Preliminary study on the efficacy and safety of eight individual and blended disinfectants against poultry and dairy indicator organisms

Zeina G. Kassaify[1], Rindala G. El Hakim[2], Edward G. Rayya[2], Houssam A. Shaib[2] & Elie K. Barbour[2, 3]

Summary

Eight individual and blended chemical disinfectants were screened for preliminary evaluation of safety, bactericidal and virucidal effectiveness against poultry and dairy organisms. The test organisms were *Escherichia coli*, *Salmonella* Enteritidis, *Staphylococcus aureus*, *Streptococcus* spp. and *Clostridium perfringens*, in addition to avian influenza virus (AIV) and Newcastle disease virus (NDV). Viable counts of surviving bacteria were determined after 30 min contact with each disinfectant and in the presence or absence of skimmed milk, to simulate the interference of organic matter. The haemagglutination test was used to assess the survival of the test viruses in the presence of the different disinfectants after propagation in 10-day-old chick embryos. In the presence of skimmed milk, a higher concentration of most of the disinfectants examined was required to exert antimicrobial effectiveness. When used individually, quaternary ammonium showed no virucidal activity against NDV and AIV; peracetic acid was not effective against *Streptococcus* spp., *S.* Enteritidis and NDV, while iodophors showed low bactericidal and inconsistent virucidal activity. The single and blended disinfectants with high microbicidal activities included phenols (high bactericidal and virucidal activity), blends of quaternary ammonium compounds (high bactericidal activity) and blends of cresols and organic acids (high virucidal activity). This suggests the use of blends of compatible compounds for disinfection operations in poultry and dairy industries since they will target a wider range of micro-organisms. None of the disinfectants had a negative effect on the development of the different organs of chicken embryos and the iodine-based disinfectant, developed for dairy-teat dipping, also showed no adverse reactions in experimental cows.

Keywords

Bactericide, Dairy, Disinfectant, Poultry, Safety, Virucide.

Studio preliminare sull'efficacia e la sicurezza di otto disinfettanti, presi singolarmente o combinati, per la bonifica di allevamenti avicoli e aziende casearie da microrganismi scelti come indicatori

Riassunto

L'efficacia e la sicurezza di otto disinfettanti chimici, usati singolarmente o miscelati, è stata testata per una valutazione preliminare della loro azione

(1) Department of Nutrition and Food Sciences, Faculty of Agricultural and Food Sciences (FAFS), American University of Beirut (AUB), P.O. Box 11-0236, Beirut, Lebanon
(2) Department of Animal Science, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon
(3) Corresponding author, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon
eb01@aub.edu.lb

battericida e virulicida contro microrganismi in aziende casearie e allevamenti avicoli. I microrganismi su cui sono stati testati sono Escherichia coli, Salmonella *Enteritidis,* Staphylococcus *aureus,* Streptococcus *spp. e* Clostridium perfringens, *oltre al virus dell'influenza aviaria (AIV) e al virus della malattia di Newcastle (NDV). La conta delle cellule batteriche vitali è stata effettuata dopo 30 minuti di contatto con ogni disinfettante ed in presenza o meno di latte scremato per simulare l'eventuale interferenza di materia organica. Per determinare la sopravvivenza dei virus testati è stato utilizzato il test di emoagglutinazione, effettuato dopo crescita in embrioni di pollo di 10 giorni, in presenza dei differenti disinfettanti. Con l'utilizzo di latte scremato è stata necessaria una maggiore concentrazione dei disinfettanti testati per stabilire l'efficacia antimicrobica. Utilizzato singolarmente l'ammonio quaternario non si dimostra un efficace virulicida nei confronti della NDV e dell'AIV; l'acido iperacetico a sua volta si è dimostrato inefficace contro* Streptococcus *spp.,* S. *Enteritidis e NDV, mentre gli iodofori mostrano una ridotta efficacia battericida e un'inconsistente capacità virulicida. I disinfettanti ad alta efficacia antimicrobica sia singolarmente sia combinati sono i fenoli (alta capacità battericida e virulicida), miscele di composti di ammonio quaternario (alta capacità battericida) e miscele di cresoli ed acidi organici (alta capacità virulicida). Da questi studi si desume che per la disinfezione in aziende casearie e allevamenti avicoli è consigliabile utilizzare miscele di composti compatibili al fine di colpire un più ampio numero di microrganismi. Nessuno dei disinfettanti testati ha avuto effetti nocivi sullo sviluppo degli organi di embrioni di pollo, ed inoltre i disinfettanti iodati, studiati nel dipping del capezzolo, non hanno mostrato reazioni negative sulle bovine.*

Parole chiave

Battericida, Caseario, Disinfettante, Pollame, Sicurezza, Virulicida.

Introduction

The modern domestic animal farm environment furnishes an appropriate medium for pathogen replication. High animal density farm operations and short downtimes contribute to high disease incidence (22). One of the most effective methods to reduce the level of pathogens includes the application of proper management and husbandry practices, such as the all-in all-out system (31) and regular cleaning and disinfection, especially before introducing a new flock or herd to the barns (9). The choice of disinfectants is critical in establishing a successful sanitation programme as not all disinfectants are effective against the major pathogens that cause economic diseases in the poultry and dairy industries (21). In addition, different families of disinfectants target specific micro-organisms (32). For instance, phenols target bacteria and viruses, quaternary ammonium, iodofor, peracetic acid, glutaraldehydes and cresols target bacteria whilst imidazole is usually used as an anti-fungal compound (11, 32). Therefore, there is not one disinfectant reported in the literature that would be efficacious against a wide spectrum of aetiological agents of economic diseases in animal farms. A rational programme based on the study of the efficacy of different disinfectants against pathogens present on the farm should be implemented (9). Moreover, special care should be taken in the application of the disinfectant as it should be safe for both animals and humans. The hardness of water, correct dilutions, contact time and the presence of organic matter are all factors that should also be taken into consideration, since they affect the efficacy and performance of the disinfectant (9, 17, 32).

Several techniques have been used to study the efficacy of disinfectants against micro-organisms (2, 22) in the presence and absence of organic matter (2, 27).

The objective of this study was to perform preliminary screening to evaluate the efficacy and safety of eight newly developed individual and blended disinfectants against different poultry and dairy indicator

organisms in the presence and absence of skimmed milk.

Materials and methods

Disinfectants

Eight newly developed disinfectants were obtained from the Oteri Company, Jal El Dib Metn in the Lebanon. The basic composition of the eight disinfectants is presented in Table I. The exact concentration was not revealed by the manufacturer.

Table I
Families of eight newly developed individual disinfectants

Disinfectant label	Family of disinfectant*
Individuals	
A	Iodofor-based
B	Phenol-based
C	Peracetic acid-based
D	Iodofor-based
Blended	
E	Phenol and quaternary ammonium compounds
F	Cresols, surfactants, and organic acids
G	Glutaraldehyde and quaternary ammonium
H	Quaternary ammonium compounds

* The chemical formulae of each disinfectant is proprietary information of the Oteri company

Indicator organisms

Indicator organisms that were used in this study to assess the efficacy of the eight disinfectants were selected based on their importance in animal and/or human diseases. The indicators were as follows: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Enteritidis, *Streptococcus* spp. and *Clostridium perfringens* (spore former). In addition, a H9N2 AIV subtype prevalent in the Middle East, Pakistan and Europe (1), and a lentogenic Newcastle disease virus (NDV) present in most poultry farms of the world, were the two viral indicator organisms used to evaluate the efficacy of the eight disinfectants.

Bacterial cultures

Bacterial isolates, namely: *E. coli*, *S. aureus*, *S.* Enteritidis, *Streptococcus* spp. were grown aerobically in tryptose phosphate broth while *C. perfringens* was grown anaerobically in thioglycollate broth to reach the exponential phase at 37°C. The count of viable organisms at this exponential phase was adjusted to 2.12×10^7 cfu/ml.

Viral propagation

The AIV and NDV were each inoculated in 10-day-old chicken embryos through the allantoic membrane route. The eggs were then incubated at 38°C at 85% humidity for 3 days. Allantoic fluids were harvested and kept at –80°C.

Evaluation of the efficacy of the disinfectants against bacterial indicators

A total of 5 ml of each bacterial culture set at 2.12×10^7 cfu/ml was placed in contact with 0.5% and 1% concentrations of each disinfectant in the presence and absence of 12.5% skimmed milk. Each test was run in duplicate and the contact time between the disinfectant and the bacterial indicator was maintained constant for 30 min at room temperature.

The viable count of the indicator bacterium was determined by the spread plate method for aerobic bacteria and the pour plate method in tryptone-sulfite-neomycin (TSN) medium for the strict anaerobe (*C. perfringens*). The media (HiMedia, Mumbai, India) used for the viable count of each bacterium were as follows: MacConkey agar (*E. coli*), Mannitol salt agar (*S. aureus*), brilliant green agar (*S.* Enteritidis) and nutrient agar (*Streptococcus* spp.). The spread plate-cultures were incubated at 37°C for 24 h and colonies were counted for the determination of percentage reduction of viable count compared to the initial count (2.12×10^7 cfu/ml) prior to contact with the disinfectant.

Evaluation of the disinfectants against viral indicators

The virus-disinfectant contact reaction was prepared in the presence and absence of 12.5% skimmed milk, following the same procedure used for the evaluation of disinfectants against bacterial indicators.

The level of viral particles propagated in allantoic fluid was 3.0×10^9 viral particles/ml. Quantitation of the viral particles is deduced from the haemagglutination (HA) titre, in which one HA titre is reported in the literature to be equivalent to 10^7 particles (29). To test the antiviral activity of the disinfectants, 0.1 ml of the reacting virus-disinfectant material was injected in duplicate through the allantoic membrane of 10 day-old chicken. The embryos were incubated for 3 days at 38°C with relative humidity at 85%. The embryos were checked for viability at the end of the incubation period. The allantoic fluid was also collected from each embryo to examine the viability of the virus and its ability to propagate to result in a detectable haemagglutinating titre, using 1% chicken red blood cells (3). Duplicate control embryos were injected with the virus without any contact with any of the eight disinfectants (positive controls); while two embryos did not receive any injection (negative controls).

Evaluation of efficacy of a newly developed disinfectant 'A' against mastitic organisms

Three test organisms were isolated from mastitic milk of dairy cows, namely: *Streptococcus* spp., *S. aureus* and *Staphylococcus* spp. Each organism was grown aerobically to the exponential phase in tryptose phosphate broth. The count of the viable organisms at this exponential phase was adjusted to 2.12×10^7 cfu/ml. Each of the three mastitic organisms was placed in contact with a 20% dilution of disinfectant 'A' (iodofor-based), as recommended by the manufacturer, in the absence and presence of skimmed milk for a period of 30 min at room temperature. The viable count of surviving bacteria was determined using the spread plate method on blood agar plates. The cultures were incubated at 37°C for 24 h and colonies were counted to determine the percentage of reduction, taking into consideration the initial count (2.12×10^7 cfu/ml) prior to contact with the disinfectant.

Safety testing

The safety of the eight individual and blended disinfectants presented in Table I was tested by delivering each disinfectant at levels of 0.5% and 1% concentration through the allantoic membrane of three 10-day-old chick embryos, in a volume of 0.1 ml. This test was intended to ensure the safety of the disinfectants should traces or remaining residues come into contact with or be ingested by animals. Hence, embryos were used for this purpose as they are the most sensitive. In the procedure, three embryos were not injected with any disinfectant (controls). The embryos were incubated for three days at 38°C at 85% relative humidity. Candling was performed on a daily basis throughout the three-day incubation period to record embryo mortalities. At the end of the third day of incubation, the embryos were observed for organ (heart, liver, membranes surrounding the embryo) development and pathological signs, growth retardation and viability.

Four milking dairy cows (Holstein) were subjected to the six-day teat dipping procedure using disinfectant 'A' at a concentration of 20%. Milk was collected from the four teats of each of the four cows just before the beginning of treatment and milk collection was repeated at the end of the treatment. The epidermis of the teat was observed for erythema and irritation before and at the end of the treatment. In addition, a somatic cell count per millilitre of the collected milk samples was performed using Newman-Lampert's stain test (15, 19). Briefly, a volume of 10 µl of each milk sample was spread and dried on a microscopic slide. Slides were then flooded with the stain and left for 2 min, followed by draining of stain, dipping in water and drying.

The smears were finally viewed under a Leica DME microscope (Leica Microsystems Inc., Buffalo, New York), the somatic cells were counted at 1 000× magnification and the count was multiplied by a factor of 3.9×10^5. This factor was implemented according to the method of Pusch *et al*. (19), enabling the calculation of the number of somatic cells/ml of milk.

Results

Efficacy of disinfectants

The results of the efficacy testing of the individual and the blended disinfectants are presented in Tables II, III and IV. Quaternary ammonium compound, namely disinfectant 'H', exhibited the highest antibacterial activity, at the concentrations recommended by the manufacturer (Oteri Company, Lebanon) in the presence of the skimmed milk (Table II). However, disinfectant 'H' did not exhibit any virucidal activity against H9N2-AIV or NDV (Table III). Blends of quaternary ammonium and glutaraldehyde (disinfectant 'G'), was the second most effective disinfectant against the five bacterial organisms (Table II), also showing anti-viral activity at different concentrations in the absence of skimmed milk (Table III). Disinfectant 'F', a synergetic blend of cresols and organic acid had less effect on bacteria in the presence of skimmed milk (Table II) but showed greater activity against NDV and H9N2 at 1% concentration, irrespective of the presence or absence of skimmed milk (Table III). The phenol-based disinfectant (disinfectant 'B') had no anti-*E. coli* or anti-*Streptococcus* spp. activity in the presence and absence of the skimmed milk, respectively, at a concentration of 0.5% (Table II). Disinfectant 'E', a blend of quaternary ammonium and phenols, displayed an inhibiting effect on some bacterial indicators in the absence of skimmed milk (Table II). At a concentration of 1%, disinfectant 'E' was effective against NDV, only in the absence of the skimmed milk, but was effective against H9N2 in most conditions (Table III), whereas, disinfectants 'C' (a peracetic compound) and D, iodosan (an iodophor), showed bactericidal effects and showed anti-AIV (H9N2) activity regardless of the concentration used, but only in the absence of skimmed milk. Finally, disinfectant 'D' had no effect against any of the bacterial indicators (Table II), but did display anti-NDV activity in the absence of skimmed milk, regardless of concentration.

Data related to the evaluation of the efficacy of disinfectant 'A' as an anti-mastitic disinfectant is shown in Table IV. This disinfectant had insignificant antimicrobial activity.

Safety testing

None of the disinfectants had a negative effect on the development of the different organs of the chicken embryos; there was no growth retardation in comparison to controls. No mortality was observed in any of the embryos that had been injected with disinfectants.

The safety of disinfectant 'A' was also observed on the epidermis of the dipped teats that showed no signs of irritation, erythema or congestion. A comparison of the mean somatic cell count (SCC)/ml of milk at the beginning of the teat dipping treatment (31 SCC×10^5/ml) versus the mean count after the treatment (11 SCC×10^5/ml), showed a reduction (p>0.05) to almost one third of the initial count.

Discussion

In this study, all disinfectants were safe and did not affect the development of chicken embryos or their viability; in addition, the iodophor-based disinfectant 'A' showed no signs of irritation, erythema or congestion on the epidermis of the dipped teats; these results were in agreement with results obtained by Bermudez and Stewart-Brown, Kitis and McDonnell and Russell (6, 14, 17) who focused on the safety of disinfectants as a paramount condition prior to use in environmental or agricultural systems. On the other hand, different families of disinfectants showed a wide range of efficacy against viruses and bacteria. Quaternary ammonium compounds, namely disinfectant 'H', showed high antibacterial efficacy which was in agreement with the works of McDonnell and Russell (17) demonstrating the complete effectiveness of quaternary ammonium compounds against Gram-negative and Gram-positive bacteria. However, these compounds showed no antiviral effectiveness in our study unlike the results obtained by the above authors; this

Table II
Percentage reduction in viable count of five indicator bacteria by eight disinfectants used at two concentration levels in the presence and absence of 12.5% skimmed milk

Disinfectant		Reduction in five indicator bacteria[a] by different levels of disinfectants in the presence or absence of skimmed milk[b] (%)									
		Presence of skimmed milk					Absence of skimmed milk				
Label	Level	EC	SA	SE	Str	CP	EC	SA	SE	Str	CP
A	0.5%	IR	IR	IR	100.00	IR	IR	99.99	IR	MO	IR
	1.0%	99.98	99.99	IR	99.99	100.00	100.00	100.00	IR	MO	100.00
B	0.5%	IR	100.00	100.00	100.00	100.00	100.00	100.00	100.00	IR	100.00
	1.0%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
C	0.5%	IR	IR	99.99	IR	100.00	99.98	IR	100.00	IR	100.00
	1.0%	99.97	IR	100.00	IR	100.00	99.98	IR	100.00	IR	100.00
D	0.5%	IR	IR	IR	MO	IR	IR	IR	IR	IR	IR
	1.0%	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
E	0.5%	IR	IR	IR	IR	100.00	IR	100.00	100.00	100.00	100.00
	1.0%	100.00	100.00	IR	100.00	100.00	100.00	100.00	100.00	100.00	100.00
F	0.5%	99.99	99.99	99.99	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	1.0%	99.99	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
G	0.5%	99.97	100.00	IR	100.00	100.00	99.99	100.00	100.00	100.00	100.00
	1.0%	99.99	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
H	0.5%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	1.0%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The initial viable count of each indicator bacterium before the contact with disinfectants was 2.12 x 10^7 cfu/ml

(a) indicator bacteria were as follows:
EC *Escherichia coli*
SA *Staphylococcus aureus*
SE *Salmonella* Enteritidis
Str *Streptococcus* spp.
CP *Clostridium perfringens*

(b) skimmed milk when present will be at a level of 12.5%

IR insignificant reduction corresponds to a count of >300 cfu of each bacterium/100 µl of reacting mixture

MO missed observation

A iodofor-based
B phenol-based
C peracetic acid-based
D iodofor- based
E blend of phenol and quaternary ammonium
F blend of cresols, surfactants and organic acids
G blend of glutaraldehyde and quaternary ammonium
H blend of quaternary ammonium

could be due to the nature of viral indicators used in this study. However, the addition of glutaraldehyde to quaternary ammonium in disinfectant 'G' resulted in both high antibacterial and antiviral effectiveness that was probably due to the additive antiviral effect of glutaraldehyde, which is known to be very effective against both the encapsulated and naked viruses (5, 18, 23).

On the other hand, the use of quaternary ammonium compounds in combination with phenols (disinfectant 'E') enhanced the virucidal effect in association with bactericidal effectiveness at higher concentrations. This could be due to the known additional bactericidal effect of quaternary ammonium compounds and the virucidal and bacterial effect of phenols documented in the works of Dwyer, McDonnell and Russell and Rodgers *et al.* (10, 17, 21). These workers demonstrated that phenol exerts both bactericidal effects through membrane degradation and progressive leakage of intracellular constituents, and virucidal effects through degradation of the capsid.

Table III
Virucidal effect of eight disinfectants used at two different concentrations against avian influenza virus (H9N2) and Newcastle disease virus in the presence and absence of 12.5% skimmed milk

Disinfectant	Label level	Virucidal effect of disinfectants			
		Presence of skimmed milk		Absence of skimmed milk	
		Newcastle disease virus	Avian influenza virus H9N2	Newcastle disease virus	Avian influenza virus H9N2
A	0.5%	NE	EF	NE	EF
	1.0%	NE	EF	NE	EF
B	0.5%	EF	NE	EF	EF
	1.0%	EF	EF	EF	EF
C	0.5%	NE	NE	NE	EF
	1.0%	NE	NE	NE	EF
D	0.5%	NE	NE	EF	NE
	1.0%	NE	NE	EF	NE
E	0.5%	NE	EF	NE	EF
	1.0%	EF	EF	EF	EF
F	0.5%	NE	EF	NE	EF
	1.0%	EF	EF	EF	EF
G	0.5%	NE	NE	EF	EF
	1.0%	NE	EF	EF	EF
H	0.5%	NE	NE	NE	NE
	1.0%	NE	NE	NE	NE

A iodofor-based
B phenol-based
C peracetic aid-based
D iodofor-based
E phenol and quaternary ammonium blend
F cresols, surfactants and organic acids blend
G glutaraldehyde and quaternary ammonium blend
H quaternary ammonium blend

NE not effective, with confirmation of presence of replicating virus in allantoic fluid of chicken embryos
EF effective, with complete absence of replicating virus in allantoic fluid of chicken embryos

The peracetic acid compound (PAA), present in disinfectant 'C', had only a virucidal effect against H9N2 at a high concentration, probably through its oxidising effect, as cited by Swayne and Halvorson (26), who stated that AIV are susceptible to oxidising agents and dilute acids especially after the removal of skimmed milk. However, no bactericidal or NDV-virucidal effect was exerted by this compound although many have emphasised the use of peracetic acid as a highly effective disinfectant against bacteria (11) and viruses (17).

The cresol and organic acid blends in disinfectant 'F' showed a high bactericidal effect (Tables III and IV). Both compounds are known to have a wide spectrum of bactericidal effectiveness, as stated by Varley and Reddish (28), proving that a low concentration of cresol disinfectant had a high anti-bacterial effect, while Russell, Chaveerach *et al*., Harris *et al*. and Russell *et al*. (8, 12, 24) emphasised the

Table IV
Percentage reduction of different mastitis-causing organisms by disinfectant 'A' (iodofor-based) in the presence and absence of 12.5% skimmed milk

Mastitis-causing organisms	Presence of skimmed milk	Reduction in bacterium (%)
Streptococcus spp.	Yes	IR
	No	IR
Staphylococcus aureus	Yes	IR
	No	IR
Staphylococcus spp.	Yes	IR
	No	IR

IR insignificant reduction corresponds to a count of >300 cfu of each bacterium/100 µl of reacting mixture

effectiveness of organic acids against Gram-negative and Gram-positive bacteria, probably through the loss of the outer membranes observed in organic acid-treated bacterial populations present in water and feed. The virucidal effectiveness could have been exerted by cresols as deduced by Saknimit *et al*. and Watanabe *et al*. (25, 30), who found that 1% saponated cresol was able to inactivate laboratory animal RNA-viruses, especially the enveloped ones.

Iodophors of disinfectants 'A' and 'D' showed an inconsistent and low anti-microbial effect although many have reported the high anti-Gram-positive bacterial effect of iodine (7, 13), high anti-Gram-negative bacterial effect (16) and high virucidal effect (6, 17), especially against myxoviruses, namely NDV and AIV (4). The anti-microbial effect of iodophors is mainly due to the high affinity of iodine to membrane lipids. This disagreement between our work and that of others could be due to a lack of surfactants in disinfectants 'A' and 'D' needed to form iodine-surfactant complexes that enhance the anti-microbial activity of iodophors (6).

The difficulty in comparing results of different laboratories is mainly due to a lack of harmonisation between the few countries that possess official methods to test disinfectants (20). Furthermore, the presence of organic matter in the contact medium between disinfectants and organisms usually has a negative impact on the disinfectant, thus affecting the levels of efficacy reported (27, 32). Moreover, most disinfectants require higher concentrations when used in the presence of organic matter which limits their application to clean surfaces after the removal of excessive organic matter (22).

Conclusion

No single developed compound, except for phenols, showed high bactericidal and virucidal activity, while the addition of either phenol or glutaraldehyde to quaternary ammonium showed additional virucidal activity against H9N2 and NDV. Based on the above results, the use of a blend of compatible compounds will be more effective, since it will target a wider range of micro-organisms, taking into consideration the compatibility of ingredients used and the nature of the targeted microbial species (22). Most of the data on the different disinfectant families agree with that reported in literature. The disagreement between data from different regions of the world highlights the need for the animal industry to be aware of this fact, as this is an indication that results obtained from one region may not be applicable to all countries. Studies on the efficacy and safety of disinfectants developed against prevalent pathogens on farms of countries and regions should be considered on an individual basis. Future research should target the comparison of the most effective compounds revealed in this preliminary screening to the classic disinfectants used in the region, using standard evaluation methods.

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