontamination of laboratory isolation cubicles. Formalin (37% formaldehyde) was delivered by a commercial insecticide fogger at a flow rate of 40 ml per minute and a volume of 36 ml per cubic meter of space. Fumigated cubicles were left sealed for 18 hr (cycle 1) before viruses were sampled, or were then exposed to a second fumigation and left sealed for an additional six hour period (cycle 2) before viruses were titrated (commencing at a 1:10 dilution) for residual infectivity.

Although the infectivity of all viruses was reduced by over 99% by one fumigation cycle, the second cycle was necessary for reduction of Newcastle disease and reoviruses to nondetectable (no infectivity demonstrated in a 1:10 dilution of fumigated virus) levels.

RÉSUMÉ

Cette expérience visait à soumettre à la fumigation à la formaldéhyde les virus aviaires suivants: l'agent infectieux de la bourse de Fabricius, le réovirus, l'adénovirus, le virus de la bronchite infectieuse, le virus de la maladie de Newcastle, le poxvirus, le virus de l'encéphalomyélite aviaire et celui de la laryngo-trachéite infectieuse. On utilisa à cette fin des suspensions de ces virus et des déjections, préalablement diluées dans une solution tampon. On tentait ainsi de déterminer l'efficacité de la fumigation comme moyen de désinfecter les compartiments d'isolation de laboratoire. On utilisa un vaporisateur commercial d'insecticides pour obtenir un débit de formaldéhyde 37% de 40 ml à la minute et un volume de 36 ml par mètre cube. Après la fumigation, on laissa les compartiments d'isolation fermés hermétiquement pour une période de 18 heures; on procéda ensuite à la recherche d'infection résiduelle dans certains d'entre eux, tandis qu'on soumit les autres à un seconde fumigation et qu'on les laissa fermés hermétiquement pour une période additionnelle de six heures avant d'y procéder à la recherche d'infection résiduelle dans les suspensions virales qu'on dilua à partir de 1:10.

Même si la première fumigation réduisit l'infectivité de tous les virus expérimentaux dans une proportion d'au delà de 99%, la seconde s'avéra nécessaire pour obtenir une quantité non décelable du virus de la maladie de Newcastle et du réovirus, à la dilution 1:10.

INTRODUCTION

Several detailed reports are available on the use of formaldehyde as a disinfectant (2, 6, 9, 15) but little has been published on the quantitative sensitivity of avian viruses to this method of fumigation. For example, Newcastle disease virus was inactivated by a 180 minute exposure to 37% formaldehyde at a concentration of approximately 25 ml/m^3, but the prefumigation titre of the virus suspension was not given (1). Fowlpox virus was inactivated by a 90 minute exposure to 37% formaldehyde (approximately 7 ml/m^3), but the titres of prefumigation or control virus

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Submitted October 20, 1978.

suspensions were not stated (7). A more recent report (6) recorded inactivation of an avian adenovirus exposed to 37% formaldehyde (10 to 40 ml/m³) as a dried film of infective tissue culture fluids but it is not clear what titre of virus could have been recovered from dried preparations which had not been fumigated. Conversely, other avian viruses have demonstrated some resistance to formaldehyde. For example, feather-associated Marek's disease virus was not inactivated in one of four samples exposed to 37% formaldehyde (approximately 12 ml/m³) for 15 minutes at 37°C and 80% humidity (3) and avian reovirus strains 24, 25 and 59 at 2,000 EID_{50}/0.2 ml remained infectious for chick embryos after exposure to an equal volume of a 1:400 dilution of formalin for five hours (4).

The following work was conducted to determine the effect of formaldehyde fumigation on several avian viruses. This was designed to establish a standard fumigation method for use in isolation rooms at this laboratory.

MATERIALS AND METHODS

Isolation Rooms and Fumigation Methods

Rooms were of epoxy painted smooth concrete block construction with cement floors and painted plywood ceilings and an internal volume of approximately 33 m³. Prior to fumigation, exhaust fans and doors were sealed with tape and the room temperature was adjusted to between 20 and 22°C. Formalin (37% formaldehyde[1]) was delivered by a commercial insecticide fogger (vaporizer) at a flow rate of 40 ml per minute and a volume of 1,200 ml per room, giving an initial concentration of 36 ml/m³. Actual air concentrations of formaldehyde during fumigation were not determined. The temperature readings of dry and wet-bulb thermometers within the room were made via an inspection port and the relative humidity was calculated from these values (8).

Viruses

Viruses used and their sources are presented in Table I. Infectious bursal agent (IBA) strains S40747 and Sk-1 were passaged by chorioallantoic-membrane (CAM) inoculation of ten day old eggs. Embryos were harvested five days later, homogenizto a 10% suspension in phosphate buffered saline (PBS) (14), clarified by centrifugation at 5000 g for 30 min and the supernatant used as the virus suspension. The vaccine strain of IBA ("Bursa Vac") was used after reconstitution as described by the manufacturer. Pools of reovirus, infectious laryngotracheitis (ILT) and poxvirus strains were prepared as a 10% suspension in PBS of infected CAM's harvested five days after inoculation by that route. Adenovirus infectious bronchitis (IB) and Newcastle disease (ND) strains were passaged by inoculation into the allantoic cavity (ALC) of ten day old eggs, from which allantoic fluid (ALF) was harvested between three and five days later. Avian encephalomyelitis virus was prepared as a PBS homogenate of brains from 18 day old chick embryos which had been inoculated via the yolk sac on the sixth day of embryonation. Titrations of fumigated virus suspensions were conducted in eggs commencing at a 10^{-1} dilution (to avoid the toxic effect of residual formalin) using between three and five eggs per dilution. The IBA strains were titrated by CAM inoculation and endpoints were based on gross lesions in the embryo. Reoviruses, poxvirus and ILT viruses were also titrated by CAM inoculation but endpoints were based on CAM lesions seven days later. Adenoviruses, ND viruses and IB viruses were titrated by ALC inoculation and endpoints were based on demonstration of precipitating antigen, hemagglutination or gross lesions, respectively (10).

Experimental Design

Experiment 1 — In the first experiment, virus suspensions in PBS were placed as 0.3 ml volumes (depth approximately 9 mm) in the peripheral wells of a 96-well microtitre plate[2]. A plastic lid was placed

[1]USP formaldehyde solution, Anachemia Chemical Products Ltd., Mississauga, Ontario.

[2]Falcon Plastics, Becton, Dickinson & Co. (Canada) Ltd., Mississauga, Ontario.

TABLE I. Virus Strains Used and Their Sources

Virus	Isolate	Source
Infectious bursal agent	S40747	American Type Culture Collection
	Bursa Vac	Sterwin Laboratories, Aurora, Ontario
	Sk-1	Local isolate
Reovirus	2937 and Crawley	Dr. N.A. Olson, University of West Virginia
Adenovirus	CELO Phelps and Indiana C	Dr. R. W. Winterfield, Purdue University
Infectious bronchitis	M-41	Dr. R.W. Winterfield, Purdue University
	Beaudette	Virology Section, A.D.R.I. (E), Ottawa
Newcastle	B-1 and GB	Virology Section, A.D.R.I.(E), Ottawa
Poxvirus	B-5601	Virology Section, A.D.R.I. (E), Ottawa
Avian encephalomyelitis	van Roekel	Virology Section, A.D.R.I. (E), Ottawa
Infectious laryngotracheitis	Lederle	American Type Culture Collection

over the plate to prevent large droplets of formalin from falling directly into the wells, but was lifted from the surface of the plate by approximately 4 mm to allow adequate space for vapor diffusion under the lid. The plate was placed in the center of the room about two feet above the floor but was not in the trajectory of the vaporizer. Duplicate aliquots of each virus suspension were similarly placed in a microtitre plate in an identical adjacent room but these were exposed to vaporized water instead of formalin. Eighteen hours later, rooms were ventilated for one hour and the virus suspensions were removed for titration.

Experiment 2 — This was conducted to determine the effect of a second fumigation cycle on the residual infectivity, detected in suspensions of reovirus and Newcastle disease viruses, after the single fumigation cycle described in Experiment 1.

In this method, virus suspensions were removed from the fumigated room after the first fumigation cycle, transferred to a new microtitre plate and then refumigated as described, but only for a six hour period. Virus preparations in the control (nonfumigated) isolation room were similarly handled but were not exposed to formaldehyde.

Experiment 3 — This experiment was conducted to determine the effect of organic material on the sensitivity of the test viruses to formaldehyde. Litter (feces and uneaten feed but no bedding) from a pen of uninoculated three week old chicks was homogenized with 0.1 M PBS pH 7.2 form a wet slurry which was autoclaved in 20 g aliquots and then stored at 4°C. A 10 ml volume of each virus suspension diluted to 1:10 or 1:100 in PBS was homogenized with 20 g of the slurry and poured into a Petri plate to a depth of 1 cm. This material was then exposed to formaldehyde in a single 18 hour cycle as described in Experiment 1, above. Similar mixtures were placed in the formaldehyde-free control cubicle. After fumigation, approximately 2-3 g was taken from each plate and diluted in PBS at a ratio of 2 ml PBS per g of slurry prior to titration.

RESULTS

The relative humidity in isolation rooms was between 50 and 60% prior to fumigation but rapidly rose after the vaporizer was switched on and peaked at between 80 and 90% within the first 20 minutes. However, levels fell rapidly and by 35 minutes, humidity had fallen to just over 60%, the decline following termination of formalin delivery by the fogger.

Titres of virus suspensions in PBS after exposure to a single fumigation cycle (Experiment 1) are compared to control titres in Table II. Inactivation by over 99% occurred in all cases but residual infectivity was detected in suspensions of the Crawley strain of reovirus and both Newcastle disease virus strains.

Titres of virus suspensions in PBS after exposure to two fumigation cycles (18 hr and six hours duration, respectively) are shown in Table III. The infectivity of the viruses tested (reovirus and Newcastle disease virus) was reduced to a nondetect-

TABLE II. The Effect of Formaldehyde Fumigation in Infectivity of Some Avian Viruses

Virus	Isolate	Infectivity of Virus: Control[a]	Infectivity of Virus: Formaldehyde-treated[b]
Infectious bursal agent	ATCC S40747	3.0[c]	⩽0.5
		5.3	⩽0.5
	Bursa Vac	3.4	⩽0.5
		3.3	⩽0.5
	Sk-1	4.3	⩽0.5
Reovirus	WVU 2937	5.8	⩽0.5
		3.8	⩽0.5
	Crawley	>6.0	0.6
		6.0	1.3
Adenovirus	CELO	6.5	⩽0.5
		>7.5	⩽0.5
	Indiana C	5.3	⩽0.5
		5.0	⩽0.5
Infectious bronchitis	Massachusetts (M41)	6.0	⩽0.5
	Beaudette	4.5	⩽0.5
Newcastle disease	B-1	5.5	1.5
		>6.5	0.8
	GB	4.5	1.0
		4.3	⩽0.5
Poxvirus	B-5601	5.5	⩽0.5
		5.8	⩽0.5
Avian encephalomyelitis	van Roekel	3.0	⩽0.5
Infectious laryngotracheitis	ATCC	3.3	⩽0.5
		4.0	⩽0.5

[a]Virus suspension in PBS held in nonfumigated isolation room for 18 hours prior to titration
[b]Virus suspension in PBS exposed to formaldehyde for 18 hours
[c]Reciprocal of $\log_{10}$ EID_{50}/0.2 ml. Except for isolates Sk-1, M41, Beaudette and van Roekel, as shown, the formaldehyde sensitivity of all viruses was tested twice in separate experiments
⩽0.5 = no infectivity demonstrable in a 10^{-1} dilution of virus suspension

TABLE III. The Effect of Two Formaldehyde Fumigation Cycles on Viruses which Demonstrated Residual Infectivity After One Fumigation Cycle

Virus	Isolate	Infectivity of Virus: Formaldehyde-treated A	Infectivity of Virus: Formaldehyde-treated B	Control 24 hr
Newcastle disease	B-1	0.8[a]	⩽0.5	7.0
	GB	⩽0.5	⩽0.5	7.0
Reovirus	Crawley	3.0	⩽0.5	6.3

A = Virus suspension exposed to formaldehyde for 18 hours (cycle 1) prior to titration
B = Virus suspension exposed to formaldehyde for an additional six hours (cycle 2) after cycle 1, prior to titration
[a]Reciprocal of $\log_{10}EID_{50}$/0.2 ml virus suspension
⩽0.5 = no infectivity demonstrable in a 10^{-1} dilution of virus suspension

able level (reduction in titre by over 5 log 10 EID_{50}) by two fumigation cycles.

Titres of viruses exposed to one cycle of fumigation while suspended in fecal slurry are shown in Table IV. Of the viruses tested, only the Sk-1 strain of IBA was infective after treatment. This virus was reduced in titre by ⩾ 3.8 $\log_{10}EID_{50}$ when suspended in PBS during fumigation, but only 2.0 $\log_{10}EID_{50}$ when fumigated in slurry. The Crawley strain of reovirus and the CELO strain of adenovirus were reduced in titre by approximately 5.0 and 6.0 $\log_{10}EID_{50}$, respectively, whether fumigated in slurry or PBS, the high organic medium having little effect on the degree of in-

TABLE IV. Formaldehyde Fumigation of Avian Viruses in Fecal Slurry

Virus	Isolate	Control[a]	Formaldehyde-treated[b]
Infectious bursal agent	Bursa Vac	1.8[c]	$\leqslant 0.5$
	Sk-1	3.0	1.0
Reovirus	Crawley	5.3	$\leqslant 0.5$
Adenovirus	CELO	6.5	$\leqslant 0.5$
Infectious bronchitis	Beaudette	3.5	$\leqslant 0.5$
Newcastle disease	B-1	4.0	$\leqslant 0.5$
	GB	2.3	$\leqslant 0.5$

[a]Virus suspension in fecal slurry held for 18 hours in nonfumigated isolation room prior to titration
[b]Virus suspension in fecal slurry exposed to formaldehyde for 18 hours
[c]Reciprocal of $\log_{10}EID_{50}/0.2$ ml

activation. Other viruses were also reduced in titre to nondetectable levels after fumigation in slurry but as control titres were considerably lower in the slurry experiments than in the PBS experiments (due to dilution of virus suspensions prior to mixing with slurry) a comparison of inactivation levels in the two media cannot be made. For example, Newcastle disease virus strains G-B and B-1 were reduced in titre by approximately 3.7 and 4.8 $\log_{10}EID_{50}$ (mean values for two experiments shown in Table II) in PBS, and by $\geqslant 1.8$ and $\geqslant 3.5$ $\log_{10}EID_{50}$ in slurry. As the control titres in the latter experiment were low, it is not known if these viruses were more resistant to fumigation when suspended in slurry. Similar results were obtained with infectious bronchitis virus (Table IV).

DISCUSSION

A volume of approximately 35 ml of 37% formaldehyde per cubic meter of space at a minimum temperature of 23.9°C, 70% humidity and a holding period of eight to ten hours has been recommended for general fumigation (12), but it is often impossible to fulfill all these criteria under field conditions (15). Shorter holding periods may be effective (2, 11) although this is dependent upon various factors, including the amount of organic material in the fumigated area (2, 15), the type of material in which the contaminant is located (2, 5), the concentration and method used for generation of the formaldehyde (15), the humidity of the fumigated area (2) and, of course, the formaldehyde sensitivity of the contaminating agent itself (13).

The results reported in this paper indicate that the infectivity of most of the viruses tested could be reduced to a nondetectable level by a single formaldehyde fumigation cycle as described, but that two serial fumigation cycles may be required for inactivation of some viruses. A single cycle of formaldehyde fumigation inactivated viruses in a liquid litter slurry approximately 1 cm in depth although the Sk-1 strain of IBA exhibited residual infectivity after such treatment (Table IV). It should be noted that as titrations of fumigated virus suspensions were started at a 1:10 dilution possible residual infectivity in undiluted fluids might not have been detected.

The effect of formaldehyde fumigation on dried virus preparations was not determined but, as the relative humidity was only transiently increased by the use of a fogger for formalin delivery and as relative humidity of over 60% should probably be maintained for at least three hours (2), dried virus preparations fumigated as described may not be totally inactivated. This problem may be avoided by thoroughly wetting all surfaces in isolation rooms prior to fumigation. As viruses established in impervious organic deposits may be highly resistant to fumigation suitable clean-up must accompany such disinfection methods (2). It was apparent that under the conditions of these experiments suitable delivery of formalin was effected by the use of a mechanical fogger. Scarlett and Mathewson (15) did not obtain satisfactory results with such equipment in commercial calf houses, although levels of formalin used (6.7 to 10 ml/m³/24 hr) were lower than those used in this work (36 ml/m³).

ACKNOWLEDGMENTS

The excellent technical assistance of Mr. M. Briscoe, Mr. D. Rayworth and Mrs. L. Griffin is gratefully acknowledged.

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