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Short Communications

Inactivation of foot-and-mouth disease virus by heat, formaldehyde, ethylene oxide and γ radiation

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HANDLING of foot-and-mouth disease virus (FMDV) is, in most countries, only allowed in high containment laboratories. Most materials leaving such a laboratory are decontaminated at a high temperature ($>100^{\circ}\text{C}$) and a high relative humidity (RH). However, because some materials will not withstand these conditions the security standards for foot-and-mouth disease (FMD) laboratories in Europe, as laid down in AGA:EUFGMD/93/2 (Anon 1993), allow sensitive instruments and paper to leave a high containment laboratory after decontamination by dry heat at 50°C for two days. This procedure is based on experiments using virus suspensions (Bachrach and others 1957, Doel and Baccarini 1981). However, as virus-containing droplets spilt on paper dry in air, the proteins present in the virus suspension concentrate and may protect FMDV from inactivation. It has been shown that FMDV can withstand high temperatures when protected by proteins, for example, from milk (Hyde and others 1975, Walker and others 1984). The inactivation of air-dried FMDV by dry heat and three alternative methods, formaldehyde, ethylene oxide and γ irradiation was therefore examined.

Primary and secondary porcine kidney cells were used to grow and titrate FMDV. To obtain primary porcine kidney cells, kidneys of a specific pathogen-free piglet, six to 10 weeks old, were treated with 0.06 per cent trypsin. After filtering and washing the cells, they were grown in medium containing Hanks salts, 0.5 per cent lactalbumin hydrolysate, 5 per cent fetal bovine serum and antibiotics.

FMDV strains A₁₀Holland, O₁BFS, C₁Detmold, and Asia-1 SAU 32/92 were passaged several times on primary porcine kidney cells. Volumes of 50 μl of each virus type were distributed on to 32 \times 9 mm coverslips and dried under vacuum in a desiccator containing silica gel. For each virus type a sufficient number of coverslips carrying air-dried virus were made at the same time and stored at -70°C .

The coverslips with air-dried FMDV were heat treated at 37°C , 50°C and 80°C in an incubator in which the temperature was monitored continuously. During heat treatment one coverslip for each virus type was removed at set times in order to titrate surviving

FMDV. Suspensions of each of the four FMDV types were treated similarly. Coverslips with each of the four FMDV types were also treated with formaldehyde, ethylene oxide and three different doses of γ radiation. Formaldehyde inactivation was performed at room temperature (85 to 90 per cent RH) and involved evaporating formalin up to a final concentration of 3 g/m^3 for 12 hours, followed by exposure to ammonia at 3 g/m^3 for three hours to neutralise the formaldehyde. Ethylene oxide inactivation was performed in an ethylene oxide autoclave (Linden), at $52 \pm 3^{\circ}\text{C}$ (30 per cent RH), using 570 g ethylene oxide gas per m^3 for 160 minutes, followed by 70 cycles of evacuation and aeration within 18 hours to remove remnants of ethylene oxide gas. γ irradiation was performed at Gammaster BV (Ede). During the irradiation experiments, sealed tubes containing the coverslips were kept on dry ice. The package still contained dry ice when it was returned to the laboratory. Three doses of γ radiation (30 kGy, 40 kGy, and 60 kGy) were used. In the formaldehyde, the ethylene oxide and the γ irradiation inactivation experiments, it was not possible to collect samples during the process and the effect of these treatments on suspensions of FMDV was not studied.

After the different treatments the coverslips were broken with a sterile pipette and submerged in 1 ml of cold medium. Each sample was titrated by incubating 200 μl of 10-fold dilutions, made in Earle's minimal essential medium (MEM) containing 5 per cent fetal bovine serum and antibiotics, for one hour on monolayers of secondary porcine kidney cells in duplicate wells of a six-well plate. After one hour 2.5 ml of Earle's MEM containing 5 per cent fetal bovine serum, 1 per cent methylcellulose and antibiotics were added to each well. Two days later the plates were dipped in 1 per cent citric acid, the monolayers were rinsed with tap water, fixed and stained with amido black (0.1 per cent amido black in 1 M acetic acid, 0.09 M sodium acetate, 10 per cent glycerol). Plaques were counted macroscopically. All incubations were made at 37°C in a humidified atmosphere containing 5 per cent carbon dioxide.

Repeated titration of untreated coverslips showed variation in the titre of virus recovered resulting in large standard deviations of 0.35, 0.96, 0.60, 0.73 (\log_{10} plaque forming units/ml) for types A, O, C and Asia-1, respectively. Fig 1 shows the kinetics of inactivation at 37°C , 50°C and 80°C . The reduction in titre due to drying varied greatly between the different types of FMDV. Titres for type C and Asia-1 quickly fell below the detection limit. Type A seemed to be more stable than the other three types. Nonetheless, inactivation at 37°C for 14 days, 50°C for two days or 80°C for one hour was not sufficient to inactivate completely three of the four types of FMDV.

No infectious virus was detected in the virus suspensions heated at 37°C for seven days, 50°C for two days or 80°C for 3-75 minutes. Virus dried on coverslips was inactivated to levels below the detection limit by treatment with formaldehyde, ethylene oxide, and by γ irradiation using a dose of 40 or 60 kGy. A reduction of 4.6 \log_{10} was found for type A using γ irradiation at 30 kGy.

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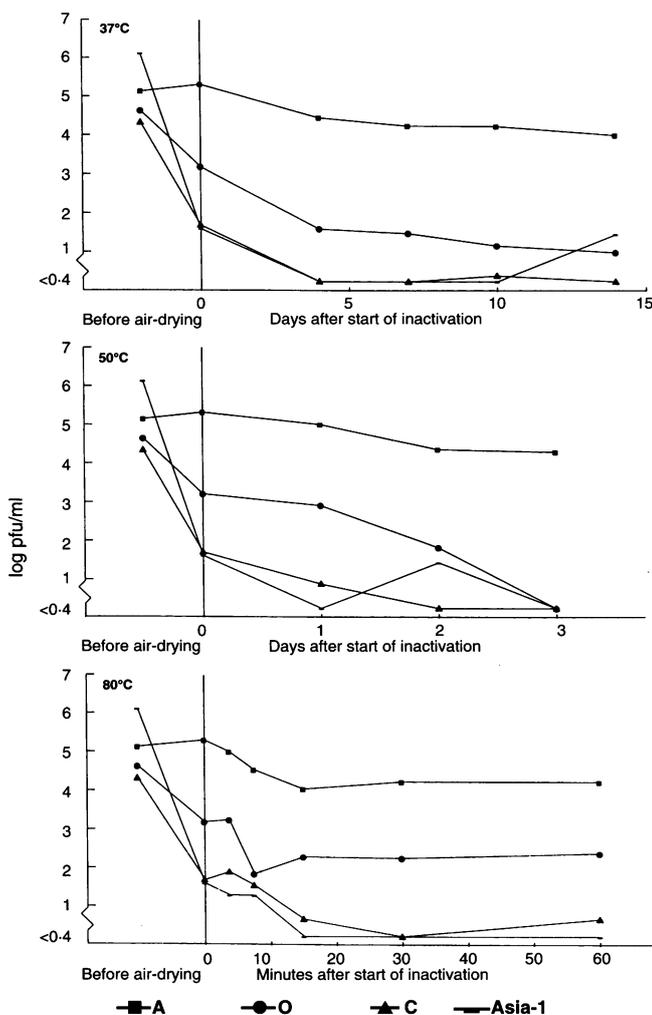


FIG 1: Kinetics of inactivation of FMDV type A, O, C and Asia-1 air-dried on coverslips at 37°C, 50°C and 80°C

These experiments confirm the observations of Bachrach and others (1957) that FMDV in suspension can be inactivated by dry heat at 50°C in two days. On the other hand, FMDV that was dried on coverslips was found to be highly resistant to heat treatment. This method of inactivation was insufficient in at least three of the four types of FMDV examined (Fig 1). In some cases, higher titres were found later, which was due to variation in titre between the different coverslips, variation in titre reduction and because only one coverslip per type was titrated each time. Taking replicates to determine the variation in titre reduction for each virus type was not worthwhile because of the large variation in titres of the untreated coverslips. The differences in titre within one batch might have been caused by the fact that coverslips in the centre of the desiccator dried quicker than those near the edges of the desiccator. These results demonstrate that dry heat is an inadequate method of inactivation of air-dried FMDV.

The failure of dry heat to adequately inactivate air-dried FMDV is probably due to the high concentration of protein remaining after the virus has been dried. Ferris and others (1990) showed that the addition of sucrose and lactalbumin, or skimmed milk powder, stabilised FMDV efficiently during and after freeze-drying. A reduction of only 0.4 to 1.1 \log_{10} in virus titre was found at 37°C after seven days using 5 per cent skimmed milk powder as stabiliser. This reduction in titre is comparable to the result found in the present experiments. Air-dried FMDV is more stable than FMDV suspended in milk. De Leeuw and others (1980) reported a reduction of 4 \log_{10} for FMDV in primary infected milk at 60°C within one minute, whereas a reduction of only 1 to 2 \log_{10} at 80°C after one hour was found in the present experiments. Groneman and others (1977) showed that the dose-response curve is linear during inactivation of FMDV by γ irradiation. They calcu-

lated a D_{10} (the dose required to reduce infectivity by one \log_{10}) of 4.3 to 4.7 and 6.5 kGy for FMDV in culture fluid and sewage, respectively. The use of 30 kGy resulted in a 7.0 to 6.4 and 4.6 \log_{10} reduction, respectively. Thus, the titre reduction found in the present experiment (4.6 \log_{10}) is comparable to the titre reduction in sewage (4 per cent solids w/w). These results have led the author's institute to abolish heat inactivation at 37°C and 50°C for materials leaving the high containment laboratory. However, treatment with formaldehyde, ethylene oxide and γ irradiation using more than 40 kGy gave satisfactory results. The results of this study suggest that the current security standards for FMD laboratories in Europe as laid down in AGA: EUFMD/93/2 (Anon 1993) are inadequate. The use of ethylene oxide (570 g/m^3 for 160 minutes at 52°C and 30 per cent RH), formaldehyde (3 g/m^3 for 12 hours at room temperature and 85 to 90 per cent RH), and γ radiation (40 kGy) may be more appropriate measures.

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Activity of moxidectin against bots and lungworm in equids

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MOXIDECTIN, a milbemycin, is highly active against many species of nematodes in farm animals including small and large strongyles in horses (Lyons and others 1992, Bello and Laningham 1994, Xiao and others 1994, Corba and others 1995, Monahan and others 1995). The present study was undertaken to extend information on its activity and specifically to confirm efficacy against the bot, *Gasterophilus nasalis*, (Lyons and others 1992) and the lungworm, *Dictyocaulus arnfieldi*.

A previous study at a horse abattoir had shown that Dartmoor ponies are usually infected with *G. nasalis* (G. C. Coles, unpublished observations). Therefore, eight randomly selected ponies were treated orally with 0.4 mg/kg moxidectin 2 per cent gel in January and eight remained untreated. The bodyweight of each animal was estimated with a heart girth tape before treatment. The ponies were then held together on a paddock and fed hay ad libitum. Temperatures were too low for significant nematode transmission. Thirty-four days after treatment the ponies were humanely slaughtered and the stomachs and large intestines removed. Bots

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