A COMPARISON OF CITRIC ACID, SODIUM HYDROXIDE AND SODIUM HYPOCHLORITE AS DISINFECTANTS FOR THE EQUINE RHINOVIRUS A (ERAV) AND PHAGE PHIX 174

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Introduction:

Disinfection plays a central role in controlling viral epidemics. Testing important animal pathogens is, however, difficult, since work with these in the laboratory requires extreme safety measures as well as expensive equipment and a great deal of expertise. It is therefore necessary to use model viruses in order to test the effectiveness of given disinfectants. We tested two different viruses as possible models for foot and mouth disease virus (FMDV) in standard disinfection tests according to the guidelines of the Deutsche Veterinärmedizinische Gesellschaft (DVG, 2000). Equine rhinitis A virus (ERAV) was chosen because of its close relation to FMDV. Both belong to the family *Picornaviridae*, genus *Aphthovirus*. As a possible further simplification of testing procedures, the bacteriophage ΦX 174 was tested in the same way as ERAV. Disinfection tests were carried out with citric acid, sodium hydroxide and sodium hypochlorite. Concentrations that were effective with ERAV were tested on ΦX 174 and results were compared.

Material and methods

All tests were carried out according to the DVG (2000). This guideline includes a suspension test with and without protein and a wood carrier test. For the suspension test with protein, a solution with 1ml ERAV, 0.8ml Phosphate Buffered Saline (PBS) and 0.2ml of 10fold concentrated disinfectant was mixed and incubated in a water bath at 20-22°C In the suspension tests with protein, the PBS was replaced by 0.8ml fetal calf serum (FCS). 0.1ml aliquotes of this solution were taken after 15, 30, 60 and 120 min. and diluted 1:100 in 9.9ml of PBS. Serial 10fold dilutions were then carried out in Dulbecco Modified Eagle's Medium (DMEM) until 10^{-7}. The virus was added to Rabbit- Kidney (RK)-13 cell monolayers seeded into 96-well plate and incubated at 37°C with 5% CO_2. Each dilution step was inoculated onto four wells. Plates were read under a light microscope after 4-5 days of incubation.

For the wood carrier test, a suspension of 1ml ERAV, 0.8ml foetal calf serum (FCS) and 0.2ml DMEM was prepared. The wood carriers were 2cm² large pieces of poplar wood

1mm thick. The carriers were sterilized before use. 0.1ml of virus suspension was dropped onto each wood carrier. The carriers were dried in a sterile Petri dish for 60min. at room temperature. The infected carriers were then placed in tubes with the disinfectant in its final concentration and left there for 15, 30, 60 and 120min. Afterwards, each carrier was cut into pieces, placed in 9,9ml PBS and treated by ultrasound at 30-40 W for 1min. After centrifuging the samples at 3000U/min for 15min. at 4°C, the samples were titrated as described above. All tests were repeated at least once.

Tests with ΦX 174 were carried out as described above with the following modifications: The titrated samples were mixed into test tubes with 10ml sterile tryptone soya broth (TSA) to which 1 ml E. coli 140 suspension and an indicator (TTC) had been added first. The TSA was kept in a water bath at 45°C to keep it liquid. Finally, each sample was poured onto a sterile Petri dish 12cm in diameter and incubated at 37°C for 24h to view results.

Results

The suspension test with and without protein done with citric acid and ERAV showed a reduction of between 4 and 5 decimal powers at concentrations of 0.2 and 0.3%. A reduction of 1 decimal power was observed at a concentration of 0.1% citric acid. The pH measured was between 6.24 at 01% and 3.90 at 0.3% citric acid.

The wood carrier test with citric acid and ERAV showed a reduction of between 3 and 4 decimal powers at concentrations of 0.2% and 0.3%. At a concentration of 0.1% citric acid, a reduction of 1 up to 4 decimal powers could be observed. The pH measured was between 3.02 at 0.1% and 2.39 at 0.3% citric acid.

The suspension test with and without protein done with sodium hydroxide and ERAV showed a reduction of between 4 and 5 decimal powers at concentrations of 0.5 and 0.7%. At a concentration of 0.1% sodium hydroxide, a reduction of 1 decimal power was observed. The pH measured was between 6.24 at 0.1% and 3.90 at 0.3%.

The wood carrier test with citric acid and ERAV showed a reduction of between 3 and 4 decimal powers at concentrations of 0.5% and 0.7%. At a concentration of 0.1% sodium hydroxide, a reduction of between 1 and 4 decimal powers was observed. The pH measured was between 3.02 at 0.1% and 2.39 at 0.3% sodium hydroxide.

The suspension test without protein done with sodium hypochlorite and ERAV showed a reduction of 4 decimal powers at 1.2%. The suspension test with protein and ERAV showed a reduction of 1 decimal power at 1.2%. At all lower concentrations of sodium hypochlorite, no remarkable reduction could be observed in either type of suspension test.

The pH measured was between 8.14 at 0.1% and 9.15 at 1.2% of sodium hypochlorite. The wood carrier test with sodium hypochlorite and ERAV showed a reduction of 4 decimal powers at concentrations of 1.2%. Similar reductions were noted in some of the tests carried out with 1.0%. A reduction of 1 to 4 decimal powers was observed at lower concentrations. The pH measured was between 8.21 at 0.1% and 10.55 at 1.2% sodium hypochlorite.

The suspension test with and without protein done with citric acid and ΦX 174 showed a reduction of 1 decimal power or less. The pH measured was between 6.01 at 0.1% and 4.02 at 0.3% citric acid. The wood carrier test with citric acid and ΦX 174 showed a reduction of up to 4 decimal powers at 0.3%. A reduction of 1 or less decimal powers was observed at lower concentrations of citric acid. The pH measured was between 3.24 at 0.1% and 2.55 at 0.3% citric acid.

The suspension test with and without protein done with sodium hydroxide and ΦX 174 showed a reduction of 4 decimal powers at concentrations of 0.3, 0.5 and 0.7%. No remarkable reduction was observed at a disinfectant concentration of 0.1%. The pH measured was between 9.18 at 0.1% and 12.95 at 0.7% sodium hydroxide. The wood carrier test with sodium hydroxide and ΦX 174 showed a reduction of up to 6 decimal powers at 0.7%. A reduction of between 1 and 5 decimal powers was observed using lower concentrations of sodium hydroxide. The pH measured was between 12.15 at 0.1% and 12.97 at 0.3% sodium hydroxide.

The suspension test without protein done with sodium hypochlorite and ΦX 174 showed a reduction of 4 decimal powers at 1.0% and 1.2%. The suspension test with protein and ΦX 174 showed a reduction of 1 decimal power at 1.2%. No remarkable reduction was observed in either type of suspension test at lower sodium hypochlorite concentrations. The pH measured was between 6.46 at 0.1% and 8.52 at 1.2% sodium hypochlorite.

The wood carrier test with sodium hypochlorite and ΦX 174 showed a reduction of up to 1 decimal power at all concentrations. The pH measured was between 7.91 at 0.1% and 9.86 at 1.2% sodium hypochlorite.

Discussion

Both of the viruses used in this study are possible model viruses. The purpose of such model viruses in disinfection testing is to use organisms that are easier and less dangerous to deal with in the laboratory than certain pathogens would be. The model viruses should be at least as resistant to inactivation as the actual pathogens under consideration. A margin of error should, however be given, so that if the model virus is inactivated, it is certain that the pathogens in question will also be inactivated. ERAV has been shown to be similar to FMDV in its resistance to environmental factors including its sensitivity to changes in pH. It is therefore considered a good model for FMDV in inactivation studies.

The disinfectants used in this study were chosen because of their presence in literature dealing with FMD outbreaks. Citric acid can be used to test the virus reaction to lowered pH. Sodium hydroxide has been described directly for use in FMD outbreaks. Sodium hypochlorite is a commonly used disinfectant in some countries (particularly the USA) and is often suggested for disinfection in the US American literature. Both citric acid and sodium hydroxide disinfected ERAV sufficiently in the concentrations suggested in the literature that these should be considered sufficient in the case of an FMD outbreak. Sodium hypochlorite, however, was unable to disinfect ERAV sufficiently at the concentrations used in this study. It also has a very great protein error, so that sufficient disinfection under field conditions would be extremely difficult.

The phage ΦX 174 was tested here as another possible model virus for FMDV. Work with phages does not require the same amount of expensive laboratory equipment, sterile conditions and expertise that work with vertebrate viruses does. Use of these viruses would therefore save a great deal of money and time in disinfection testing. Phage ΦX 174 has been described as similar in resistance to inactivation as members of the families *Picornaviridae* and *Parvoviridae*. The results of our study show that this virus is, however, a great deal more resistant to inactivation than members of genus *Aphthovirus* of the family *Picornaviridae*. Although we did not determine the exact amount of disinfectant necessary for the inactivation of these viruses in this study, it is clear that it cannot be a sensible model for FMDV. It would, however, be interesting to examine this virus further as a possible model virus for the inactivation of more stable viruses such as members of the families *Parvoviridae* or *Circoviridae*.