

Disinfection in poultry medicine – aims and means¹

E. F. Kaleta, Giessen

Introduction

Veterinary activity is focused simultaneously on the animal itself and the suitable environment. Animal care includes detailed diagnostics, prophylactic immunization and, in some cases at least, effective treatment. In the environment, practically all plants, animals and humans are colonized by bacteria, fungi, viruses, prions but also by single cell coccidia and multicellular parasites. As a logical consequence, a permanent fight exists to control such agents and their debilitating effects on animal health, welfare and productivity. Effective measures are essential to combat these pathogenic microorganisms with the target to reduce or even to eliminate their deleterious effects. Such measures are applied on non-living objects and generally summarized under the term “disinfection”.

Highly desired effects of disinfection are (i) all pathogenic microorganisms, all oocysts of coccidia, all eggs of internal and external parasites are totally destroyed in all areas, (ii) re-introduction of pathogens is permanently prevented, (iii) losses due to transmissible diseases are minimized, (iv) profitability of animal farming is enhanced and (v) a positive cost-benefit balance is assured.

Proper disinfection is usually associated with undesired effects. These effects may be (i) irritation of skin and eyes of workers, (ii) slippery surfaces may cause accidents, (iii) persistence of disinfectants in rooms and on surfaces, (iv) some disinfectants discolour painted walls, (v) spill of disinfectant into the environment, open waters, creeks etc., (vi) toxicity for fish, crustaceans, arthropods, plants etc., (vii) damage to electrical wires, to engines, equipment and (viii) corrosion on metal surfaces.

Various definitions have been given to characterize “disinfection” and to discriminate it from related or similar measures. The following delineates some selected definitions.

Disinfection is a

- method applied to prevent transmission of disease causing agents (Schließer, 1981)
- procedure used for inactivation of certain microorganisms (Böhm & Straub, 2002)
- abolition (abrogation) of disease causing agents (Duden, 2006)
- method that destroys infection-producing agents (Blood *et al.*, 2007)

It is clear from these four selected definitions that the prevailing target is the elimination or at least reduction of such agents that (may) cause disease. It is never attempted to obtain an environment that is devoid of virtually all microorganisms. Also, the aim is prevention of transmission and infection by destroying the vital properties of pathogenic organisms.

The term “disinfection” requires demarcation from other more or less related technical means and procedures.

Some examples are:

- cleansing** means mechanical removal of dirt, manure, dust, vermin etc.
- sterilization** is the application of dry heat for several hours on solid materials
- filtration** means separation of organic matter in a liquid phase by membrane or asbestos filters of variable pore size
- pasteurization** includes repeated cycles of heating and cooling of liquid matter for variable times and temperatures
- antiseptics** is the application of germicidal substances on and in animals

¹ Based on a presentation at the annual meeting of the Poultry Veterinary Study Group in Vilnius, Lithuania, on May 27, 2013

On the history of disinfection

Some historical remarks should be added at this point on means to correct environmental pollution of air and surfaces and on preservation of human food. Elevated but appropriate room temperature for hatching chicks was achieved in old Egypt in so-called Pharaoh hatcheries by burning of plant material. The developing smoke contains phenolic compounds that condense at surfaces in the hatchery compartments and exert their disinfecting effects. Another example: The common plant thyme (*Thymus vulgaris*) is frequently used in herbal medicine. It contains high concentrations of thymol which is chemically o-isopropyl-m-cresol, a substance with a high disinfecting property. Thirdly, even in contemporary times smoke and smell are generated by smouldering of the resin derived from the tree *Boswellia serrata* which contains (among many others) a high concentration of phenolic substances and terebinthene which have disinfecting properties and also emit a pleasant smell. Frankincense is commonly used in religious ceremonies e.g. in catholic churches - without realizing the real disinfecting effects of this practice.

I mention these practices to illustrate different germ reduction strategies which have been used for centuries without realizing the basic modes of chemical reaction and without realizing and using the contemporary term “disinfection” which means the opposite of “infection”.

In the past and in some areas even today, various means are practised to maintain and to preserve edible food for human consumption. Such measures include reduction of the water content of food by drying and salting or by adding sugar or keeping food on ice.

Another example of traditional “disinfection” is the rather common use of sunshine to dry and bleach cloths etc. on green meadows. Housewives interpret this as an effective measure to turn the greyish appearance of cotton to splendid white and to obtain dry and nice smelling cloths. Actually, the most important effect of “drying” in sunshine is directly associated to inactivation of the infectivity of microorganisms. It is well known that UV-light destroys the genome of pathogenic microorganisms within a short time, and drying and bleaching are just welcome additional side effects.

Historically, the most important prerequisite of disinfection was the development of techniques for isolation and identification of microorganisms, especially bacteria and viruses. Pioneers in this field were Louis Pasteur (1822-1895) in France (*Pasteurella* spp.), Robert Koch (1843-1910) in Germany (*Mycobacterium tuberculosis*), Theodor Escherich (1857-1911) in Austria (*Escherichia* spp.), Georg Theodor August Gaffky (1850-1918) in Germany (*Salmonella* spp.) and Friedrich Loeffler (1852-1915) in Germany (*Foot and mouth disease virus*). These and many other outstanding scientists paved the way to microbiology and fundamentals of the theory of infectious diseases.

Equally important for the promotion of microbiology and disinfection were chemists who isolated and identified chemicals and developed techniques for their synthesis which might be useful as disinfectants. Outstanding in this respect is August Wilhelm Hofmann (1818-1892) who discovered in 1867 (among many other inventions) the structure of formaldehyde and developed the synthesis of this chemical.

Initial studies on inactivation of microorganisms by available chemicals were undertaken some hundred years ago. Robert Koch was the first to test phenol for the inactivation of spores of *Bacillus anthracis* and published his results in 1884. He contaminated threads of silk by sporulated bacteria that cause anthrax, dipped these threads into a phenol solution and tried to re-isolate these bacteria after several time intervals. More recently, the German Society for Hygiene and Microbiology produced the first guideline in 1958 which contains already suspension and carrier tests and selected bacteria and fungi as test organisms.

Currently, the fourth edition of the guidelines provided by the German Veterinary Society (DVG) forms the basis for any testing of disinfectants in Germany. Besides suspension and carrier tests, a reference disinfectant and a reference test virus are included. All disinfectants must be examined by at least two independent persons who were approved by the board of the DVG. On an international level, various organisations are involved in the development of testing procedures for the evaluation of disinfectants.

In the following, past and current procedures for testing and evaluation of chemicals for disinfection in the veterinary field are reviewed. Various governmental and non-governmental organisations

developed guidelines for this purpose. Internationally operating organisations include OECD, FAO, EFSA, FDA, national organisations include the British Standards Institute (BSI); in Germany the Robert-Koch-Institute (RKI), the Veterinary Society (DVG), the Society for Applied Hygiene (VAH) and the German Agricultural Society (DLG). National organisations for testing and evaluation of chemical disinfectants exist also in The Netherlands, France and Italy, to name only a few countries. All these organisations developed procedures that are similar in testing principles, but the details of methodology, the agents used and the evaluation and subsequent recommendations differ quite markedly. Since 1989 the European Committee for Standardization (CEN) is working on harmonization of test procedures and their efficient evaluation of results.

The results obtained on the basis of a firmly established testing protocol are of paramount importance for the producer, for the testing establishment and finally also for the user of a disinfectant. Producers wish to obtain (i) reliable and reproducible test results for efficacy and safety reasons, (ii) a low working concentration of a disinfectant because low working concentrations have a strong bearing on the market price and (iii) results that are acceptable in almost all countries in order to expand their international market without re-testing of their products in various countries. The users prefer a broad spectrum of efficacy at low cost. The testing laboratories need a testing procedure which (i) yields results that must be reproduced in their own and in other laboratories, (ii) is efficient and not too elaborative and (iii) can be obtained within acceptable times.

The sub-committee for disinfection of the German Veterinary Society (DVG)

Around 1970 Theodor Schließer in Giessen promoted the development of guidelines for testing of chemical compounds as disinfectants for virucidal, bactericidal, levoricidal and antiparasitic efficacy. The initial testing protocols were further refined during the following decades. Currently, the fourth edition (2004) of the guidelines for testing and evaluation of chemical compounds for application in the fields of veterinary medicine and food production are in use. Both guidelines require suspension and carrier tests and contain lists of reference bacteria, viruses, fungi and parasites. The results of testing that were generated by two laboratories are evaluated by the sub-committee and subsequently published at regular intervals in the *Deutsches Tierärzteblatt* and are accessible on the internet. Two separate lists are currently produced. One list contains data on disinfectants intended for use in the field of animal production; the second list aims at use in food hygiene. Both lists contain all essential background information (concentration, time, temperature) and the proven efficacy against viruses, bacteria, fungi and parasites.

The European Committee for Standardization (CEN)

This Committee started its work in 1989 during the first meeting in the building of the British Standards Institution (BSI) in London, UK. The committee is composed of a maximum of three delegates per European country. In Germany, the delegates are selected and approved by the German Institute for Standardization (DIN) in Berlin.

The CEN has the following structure:

Technical Committee TC 216 – evaluates content and form of all drafts for standards and makes final decisions. The TC 216 inaugurates and installs three working groups:

Working group 1 is responsible for drafts on standardization in human medicine

Working group 2 is responsible for drafts on standardization in veterinary medicine

Working group 3 is responsible for drafts on norms in areas of food, drinks, cosmetics, hospitals, kitchens etc.

The Technical Committee 216 may create Special Task Groups for defined topics and workloads such as “surface test task group”, “sporocidal test task group” or “virucidal task group”. Such groups have to perform specified experiments and to report on the results to the Technical Committee 216 within a fixed deadline.

General duties of the CEN are to further improve guidelines that contain detailed prescriptions for test organisms (including proven purity, identity and infectivity titres), necessary diluents for test

organisms, materials, size and surface structure of germ carriers (e.g. poplar or linden wood, steel, glass, plastics etc.).

Even now a large number of questions on the testing procedure are still open to debate. In order to answer at least some of the very pressing questions, members of the Committee are asked to perform specified experiments “at home” and report the obtained results to all members of the Committee prior to each session, to comment on the results in writing and answer questions during the sessions. It appears that all members consider this procedure as essential to obtain further progress.

The content and structure of the testing protocol is also important. Required are data on the method used, the microorganisms, the organism and disinfectant used as references.

So far, the CEN produced a large number of preliminary reports. The circulated drafts are termed “preliminary European Norm, preEN” and an approved final text represents a European Norm (EN). Quite a number of such ENs exist already and are in full use in Europe. Comprehensive texts of all approved EN are available on the internet.

At any time in the future the work on standardization of testing of disinfectants and evaluation of procedures for disinfection will be completed and all results are then published. At this future stage further work is planned for harmonization of aims and means in cooperation with non-European countries (e.g. USA, Japan, India) and internationally operating organisations such as OECD, FAO, WHO.

Areas that may need disinfection

Practical experience and solid scientific evidence prove the existence of highly different microbial populations at different locations. In the veterinary field, such locations require different disinfectants and carefully selected modes of application.

Such locations may be:

- Stables, including roads and gateways
- Liquid and solid manure
- Hatching eggs, hatcheries, chick trucks
- Slaughterhouse, transport vehicles
- Milk production: teats, tubes, vessels
- Breweries: in process applications
- Hands, towels, offices, door locks, ...
- Oil production and conservation
- Machineries lubricating oils, cooling devices, ...

These and most likely other “locations” require correct disinfection in specified situations. The selection of the most appropriate chemical disinfectant for any contaminated location leads to the question of prevalence of specific pathogenic microorganisms and available chemical compounds.

Microorganisms as test models

Two conditions must be considered for the use of a microorganism for testing purposes. These are the multiplication of an organism to high concentrations (titres) under laboratory conditions and the relative resistance to chemical inactivation which is known from the results of previous experiments. After long debates during committee meetings of the DVG, DIN and CEN the following mandatory test organisms were approved and published in several EN for quantitative suspension and carrier tests:

Bacteria: *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Enterococcus hirae*, *Staphylococcus aureus*

Mycobacteria: *Mycobacterium avium*

Levuricidy: *Candida albicans*, spores of *Aspergillus niger*

Viruses: Newcastle disease virus, vaccinia virus, reovirus, bovine enterovirus type 1, others if desired

Parasites: *Ascaris suum* eggs, *Eimeria tenella* oocysts

In addition to these approved test organisms additional agents of interest may be tested on an optional basis. More recently, these are avian influenza A viruses (subtypes H5N1, H1N1, H6Nx H9Nx) West Nile virus, calicivirus, strain Norwalk. For these and any other optional viruses the CEN-approved testing procedure must be followed. The general pathway consists of the following phases and steps:

- Phase 1: laboratory testing of chemicals for antimicrobial efficacy
- Phase 2, step 1: quantitative suspension test
- Phase 2, step 2: quantitative carrier test
- Phase 2, step 3: quantitative surface test under field conditions

Basic tests on chemical disinfectants

All active substances intended for use in disinfectants must be listed in Annex I or IA to Directive 98/8/EC. The commercial product is classified as product type 3 (Veterinary hygiene biocidal products).

For conformity, the following information and material is required:

- Chemical composition of the disinfectant, identity and quantity of the active substance, minimum purity of the active substance, absence of impurities.
- Physical and chemical properties (e.g. pH at various concentrations in water of standardized hardness; solubility in water of standardized hardness)
- Freedom of contamination by infectious agents and other cell type of primary or permanent cell cultures. Permanent cell lines can be obtained from the American Cell Culture Collection, Manassas, Virginia, USA, the Cell Culture Bank, FLI, Insel Riems or from well-known virological laboratories. Primary cell cultures are produced from SPF chicken eggs, VALO, Cuxhaven.
- Pure and well characterized microorganisms (viruses, fungi and bacteria) are maintained and distributed by National Reference Laboratories, the American Type Culture Collection, and in Germany by the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig. Additional sources are published by the Deutsche Veterinärmedizinische Gesellschaft e. V., Giessen in the 4th edition of the "Richtlinien für die Prüfung chemischer Desinfektionsmittel".
- There is no formal source for ascaride eggs. These eggs are collected from the intestine of pigs at slaughter. Oocysts of *Eimeria tenella*, strain Houghton, are maintained and distributed on formal request by the Institute for Animal Health, Compton Laboratories, UK.
- Toxicological profile for man and animals including metabolism (e.g. determined cytotoxicity in approved cell cultures)
- Effectiveness against target organisms (e.g. minimal inhibitory concentration of test microorganisms)
- Ecotoxicological profile including environmental fate and behaviour
- Measures necessary to protect man, animals and the environment

If these conditions are fulfilled, a more specific question arises which refers to the intended uses of disinfection. Under field conditions, the following groups of pathogens are of major interest:

- Bacteria including *Chlamydia* spp. and *Mycoplasma* spp.
- Viruses (and possibly prions)
- Protozoa: coccidial oocysts, flagellates (e.g. *Histomonas* spp., *Trichomonas* spp.)
- Eggs of round and tape worms

The inherent biological properties of these organisms vary extremely from highly sensitive to highly resistant. Therefore, the disinfectant must be tailored according to the predominant and mostly prevalent target microorganisms. In other words, a meaningful selection of an available disinfectant is necessary.

Chemicals useful as disinfectants

Surprisingly, all currently used chemical disinfectants are "old" compounds and their chemical actions are known for many decades. These chemicals include:

- Aldehydes – formaldehyde, glutaraldehyde, glyoxal
- Aliphatic alcohols – ethanol, propanol, isopropanol

- Aromatic alcohols – phenols including alkyl-, aryl- and halogen derivatives
- Organic acids – formic, acetic, propionic, citric acid
- Hydrogenperoxide – H₂O₂
- Guanide – biguanide
- Iodophors – alcoholic solution of potassium iodide
- polyvinylpyrrolidone solutions of iodine
- Chlor – organic and inorganic compounds
- Alkalines – NaOH, CaO, Ca(OH)₂, bleach
- Peroxyacetic acid (plus alcapur)
- Quaternary ammonium compounds

Some of these chemicals are now considered as obsolete; some others are applied under limited conditions. Beside a reliable mode of fast action, presently more impetus is given to the fate of a disinfectant after its application. Desirable are (i) rapid degradation without residues in the area of application, (ii) low level of toxicity to farmed animals and men, (iii) easy to handle during application, (iv) effective across a wide range of low and high environmental temperatures and (v) low price.

In recent years organic acids and especially peroxyacetic acid (plus alcapur) apparently gained more interest and widespread use under farm conditions. Various phenol derivatives maintain a strong position as antiparasites. Almost all commercially available products contain not only an active ingredient, but also additives to enhance dispersion on surfaces (surface-active compounds).

Formalin as a traditional disinfectant

Formaldehyde is the gaseous form and its solution in water is named formalin. Formalin is on the market either at a concentration of 35 - 37% or as crude formalin at various concentrations. Since the discovery of formaldehyde by A. W. Hofmann in 1867, this compound was recognized as a disinfectant of superior value. The aqueous solution of formaldehyde was initially used to prevent fouling of animal skins prior to tanning. It was soon realized that repeated formalin exposure of the skin of tanners resulted in hypersensitivity, inflammation and pruritus. Since these early observations, formalin is considered as a potent allergenic compound. It does cause epithelial hypertrophy in the respiratory tract of rats that were experimentally exposed to high concentrations for prolonged times. More recently rumours spread that formalin may cause also cancer in experimental animals. Definite proof for this assumption is still lacking. However, the Institute for Risk Assessment in Berlin argues that a “certain risk for cancer development cannot be excluded.”

Despite this debate with pros and cons, formalin experienced a world-wide use as topical disinfectant especially for the disinfection of shells of hatching eggs. Rather recently, formalin was replaced by other compounds in hatcheries for safety reasons. It seems to be appropriate to list some of the major facts on formalin:

- 1867: first synthesis by August Wilhelm Hofmann in Giessen, Germany
- Technical synthesis today in Germany: approx. 500,000 metric tons per year by catalysis: CO₂ + H₂O = HCHO
- Automobiles in Germany emit ca. 35,000 tons CO₂ per year (2004 statistics)
- Naturally present in plants, cigarette smoke and exhaust of gasoline and diesel engines
- Overwhelming amount is used for the production of plastics, waxes, glues, insulation foams for buildings, panel wood etc.
- Small fraction of the total production is used in medicine, anatomy, histology, pathology, production of inactivated vaccines, cosmetics, deodorants, creams ...
- Polymerisation of formaldehyde to paraformaldehyde, so-called „dry alcohol“ is used by campers
- Maximal concentration at working places is fixed by the German Institute for Risk Assessment, Berlin, at 0.6 mg/m³, equal to 1 ml/m³, equal to 0.5 ppm. Experienced people cannot smell this level

To complete the list of known facts on advantages and disadvantages of formalin, the following should be kept in mind:

Advantages of using formalin as a disinfectant are the following facts:

- Effective at rather broad range of temperatures
- Effective against bacteria, fungi, yeasts, some viruses
- Effective in liquid and gaseous forms
- Effective at pH 4.0 to 9.0
- Penetrates porous surfaces, e.g. egg shells
- Oxydation to formic acid: $2 \text{HCHO} + \text{O}_2 = 2 \text{HCOOH}$
- Production is cheap, favourable cost-effect ratio

Major **disadvantages** of formalin are:

- Not listed in Annex I and IA of the Biocide Directive 98/8/EC. Legal use in the fields of medicine and animal production is not permitted anymore or requires special permit
- Causes irritation of conjunctiva and respiratory tract of humans and animals
- Causes allergic contact dermatitis after prolonged exposure
- Evaporates for long times from treated wood, panel wood, furniture etc.
- Hardens plastics – electrical cables etc.
- Polymerisation is prevented by methanol which is highly toxic
- Is inactivated by proteins
- Assumed to cause nasopharynx carcinoma after long-time exposure of rats

These and possibly more advantages and drawbacks may exist in various fields of technical and biological applications. In any case, it is worthwhile to look for promising alternatives to formalin as chemical disinfectants.

Detection of formaldehyde by its smell

Some people maintain that they are able to recognize the rather characteristic smell (odour) of formaldehyde even at extremely low levels, e.g. evaporating from newly acquired furniture made of wood panel. These people consider formaldehyde as a dangerous product to their health and wellbeing. In an attempt to confirm the ability to detect this gas, I asked my veterinary students to determine the lowest concentration which they are able to detect by smelling briefly opened petri dishes. For this purpose dilutions of formalin were produced in distilled water in the range of 10^{-2} to 10^{-5} and two millilitres of each of the dilutions were placed on filter paper in closed petri dishes. A separate petri dish was offered to the students that contained two millilitres of distilled water. Beginning with the highest dilution of formalin, the students were asked to write a protocol on their ability to detect the smell of formaldehyde in each of the petri dishes. This experiment – performed on a voluntary basis – was done with veterinary students of the fifth and seventh semester. The results are presented in the following Tables 1 and 2.

Table 1: Recognition of formaldehyde (FA) by 44 veterinary students, 5th semester. MAC $0.6\text{mg}/\text{m}^3$ (= $1 \text{ ml}/\text{m}^3$ = 0.5 ppm). N. t. = not tested

Dilution in log10	FA concentration		FA recognition	
	%	ppm	Yes	No
0	37.0	370 000	N. t.	N. t.
1	3.7	37 000	44	0
2	0.37	3 700	44	0
3	0.037	370	27	17
4	0.0037	37	29	14
5	0.00037	3.7	23	21
Control ¹	0.0	0.0	27	17

¹ – distilled water

In medicine, anatomy, histology and pathology, phosphate buffered formalin is generally used in concentration between 9% and 10%. All students detected in correct manner formaldehyde at concentrations of 3.7 and 0.37%. However, lower concentrations were not recognized by all students. The maximum working concentration (MAC) of formaldehyde is in Germany fixed at 0.5 ppm. Obviously, approximately half of the students detected this low level by smelling. Some of them believed that they smelled the odour of formaldehyde even in distilled water. Thus, the errors of correct detection increased at low levels.

The same arrangement of the test was repeated with students of the seventh semester. The results are quite similar to the first test. Again, high concentrations were recognized in a correct manner. At low concentrations the number of students increased that came up with obviously wrong results. A few students (3 of 27) recognized formaldehyde at a concentration of 3.7 ppm. In contrast to the first test, all students interpreted the control sample that consisted of distilled water in a correct manner. In conclusion, formaldehyde at levels well above to the MAC value can be detected only by a few students but the rates of failure are high and therefore unreliable.

Table 2: Recognition of formaldehyde (FA) by 27 veterinary students, 7th semester. MAC = 0.6mg/m³ (= 1 ml/m³ = 0.5 ppm). N. t. = not tested

Dilution in log10	FA concentration		FA recognition	
	%	ppm	Yes	No
0	37.0	370 000	N. t.	N. t.
1	3.7	37 000	27	0
2	0.37	3 700	26	1
3	0.037	370	20	7
4	0.0037	37	8	19
5	0.00037	3.7	3	24
Control ¹	0.0	0.0	0	27

¹ – distilled water

Alternative approaches for the inactivation or elimination of pathogens

Ironically, nothing – including pathogens – live for ever. This common saying applies also for pathogens. An inactive approach to get rid of pathogens according to the advice “just wait, time helps” may be effective under certain circumstances. However, under modern life style conditions active actions are generally preferred. Almost no commercial, but mainly hobby-type activities were and are practised in some selected alternative areas for the inactivation or elimination of pathogens. These may include the following:

Biologically:

- composting of carcasses
- production of biogas
- addition of harmless bacteria to decaying plants and carcasses
- addition of cultivated earth worms (*Eisenia foetida* or *Lumbricus terrestris*) or fungi

Physically:

- enhanced heat generation during composting
- Heat plus elevated pressure (autoclave)
- Sunshine or UV light
- Gamma radiation

Chemically:

- Oxidation by exposure to open air
- Denaturation by addition of lime stone or calcium hydroxide

These measures have their own merits and under certain circumstances and meaningful use in both, under extensive poultry production in developed and in developing countries with small scale productivity. At least some of these germ reduction strategies will still be applied in the future. Major applications of disinfection are established following approved testing of efficacy and likely side-effects. Such testing is performed in suspension and germ carrier tests. This is demonstrated by the methodology provided by the German Veterinary Society.

Quantitative virucidal suspension test

The quantitative virucidal suspension test includes

- Four test viruses: cytopathogenic Newcastle disease virus (NDV), vaccinia virus, strain Elstrey, human reovirus type 1, enteric cytopathogenic bovine orphan virus (ECBOV)
- Several temperatures, at least recommended 4 and 10°C
- With protein (40% foetal calve serum, FCS) and without protein load
- Several concentrations of disinfectant under test
- Quantitative determination of residual virus by titration
- Minimum requirement to pass: at least 4 log₁₀ reduction as compared to control

Each assay has to be performed in duplicates and the final report must contain details of all methods and a recommendation for practical use. The quantitative virucidal suspension test is considered as an initial test to provide orientation of the efficacy of the tested disinfectant. A quantitative carrier test must follow.

Quantitative virucidal carrier test

The major aim of the quantitative virucidal carrier test is to confirm or to disprove the results of the suspension test. It consists of the following components:

- Four test viruses: cytopathogenic Newcastle disease virus (NDV), Vaccinia virus, strain Elstrey, human reovirus type 1, enteric cytopathogenic bovine orphan virus (ECBOV)
- Carrier: mandatory is poplar wood, optional are linden wood, steel, concrete, others
- Mandatory are temperatures of 4 and 10°C, optional are several other and room temperatures
- Required is a protein load of 40% fetal calve serum (FCS)
- Several concentrations of disinfectant under test
- Quantitative determination of residual virus by titration
- Minimum requirement to pass: at least 4 log₁₀ reduction as compared to control

Number of listed disinfectants per indication in 13th list of DVG, 2011

The sub-committee of the DVG on disinfection in the veterinary field, division animal farming, approved in 2011 a total of 103 commercial products (Table 3). According to the intension of the producers, these disinfectants were not examined for efficacy against all test organisms. Most of the commercial disinfectants (84 products, 81.6%) were examined for virucidal efficacy. Second and third rank bactericidal and fungicidal efficacy. Rather few products were examined for their tuberculocidal and antiparasitic efficacy.

The active compounds of disinfectants intended for use in the veterinary field (animal production) are contained in Table 4. The peroxyacetic acid and combinations of this acid with other organic acids is the most frequently listed compound for the field of animal farming. Second rank aldehydes (various combinations that were not specified in the 13th list) either as mono preparation or in combination with quaternary ammonium compounds (QUAC) and alcohols. Various aldehydes – not specified in the 13th DVG list – represent also a major group of disinfecting compounds (Table 4). Aromatic circular hydrocarbons such as derivates of phenol and cresol find their prevailing applications for disinfection of parasites (eggs of round- and tapeworms and oocysts of Eimeridae). The organic acids, formic and acetic acid in particular, are now more frequently listed as compared to previous lists. Disinfectants

Table 3: Number of commercially available chemical disinfectants for use in the veterinary field according to targets of their intended use (13th DVG-list, published in 2011)

Targets for efficacy testing	No. of commercial disinfectants	% of total 103
Virus	84	81.6
Bacterium	79	76.7
Fungus	78	75.7
Tubercle	15	14.6
Parasite	13	12.6

Table 4: Active compounds in commercial disinfectants for use in the field of animal production according to the 13th DVG-list, published in 2011

Active compounds in commercial disinfectants	Total number disinfectants in the 13 th list (2011)	% of total 103 commercial disinfectants
Peroxy compounds	7	6.8
Peroxyacetic acid	16	15.5
Peroxyacetic acid + organic acids	7	6.8
Peroxyacetic acid + quaternary ammonium compounds	4	3.9
Peracetic acid + H ₂ O ₂	3	2.9
Aldehydes	17	16.6
Aldehydes + quatern. comp.*	19	18.4
Aldehydes + alcohol	1	1.0
Glutaraldehyde + quatern. amm. comp.	5	4.9
Glutaraldehyde + formalin	1	1.0
Organic acids	4	3.9
Chloramin T	2	1.9
Phenols and cresol derivates	15	14.6
Others	2	1.9

* - quaternary ammonium compounds

that contain glutaraldehyde or chloramine T are currently listed at rather low rates. Phenols and cresols are of major relevance for their antiparasitic effects and only a few producers of these compounds are listed.

Peroxyacetic acid (PAA) in higher concentrations is explosive, inflammable and corrosive on surfaces of metal. The explosiveness and corrosiveness is now prevented by alkalisation with alkali phosphates and subsequent shift of the pH to ≥ 8 . PAA exerts a broad spectrum of efficacy against bacteria, fungi and viruses. Its action is not inhibited by low temperatures and the presence of proteins (with the exception of blood). Other peroxide substances such as performic, perpropionic and perphthalic acids found so far no applications in the veterinary field. PAA alone or in combination with either hydrogen peroxide or organic acids (mainly formic and acetic acid) have a strong oxidizing power. It seems that PAA and combinations with other active compounds gained market shares compared to former years.

Both compounds are also highly germicidal against almost all pathogenic agents.

Obviously, the listed active disinfecting compounds differ markedly according to their intended fields of application in the veterinary fields as compared to food hygiene. The chemical compounds used and listed for use in the fields of food hygiene, production and processing of food are shown in Table 5. Almost half of the listed disinfectants contain quaternary ammonium compounds (QUAC) as active ingredients. This group of chemicals are soluble in water and contain positively-charged hydrophilic radicals. Distinct antimicrobial activity of QUACs is evident even at low concentrations. Most of the gram-positive bacteria are inactivated at concentrations of 50 - 100 mg per millilitre whereas gram-negative bacteria need more than 200 mg/ml. QUACs are not effective against *Mycobacteria* spp. and spores of bacteria. Their action is partially inhibited by the presence of proteins and iron. Hard water reduces the efficacy. QUACs are frequently used in food hygiene due to its easy modes of application and absence of negative effects on surfaces.

Sodium hypochloride and various aliphatic alcohols are popular for application on plane surfaces, tubes and appliances. Their effectiveness is rather broad but alcohols tend to evaporate at room and higher temperatures.

Table 5: Proportions of DVG-listed chemical disinfectants for use in the fields of food hygiene, production and processing of food

Active compound in commercial disinfectants	Total number of disinfectants in the 7 th list (2011)	% of total 220 commercial disinfectants
Quaternary ammonium compounds	108	49.1
Sodium hypochloride	30	13.6
Alcohols including 1- and 2-propanol	24	10.9
Products containing peracetic acid including and H ₂ O ₂ or organic acids	22	10.0
Alkylamines	22	10.0
Other compounds	14	6.4

Recommended disinfectants for specific indications (13th list DVG, 2011)

Most of the chemicals in disinfectants are known since many decades or even centuries. Nowadays a large number of commercially available products are present on the market. The large number of products for use in hygiene and food processing (total 220 products) and for use in the veterinary field (103 products) reflects the economic efforts of various companies, but not necessarily their creativity. Admittedly, many companies try hard to improve secondary properties of their products such as better adherence to vertical surfaces, spreading on uneven areas, and reduction of surface tension.

In contrast to the large number of available products, the search, invention and synthesis of new chemicals with germicidal properties is lacking. In addition, the effective concentrations and necessary times appear to be rather similar.

Table 6 summarizes recommended concentrations and times to achieve bactericidal, tuberculocidal, fungicidal, virucidal and antiparasitic effects for a selected number of compounds. Not mentioned in Table 6 are additions to the final product that enhance penetration, spreading on surfaces by tensides and related chemicals. Also omitted are properties which affect handling, smell, corrosiveness and other characteristics.

Table 6: Grouping of selected chemical compounds in relation to their activity against infectious agents

Active Compound	Target									
	Bacterium		Tubercle		Fungus		Virus		Parasite	
	%	h	%	h	%	h	%	h	%	h
Peroxyacetic acid	1	1	2	2	1	1	1	1	3	1
Organic acids	1	1	4	4	1	2	1	1		
Aldehydes	1	2			1	2	1	2		
Choramine T	3	2			3	2	3	2		
Quaternary ammonium compounds	3	2			6	2	2	2		
Cresols	2	2	4	3					4	2

% - required concentration of disinfectant during application
h - minimum time of effective exposure of disinfectant

Antiparasitic disinfectants

Among the targets for disinfection are also the developmental stages of round- and tapeworms and oocysts of coccidia, mainly of the family Eimeridae. All these forms have long survival times in the environment. Consolidated scientific data and practical experience have proven that only few chemicals destroy the viability of these parasitic forms. Most common and listed in the 13th list are three specified chemicals. These are p-chlor-meta-cresol, chlor-methyl-phenol and o-hydroxydiphenyl fatty acid eutectic peracetic acid (Table 7). The 13th list of disinfectants for use in the veterinary field contains in addition to these four compounds seven disinfectants without disclosure of their chemical names.

From the data in Table 7 it is obvious that rather high concentrations and at least two hours of exposure are needed to inactivate worm eggs and oocysts. It is important to note that two hours is the maximum time for listing of a product. Also, only a small number of producers were listed (see Table 5).

Table 7: Listed compounds for inactivation of eggs of flat- and round worms and coccidial oocysts

Active compound in disinfectant	Embryonated eggs of <i>Ascaris suum</i>		Embryonated oocysts of <i>Eimeria tenella</i>	
	%	h	%	h
o-hydroxydiphenyl fatty acid -eutectic peracetic acid (listed twice)	2 A + 1.5 B	2	3 A + 1.5 B	2
p-chlor-m-cresol (listed 4 times)	2 - 3	2	3 - 4	2
Chlor-methyl-phenol (listed twice)	3	2	3	2
Cresols - chemically not specified) (listed 7 times)	2	2	4	2

% - required concentration of disinfectant during application
h - minimum time of effective exposure of disinfectant

Criteria for selection of a disinfectant

Advantages and limitations of a given disinfectant should be known by all users. In addition, it is a special duty to investigate and evaluate the areas and structures of a farm prior to application of a disinfectant. Here, local specifications of buildings and their structural components (wood, aluminium, steel, plastics) and likely undesired side effects (corrosion, vapour), the presence of animals and/or humans must be considered.

Beside the structure of a farm, the specific cause of contamination is essential because bacteria, fungi, viruses or parasites demand products of different modes of action, qualities and applications. In case of the confirmed or putative presence of epidemic, notifiable diseases, legal recommendations / prescriptions must be followed.

Who should disinfect?

The selection from a large number of available products of an appropriate disinfectant and the timely and correct application is a major issue for use in practice. A consulted veterinarian may provide professional advice in this respect. Further candidates for this selection may be the farmers themselves or their employees. None of these persons will guarantee the effectiveness of the performed disinfection. More likely, they will deny any legal responsibility. To prevent any queries, it seems to be wise, to delegate the disinfection to a professional company. Such company will perform disinfection in a professional way, will guarantee the proper performance and will compensate for any possible failure. In Germany, quite recently the company *Gesellschaft für Seuchenvorsorge mbH*, Cloppenburg, Germany, has been founded which will provide professional advice and logistic support in all issues related to disinfection and monitoring of the effect.

In view of any likely failure, it is recommended to monitor the effects of disinfection right after its completion. However, this is rarely done in practice. If it is done, cotton swabs from various representative surfaces should be collected and assayed for residual infectious agents.

Common errors of disinfection

Reliable statistics on types and frequencies of errors during disinfection are not published and remain unknown to outsiders. Some more general points will draw the attention to possible failures. Among these are (i) a wrong product was used for the intended purpose, (ii) the dilution of the concentrate of a disinfectant was false, e.g. too low or too high, (iii) the exposure time was too short, (iv) applied disinfectant rinsing off or dripping off from vertical walls, (v) not all contaminated areas were reached, (vi) the environmental temperature was too low, (vii) re-contamination occurred at or soon after disinfection.

Conclusions

Disinfection is a valuable tool in disease control and should be an integral part in prevention and elimination of transmissible diseases. Any disinfection must be done professionally, preferably by experienced companies. Selection of an appropriate disinfectant must be tailored according to a recognized, farm-specific problem. Inherent properties of disinfectants must be known for the selection of the best possible product. For safety and efficacy reasons, instructions of the producer should be followed. Generate a detailed work-plan and time schedule prior to any activity. Duration, time, temperature, concentration of any process of disinfection must be realized to guarantee success. Keep records on all steps of disinfection.

Summary

Disinfection of the surroundings where farm animals and poultry are kept is an important part of maintaining and/or restoring health and production. The selection of suitable disinfectants from a large number of basic substances available and commercial products with proven benefits and their correct application in specific situations requires extensive knowledge and experience. Technical possibilities and goals for disinfection in different areas of poultry farming are described. Health risks from using disinfectants for animals, man and the environment are also addressed.

Zusammenfassung

Desinfektion als Voraussetzung für erfolgreiche Geflügelhaltung: Ziele und Methoden für den Fachtierarzt

Die Desinfektion des Lebensraums landwirtschaftlicher Nutztiere ist ein bedeutsamer Teil zur Erhaltung bzw. zur Wiederherstellung der Gesundheit und Leistung. Die Auswahl geeigneter Desinfektionsmittel aus einer großen Zahl verfügbarer Grundstoffe und als wirksam befundener Handelspräparate sowie die fallbezogene korrekte Anwendung erfordern große Sachkenntnis und Erfahrung. Beschrieben werden die technischen und räumlichen Möglichkeiten und erreichbaren Ziele von Desinfektionsmaßnahmen im Rahmen der Nutztierhaltung. Auf gesundheitliche Risiken für Tier, Mensch und Umwelt beim Umgang mit Desinfektionsmitteln wird hingewiesen.

Acknowledgment

The author wishes to thank Prof. Dr. Dietmar Flock, Mrs. Brigitte Othmar and Dr. Egon Vielitz for critical reading of the manuscript and their suggested valuable improvements.

Selected references

- Blood, D. C., V. P. Studdert, and C. C. Gay (2007): Saunders comprehensive veterinary dictionary, 3rd edition. Saunders, London.
- European Commission über die Nichtaufnahme von Formaldehyd in Anhang I, IA oder IB der Richtlinie 98/8/EG des Europäischen Parlaments und des Rates über das Inverkehrbringen von Biozid-Produkten für die Produktart 20x vom 25. April 2013.
- Rautenschlein, S. und E. F. Kaleta, (2012): Prophylaxe. Allgemeine Seuchenvorbeuge. Reinigung und Desinfektion. In: Siegmann und Neumann (Hrsg.). Kompendium der Geflügelkrankheiten, 7. Auflage. Schlütersche, Hannover.
- Schließer, T. und D. Strauch (Hrsg.), (1981): Desinfektion in Tierhaltung, Fleisch- und Milchwirtschaft. Enke Verlag, Stuttgart.
- Seemann, G. und P. Trenner (2004): Neuere Entwicklungen bei Desinfektionsverfahren in der Geflügelhaltung. Lohmann Information, Januar – März, Heft 1.
- Strauch, D. und R. Böhm (Hrsg.) (2002): Reinigung und Desinfektion in der Nutztierhaltung und Veredelungswirtschaft. Enke-Verlag, Stuttgart.

Author's address:

Prof. (em.) Dr. med. vet. Dr. h.c. Erhard F. Kaleta
Klinik für Vögel, Reptilien, Amphibien und Fische
Justus-Liebig-Universität Gießen
Frankfurter Straße 91-93
D-35392 Gießen, Germany

E-mail: erhard.f.kaleta@vetmed.uni-giessen.de