



Transmissible Diseases Handbook

VIII. GUIDELINES FOR CLEANING AND DISINFECTION IN ZOOLOGICAL GARDENS

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Introduction

Hygienic measures are a major component for prevention of infectious diseases in zoological exhibitions. A clean environment is not only a key feature in disease control, but also attractive to the public. Preventive health care consists of multiple components of equal importance including food storage, preparation and handling, insect and vermin control and cleaning and disinfection (Fowler, 1978).

The purpose of this chapter is to provide guidelines for cleaning and disinfection to the range of people who would be involved in managing a disease emergency involving a zoo, zoological garden, game park, circus or any other facility keeping exotic animals. For the information in this chapter to be effective, it is important that the described cleaning and disinfection methods are incorporated into the routine husbandry and emergency procedures. The goal is to provide individual zoos with information that can be used to develop their own specific cleaning and disinfection protocols. Each of the fact sheets in the “Transmissible Disease Handbook” contains disease specific information about prevention and control of diseases in zoos and suggested disinfectants for animal housing. Therefore, the purpose of this chapter is not to provide detailed information on specific disinfectants for the diseases listed in this handbook, but rather to provide an overview of the guiding principles of cleaning and disinfection in zoological gardens and to review the various classes of chemical disinfectants.

Defining Disinfection

The term “disinfectant” is defined as “an agent that frees from infection, usually a chemical agent but sometimes a physical one, such as x-rays or ultraviolet light, that destroys diseases or other harmful microorganisms but may not kill bacterial spores” (Block, 2000). Disinfectants are used on inanimate surfaces and are assumed to act rapidly and efficiently to kill or inhibit growth of microorganisms. In the veterinary care environment, disinfection is most effective for diseases that are not vector-borne, but are acquired by direct contact with contaminated fluids or animal products (Quinn, 2000). Therefore it is important to recognize that disinfectants play a critical, yet limited role in infection control. Insect and vermin control are equally important in preventing infectious diseases, especially for the control of insect-



borne diseases that present particular challenges in zoos. Techniques available to minimize insect-borne disease spread include the □use of anthelmintics and insecticides through direct application on at-risk species or through the □spraying of □housing of susceptible animals. The assistance of epidemiologists and entomologists should be sought to establish insect traps within the zoo for insect identification and to identify potential areas of a high-risk exposure.

For situations where resistant microorganisms are present, or animals may be susceptible to infection due to their health status or the invasiveness of the procedure to be performed, a sterilization method should be used for treatment of contaminated items or surfaces. Sterilization is achieved when all living microorganisms and bacterial endospores have been destroyed. Common methods of sterilization include, dry heat, moist heat, and exposure to specific chemical compounds. For dry heat sterilization, surfaces must be exposed to 160 C to 170 C for periods of 2 to 4 hours (Heinsohn et al., 1995). Sterilization by steam may be accomplished by exposing contaminated surfaces to moist heat at 121 C for at least 15 minutes (Quinn, 2000) This will not be sufficient for certain heat resistant organisms such as some thermophilic spores and prions. Chemical sterilization may be suitable in situations where items to be sterilized cannot withstand the temperatures and physical conditions required for dry heat or steam sterilization. Chemical sterilants may be used in the form of a vapor or gas, such as formaldehyde or ethylene oxide, or as an immersion liquid such as glutaraldehyde. These chemical compounds and the requirements for their proper use will be discussed in a later section of this chapter.

The efficacy of a certain disinfection or sterilization protocol will depend on a number of factors. The nature of the surface that is to be disinfected plays an important role. An uneven surface will be more difficult to disinfect than an even one. Dirt will interfere with the disinfection. Another important factor are the pathogens present. Some pathogens are more difficult to inactivate than others. The most difficult to inactivate are bacterial spores and prions. For disinfection, non-enveloped viruses can also be a challenge.

Role of Cleaning and Disinfection in Infection Control

Having an effective cleaning and disinfection plan is a crucial step in every biosecurity program. Such a program should be instituted for every new building and facility and should be revised after the occurrence of an infectious disease and prior to the introduction of new animals. The main purpose of a cleaning and disinfection program is to reduce the number of pathogens (disease-causing agents) in the environment and thereby to reduce the potential for diseases to occur.

The first step in an effective disease control plan is an exact identification of the routes how infectious agents may enter zoological gardens (inputs), and how they may spread throughout the zoo to other facilities and outside the zoo to become a threat to farming operations or possibly humans. Inputs into and outputs from zoological institutions may vary depending on the type of the facility.

Animal inputs may include: □animals introduced from other facilities within the same institution, animals from institutions, either from within Europe, or imported from another continent, animals confiscated by customs/quarantine officers, sick or injured animals brought in by members of the public, □free-ranging animals, which may be either native (rats, mice, birds), or feral (including cats and dogs), and □animals imported from farming



operations. Feed inputs include dry processed preparation (concentrates, hay, pellets, seed etc.) and wet feed, including fresh fruit, fish, meat, vegetables and pasture silage. Biological specimens confiscated by customs and quarantine officers are sometimes brought to zoos for identification. Semen and embryos may be imported for breeding purposes. Various vehicles move into the facility and may be contaminated. Other materials entering the facility include materials used during the importation of the animals (hay, sawdust, crates etc.). Personnel entering the premises for normal work purposes may have contact with other animals (pets/farm animals) outside of work hours. Local and international visitors pass through the premises on a daily basis and may introduce disease.

Animals may leave the facilities for a number of reasons including: exchange and sale of animals, □relocation of animals to other facilities within and outside the institution, □use of animals in public relations exercises, i.e. animals taken to shows, shopping centers, schools and television stations, □animals taken home by staff for hand rearing, □free-ranging of native and feral animals, including cats and dogs that may have had contact with animals/animal waste. Waste materials that have to be removed from the premises may include hay and composted feces, effluent, some of which has secondary treatment, and a small portion with tertiary treatment, waste-water. Biological specimens and feces are sent to laboratories for testing and biological specimens are sometimes also sent to museums, veterinary schools etc. In some institutions, offal and carcasses are taken off the property for disposal or may be sent to veterinary schools for necropsy. Vehicles, crates and packing material used in the transportation of animals are all regularly moved off the premises. Possible outputs related to people include □personnel who have been in contact with animals and waste products (contaminated clothing and footwear), and □visitors who may have been in contact with animals.

Due to the public role of zoological gardens, it will be impossible to clean and disinfect all inputs and outputs. In particular, visitors and the media would not expect the imposition of cleaning and disinfection programs onto them. Therefore a strict cleaning and disinfection program of the animal premises incorporated into daily routines becomes even more important. In addition, it is useful to identify premises according to their exposure to infectious agents to select the proper cleaning and disinfection regimes and to possibly limit the access to such premises. Areas (could be all or part of a facility) in which an infectious disease exists, is believed to exist, or which may harbor the infective disease agent should be classified as an infected premise (IP). Dangerous contact premises (DCP) are premises containing animals with no clinical signs that were exposed to an infectious disease and therefore will be subjected to disease control measures. Suspect premises (SP) are areas containing animals that show no clinical signs, but may have been exposed to an infectious disease through possible contact with infected animals or facilities, people, equipment, semen or embryos, or animals with evident disease symptoms, but no confirmed diagnosis. Using this classification in combination with specific cleaning and disinfection programs and other measures, such as insect and vermin control, as well as treatment, vaccination and quarantine of infected and exposed animals or animals at high-risk for infections, will help to control and to eliminate an infectious disease after an outbreak has occurred. In some cases, it may also be necessary to consider culling certain animals that have been exposed to disease. Disposal of animal carcasses in the case of a disease outbreak should also be incorporated into these plans.

Cleaning before Disinfection

The first step in any cleaning and disinfection program is cleaning. Cleaning is the removal of organic material (i.e., feces, urine, blood, bedding, food, dust etc.). Disease agents are often protected by such materials and can survive the disinfection process. Therefore, thorough cleaning of a building prior to the disinfection is required. The cleaning process can include a dry cleaning and a wet cleaning step. Dry cleaning physically removes the organic material before the actual wet cleaning. Wet cleaning, as the name implies, involves the use of water. There are 4 basic steps in the wet cleaning process: soaking, washing, rinsing, and drying. The use of detergents will often benefit the wet cleaning process. However, it is more important to have pressure washers with the proper pressure (500-800 psi) to ensure that all organic materials are removed from the facilities. The final step for ensuring proper cleaning is to dry the wet areas of the building quickly. If the building is not dried properly, the excess moisture can result in bacteria multiplying to higher levels than prior to cleaning. It is vital to make sure the cleaning procedure is done properly, as an improper cleaning can actually increase the load of infectious agents! If done properly, a good cleaning can remove 90% of all pathogens (Fotheringham, 1995). Special care must be taken when cleaning facilities that are contaminated with suspected zoonotic or highly contagious animal pathogens. All personnel should wear protective clothing, footwear and if necessary face masks, goggles, and headwear. After the cleaning of facilities, proper care must be taken of the protective clothing and equipment that has been used for cleaning and disposable products should be disposed as biohazards. Organic material removed during cleaning may also contain pathogens and should be treated accordingly.

The last step in a cleaning and disinfection program is the actual disinfection process that will further reduce pathogens in the facilities. Disinfection is especially useful in reducing infection risks in young animal nursery facilities, and in routine cleaning operations of animal quarters and feeding utensils. To maximize the effectiveness of a cleaning and disinfection program, it is crucial to modify such a program based on the suspected pathogens that should be eliminated or reduced. In addition, specific disinfectants may be selected for certain known microbial contaminants following an infectious disease outbreak. It is also important to remember that many disinfectants can be toxic to the animals, or may be caustic or corrosive. Animals must usually be excluded from facilities being disinfected. After a suitable environmental exposure time to such disinfectants premises should be rinsed thoroughly, before animals are allowed to return.

Levels of Disinfection

Disinfectants are tested for their bacteriocidal, tuberculocidal, sporicidal, fungicidal, virucidal (against enveloped and non-enveloped viruses), and antiparasitic (against eggs and coccidia) effects. They can generally be categorized into 3 groups based on their ability to inactivate certain microorganisms. High-level disinfectants are effective against bacterial endospores under specific conditions. Intermediate-level disinfectants include those products that can inactivate tubercle bacilli, but do not kill bacterial spores. Such products are commonly marketed by manufacturers as "tuberculocidal". Finally, normal ("low-level") disinfectants include products that kill vegetative bacteria and fungi but are not reliable for the destruction of bacterial endospores, tubercle bacilli or small non-enveloped viruses within a practical period of time (Boothe, 2000).



When selecting a disinfectant for use on items that will come in contact with patients during animal care procedures, the invasiveness of the procedure and composition of the medical device must be considered. A system for classifying such devices was developed by Spaulding in the 1970s. This system has been recognized and referenced since that time by many human healthcare organizations like the Association for Professional in Infection Control and Epidemiology (Rutala, 1996). Although this system was developed for human healthcare, there are notable similarities to procedures and devices used in veterinary care environments. These principles may be adapted for disinfectant selection purposes in zoological gardens and other exotic animal containments.

The Spaulding system categorizes devices into 3 groups based on the risk of infection involved with their use: critical, semicritical and noncritical. Critical devices are those that bear a substantial risk of acquiring infection if contaminated with microorganisms at the time of use. Devices that are introduced directly into the body such as needles, scalpels, catheters and implants fall into this category. These items must be sterilized in between animal use (Favero and Bond, 2000). When choosing a sterilization method, consideration must be given to the impact of the sterilization process on the integrity of the device. Semicritical devices are those items that come in contact with mucous membranes but do not normally penetrate the skin. Examples of these devices include endoscopes, bronchoscopes, and urinary catheters. Sterilization of semicritical devices is desirable, but high-level disinfection is a minimum requirement (Favero and Bond, 2000). High-level disinfectants may also act as cold liquid sterilants under specific conditions of use. Therefore, some products may be appropriate for both critical and semicritical devices. Product versatility should be one of the considerations for disinfectant selection. Non-critical devices are items that only come in contact with an animal's intact skin, and therefore bear the lowest relative risk of disease transmission. Stethoscopes, otoscopes, electrodes, and common animal restraining tools are a few examples of non-critical devices. Proper treatment of this category of devices in between animals will depend on the nature and degree of contamination sustained during use. Where contamination is minimal, simple scrubbing with detergent and warm water may be acceptable to assure safety. However, the use of low- or intermediate-level disinfectants may be appropriate to assure that disinfection has been accomplished (Favero and Bond, 2000).

Factors that Impact Efficacy of Disinfection

Effective disinfection can only be achieved if the following interrelated factors are taken into account:

Nature of the item or surface to be disinfected

Items with smooth, non-porous surfaces are the easiest to disinfect because they can be effectively cleaned of organic debris and this will allow for ample surface contact with the disinfectant applied. As a rule of thumb, the "rougher" the surface (i.e. crevices, damaged finish), the more difficult cleaning and subsequent disinfection will be. To account for this, increased contact time of the disinfectant or a higher disinfectant concentration on these surfaces may be beneficial (careful with increased concentrations: toxicity and corrosive or caustic effects may also be increased).



Contact time and other factors

The surface contact time is the minimum required duration of exposure of the contaminated surface to the disinfecting agent in order to achieve the level of disinfection that is claimed by the manufacturer. Therefore, careful consideration should be given to the manufacturer's recommendations and conditions of use. These recommendations will also address other factors that may impact efficacy such as pH, water hardness, performance in the presence of organic debris and optimal temperatures for product use.

Presence of organic material

The presence of organic material on surfaces to be disinfected will generally compromise disinfection. Any type of contamination will reduce the surface contact time of the applied disinfectant and therefore its effectiveness. Blood, blood products, body fluids and feces contain proteins that will bind and inactivate some disinfections or slow their action. This reinforces the importance of cleaning surfaces prior to disinfection whenever possible. If organic debris cannot be removed, an increased concentration of the disinfectant or contact time may be warranted. Furthermore there are also products available that will be effective in the presence of organic debris.

Number and type of microorganisms present

In general, items that have a higher level of microbial contamination will require a longer exposure to chemical products to achieve disinfection. Level and type of microbial contamination may be presumed by the nature of a procedure that a device was used for, the health status of the animal, or other epidemiological factors associated with the veterinary care environment.

Resistance of microorganisms

Microorganisms vary in their susceptibility to disinfectants. In general, obligate intracellular bacteria, such as Mycoplasma, are the most susceptible to disinfection. Gram-positive and Gram-negative bacteria, enveloped viruses and fungal spores are less susceptible, but are not considered to be resistant to disinfection. Highly resistant microorganisms include non-enveloped viruses and mycobacteria. Although these organisms are hearty, they are not as resistant as bacterial endospores or protozoal oocysts and even more prions, which are resistant to most disinfectants (Quinn and Markey, 2000). The level of disinfection should be based on the most resistant microorganism posing a risk in a given situation.

Type and concentration of disinfectant

As previously noted, microorganisms vary in their overall resistance to disinfection. However, resistance will also depend on the type of disinfectant used. Each class of chemical products acts differently on the cells. It is important to assure that the mode of action for the disinfectant is compatible for the type of cells to be destroyed. General modes of action for commonly used disinfectant classes are addressed in a later section of this chapter. Regarding concentration, with all other variables constant, the higher the concentration, the greater the effectiveness of the disinfection and the shorter the contact time (Favero and Bond, 2000). However, there are exceptions to this rule including alcohol and iodophors that have an optimal range of concentration. While the concept of using concentrated

disinfectants to achieve faster, more effective disinfection may seem like an attractive time-saver, caution should be exercised. Chemical disinfectants are often hazardous to human and animal health and an increased concentration is likely to equate to increased chemical exposure hazard to personnel, animals and the visiting public.

Factors for Selecting a Disinfectant

Because all disinfectants are unique in their chemical action and properties, it is unlikely that one disinfectant will fit all needs. However, for veterinary care purposes, there are a number of desirable qualities that have been identified and should be considered for product selection. These include (Quinn and Markey, 2000):

- Wide antimicrobial range
- Absence of chemical hazards (i.e. toxicity, teratogenicity, carcinogenicity)
- Compatible with a wide range of chemicals
- Non-corrosive
- Active in the presence of organic debris
- Stable at ambient temperatures
- Long shelf-life
- Effective over wide range of temperatures
- Inexpensive and readily available
- Nonpolluting and biodegradable

In Europe, there are a number of organizations that test disinfectants for use in animal husbandry. In Germany, the German Veterinary Medical Society (DVG) provides guidelines for testing disinfectants and publishes a list of disinfectants that have been tested for use in animal husbandry. The list also contains information on effectivity of the products against different categories of pathogens, and suggested concentrations and incubation times. Products in this list have also been tested under conditions meant to simulate “real life” outside the laboratory, and include effectivity in a protein-contaminated environment and on “rougher” porous surfaces. The European Committee for Standardization (CEN) is currently establishing European guidelines for disinfectant testing in the veterinary field.

Overview of Categories of Chemical Disinfectants

There are a number of compounds that have specific use as disinfectants in the veterinary care environment. An overview of the main classes of chemical disinfectants is given in this section but periodic consultation to current literature is required as new products are constantly under investigation and emerging infectious diseases continue to rise. For lists of tested disinfectants see e.g. the DVG (German Veterinary Medical Society) for products used for veterinary medicine or in the food industry or the DGHM (German Society for Hygiene and Microbiology) for disinfection in human medicine and hospitals. At this time, no general European lists are available.

Alcohols

Alcohols are inexpensive, relatively nontoxic, and colorless, and ethyl and isopropyl alcohol are most widely used. They are considered to be intermediate level disinfectants that inactivate organisms by denaturing proteins. While they are effective for destruction of enveloped viruses and tubercle bacilli, they are less effective against non-enveloped viruses



(particularly isopropyl alcohol) and are not sporocidal (Maillard and Russell, 1997). A concentration of 70% of ethyl alcohol is most effective. When used as a surface disinfectant, rapid evaporation of these compounds makes sufficient contact time difficult to achieve. Additionally, alcohols tend to harden and swell plastic tubing when used over time, and may be absorbed by rubber products that can lead to irritation of the skin and mucous membranes (Favero and Bond, 2000).

Aldehydes

Formaldehyde (available in an aqueous 37% solution) and glutaraldehyde are two common examples of this class. Formalin in solutions of 3% - 8% is effective for intermediate to high level disinfection. It is less effective than glutaraldehyde in the presence of organic matter. Despite discussions on potential carcinogenicity, skin irritation, and irritating fumes, aldehydes are common compounds in disinfectant preparations used in the veterinary field (i.e. breeding, husbandry, transport and disposal of all animals).

Glutaraldehyde, which inactivates microorganisms by alkylation, is also an intermediate to high level disinfectant. At 2% active ingredient, glutaraldehyde solutions are effective against bacterial endospores. Although minimally affected by organic matter, product effectiveness is influenced by a number of factors such as pH and temperature. Glutaraldehyde solutions are most effective at alkaline pH and effectiveness increases with temperature (Quinn and Markey, 2000). Glutaraldehyde solutions are noncorrosive and are typically used in immersion baths for high level disinfection of semi-critical devices (i.e. endoscopes) that cannot withstand steam or heat sterilization. Due to the toxic nature of this compound, special considerations must be made for proper handling and storage of solutions. Immersion baths should have tight-fitting lids and be placed in an exhaust hood to minimize personnel exposure to vapors. Additionally, to eliminate skin and mucous membrane exposure, splash goggles and appropriate chemical resistant gloves should be worn. Aldehyds should not be used at temperatures below 10 °C.

Alkalis

Sodium hydroxide (NaOH), or lye is a caustic alkali that has a wide virucidal spectrum when used at a 2 percent concentration (prepare by mixing 1/3 cup of NaOH pellets per gallon of water – careful! hot). This is effective against most bacteria, and enveloped and non-enveloped viruses, although somewhat higher concentrations may be necessary for some viruses. This concentration is effective against viruses including the causes of Avian Influenza, Rinderpest and Pest of Small Ruminants, Newcastle Disease, Malignant Catarrhal Fever, Lumpy Skin Disease and Sheep and Goat Pox, African Horsesickness and Bluetongue, Foot-and-Mouth Disease, and Swine Vesicular Disease. Sodium hydroxide should not be used on wood. Sodium hydroxide is also one of the few compounds that can be used for the inactivation of prions (in much higher concentrations) (Taylor, 2000). An overview on deactivation of prions is given below. A pH above 12 is required to for the inactivation of bacilli such as *Mycobacterium bovis*. Chemical exposure risks to personnel and animals as well as surface incompatibility are factors for the limited use of such products.

Ammonium hydroxide, a weak base, has a strong activity against coccidial oocytes (Williams, 1997). The high pH of even low aqueous solutions and intense fumes require protective clothing when using this alkali.



Sodium carbonate, containing 0.1% sodium silicate, has significant virucidal activity and is commonly used for the decontamination of aircrafts.

Biguanides

Biguanides are a group of cationic compounds commonly used for hand washing and skin preparation.

Chlorhexidine, a low-level disinfectant that is incompatible with anionic compounds, is the most commonly used compound in this group. Its activity is pH dependent and it is inactivated by organic debris (McDonnell and Russell, 1999). It is more active against gram-positive than gram-negative bacteria and *Pseudomonas spec.* and *Proteus spec.* are resistant against chlorhexidine (Widmer and Frei, 1999). Although it is active against enveloped viruses, it can not be used against non-enveloped viruses, mycobacteria and most fungal species, such as dermatophytes, i.e. *Microsporum canis*, are resistant (DeBoer et al., 1995) Because chlorhexidine has a longer residual activity on teat skin than most other disinfectants, it is widely used for mastitis control (Quinn and Markey, 2000).

Halogens

Chlorine Compounds

Chlorine and chlorine releasing compounds, such as hypochlorite, chlorine dioxide, sodium dichloroisocyanurate, and chloramine-T, are used in some countries for disinfection of surfaces, equipment, buildings and vehicles as well as disinfection of milking installations and in the food industry.

Sodium hypochlorite (NaOCl or household bleach) solution is an intermediate-level disinfectant when properly diluted. The mode of action for this solution is dependent on the formation of undissociated hypochlorous acid that will oxidize peptide links and denature proteins (Maris, 1995). Solutions diluted to final concentration must be prepared directly before use and should not be stored for more than 1 day. At a concentration of 0.1%, this compound is effective against microbial agents of diseases, including enveloped viruses and Foot-and-Mouth Disease virus. It can be prepared at the time of use by adding approximately 30 cc (ml) of household bleach to a gallon of water (or 1 gallon of bleach plus 50 gallons of water). As pH decreases microbicidal activity (as well as corrosivity) increases. In concentrated form chlorine-based disinfectants are usually unstable, and affected by light and heat. They can lose 50% of their concentration in a month when left in an open container (Widmer and Frei, 1999). Additionally, the corrosivity of active solutions can be damaging to surfaces and equipment and irritating to the skin and eyes. Bleach solutions are readily inactivated by organic debris, limiting their effectiveness to situations where devices or surfaces have been thoroughly cleaned prior to disinfection. In areas heavily contaminated with secretions, excretions, and soil, there is a considerable organic demand for available chlorine and disinfection should be repeated at least once. Under such circumstances a 3% solution of sodium hypochlorite should probably be used (3 liters of bleach to 2 liters of water). This concentration is effective against a variety of agents of viral diseases including Avian Influenza, Rinderpest and Pest of Small Ruminants, Malignant Catarrhal Fever, Lumpy Skin Disease and Sheep and Goat Pox, and African Horsesickness and Bluetongue as well as other non-enveloped viruses. At concentrations used for water

treatment it is not effective against *Cryptosporidium parvum* (Fayer, 1995). Halogens rapidly lose efficacy on rough surfaces such as concrete.

Iodine Compounds

Iodine compound disinfectants are more effective in the presence of organic matter than chlorine compounds, and less chemically reactive. Inorganic iodine and iodophors, compounds in which iodine has been complexed with polymers to sustain the release of free iodine and to increase water solubility, have both been used for disinfection. However, the instability of inorganic iodine in the environment, skin irritations, hypersensitivity reactions, and intense staining of contaminated surface limit its use. At appropriate dilutions, iodophors are bactericidal, mycobactericidal, sporicidal, fungicidal, and virucidal. They are also widely used as teat dips (Saran, 1995). Because the activity of iodophors is greater at a low pH, the compound should not be used in alkaline conditions or mixed with other compounds.

Certain acidified iodophor compounds that contain a generic formulation of polyethoxy-substituted polypropoxy-ethane complex are extremely effective disinfectant compounds with strong virucidal activity (Maillard and Russell, 1997). The active ingredient must provide a minimum use concentration of 0.02% titratable iodine.

Organic and Anorganic Acids

Acids inhibit the growth of microorganisms and a number of compounds are commonly used as preservatives in the food industry. Acetic acid has been used for the treatment of wounds infected with *Pseudomonas spec.* (Lemarie and Hosgood, 1995). The use of acids may be warranted under certain circumstances in the animal care environment. Formic acid is the most effective of these substances and should be used at a concentration of 4%. Citric acid, formic acid, and strong mineral acids, such as phosphoric acid, are effective for inactivating the Foot-and-Mouth Disease virus (Quinn and Markey, 2000). Two mineral acids, hydrochloric acid and sulfuric acid, are sometimes used for cleaning and disinfection of animal housing. A 2.5% concentration of hydrochloric acid can be used to inactivate endospores of *Bacillus anthracis* on the skin and is also effective against Rotaviruses and Vesicular Stomatitis virus (Maillard and Russell, 1997). However, the activity of these compounds is highly pH dependent and these compounds are corrosive and hazardous to workers and animals. To minimize chemical exposure risk to personnel, citric acid should be used when possible.

Peroxygen Compounds

Strong oxidizing agents such as hydrogen peroxide and peracetic acid, have a broad antimicrobial spectrum. Hydrogen peroxide is fast acting, nonpolluting, and decomposes to oxygen and water. Because it is unstable in solution, stabilizers are usually added. It is bactericidal, fungicidal and virucidal, but should not be used against Mycobacteria (Maillard and Russell, 1997). It is quickly inactivated by protein contamination of treated surfaces. Peracetic acid is a strong oxidizing agent and an even stronger disinfectant that has algicidal, bactericidal, fungicidal, virucidal and sporicidal activity. Unfortunately it can corrode most metals, rubber and has been suspected to act as a cocarcinogen (Sattar and Springthorpe, 1999). Oxidising agents rapidly lose efficacy on rough surfaces such as concrete.

Phenolic Compounds

Phenol is cited in the literature as being the original standard against which other disinfectants were compared (Quinn and Markey, 2000). Due to its toxicity and unpleasant odor, the use of phenol for disinfection purposes has been largely replaced by the use of synthesized phenolic compounds. Examples include *ortho*-phenylphenol and *ortho*-benzyl-*para*-chlorophenol. These compounds are a complex group of chemicals often found in various combinations and concentrations in disinfectant products. The effectivity of phenolic compounds increases with the length of the alkyl chains and number of halogen atoms in the molecule, but this also increases the sensitivity of the compounds to organic material, lowers their water solubility and increases their toxicity, so that these compounds are not frequently found in disinfectants for veterinary medical use. These products are considered to be intermediate to low level disinfectants. For veterinary care purposes, it is important to note that these compounds generally leave a residue on surfaces that may increase chemical exposure for personnel and animals. Additionally, pigs and cats are susceptible to the toxic effects of these compounds (Quinn and Markey, 2000). Also available are substituted phenolic compound, that have potent virucidal activity when prepared as a 1% solution of stock disinfectant. These types of compounds are effective against *Eimeria tenella* (Williams, 1997) and the viruses of African Swine Fever, Avian Influenza, Hog Cholera, and Velogenic Newcastle Disease, but not effective against the viruses of Foot-and-Mouth Disease and Swine Vesicular Disease (Widmer and Frei, 1999).

Quaternary Ammonium Compounds (QACs)

Quaternary ammonium compounds (QACs) are cationic detergents that may be used for low level disinfection, especially surface disinfection. Benzalkonium chloride and dodecyl dimethyl ammonium chloride are common examples of this group. QACs are relatively non-toxic making them appropriate for general cleaning purposes. However, QACs are inactivated by organic debris, metal salts in water (i.e. hard water) and anionic detergents (Heinsohn et al., 1995). Even some gram-negative bacteria, such as *Pseudomonas spec.*, can survive disinfection with QAC and may grow in QAC solutions. Such limitations must be considered when developing procedures for the use of the products in the animal care environment. QACS are found in many disinfectant formulations used in the food industry.

Inactivation of prions

Because of the political impact transmissible spongiform encephalopathies have had in the past few years and the public perception of these diseases, this paragraph has been included. Prions are characterized by extreme resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals (formalin, betapropiolactone, alcohols). While prion infectivity in purified samples is diminished by prolonged digestion with proteases, results from boiling in sodium dodecyl sulfate and urea are variable. Sterilization of rodent brain extracts with high titers of prions requires autoclaving at 134 C at 3 bar for 1 h . Denaturing organic solvents such as phenol or chaotropic reagents such as guanidine isothiocyanate or alkali such as NaOH can also be used for sterilization. Prions are inactivated by 1 N NaOH (>1 h), 4.0 M guanidinium hydrochloride or isocyanate, 2,5 % sodium hypochlorite (2% free chlorine concentration; >1 h), followed by heating in an autoclave at 134 C at 3 bar for ≥ 1 h (Anonymus, 2003). It is recommended that dry waste be autoclaved at 134 C at 3 bar for at least 1 hour (depends on the thickness of the material) or incinerated. Large volumes of infectious liquid waste



containing high titers of prions can be treated with 1 N NaOH (final concentration) before autoclaving at 134 C at 3 bar for 1 hour. Disposable plasticware, which can be discarded as a dry waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, biosafety cabinets must be decontaminated with 1 N NaOH, followed by 1 N HCl, and rinsed with water. Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids. Formaldehyde-fixed and paraffin-embedded tissues, especially of the brain, remain infectious.

Considerations for Disinfectant Use

Disinfectant products, as well as range of applications vary widely. However, careful consideration of manufacturer's product information in conjunction with a basic understanding of limitations to the disinfection process will go a long way in assuring that disinfection is an effective part of an infection control program.

As previously addressed, selecting the proper disinfectant for specific needs is essential. Before purchasing a product, request and review information from the manufacturer. Many manufacturers have product sheets that will go into more detail than the information on the product label and may even include efficacy studies that could impact the decision-making process. Review the material safety data sheet (MSDS) for the product to determine the nature of hazards associated with product use and specific product disposal requirements. Remember that disinfectants are designed to destroy living cells. Therefore, all disinfectants are likely to be hazardous in one way or another to personnel and to client animals if the product has residual properties or is improperly used.

Before disinfecting surfaces or devices, it is essential to clean the surface first if at all possible. Organic debris can inactivate many disinfectants. Additionally, feces and other body excretions can shield pathogens from contact with the disinfectant thus hindering disinfection.

When using a disinfectant, it is essential that the product be used in accordance with the manufacturer's instructions. Note that the manufacturer's efficacy claims are based on its prescribed dilution ratio, method of preparation, method of application, surface contact time and shelf-life. To clarify, contact time is the minimal amount of time that a product must be in contact with the surface to be disinfected in order to achieve the level of disinfection claimed by the manufacturer. Shelf-life is the amount of time that a diluted product is actively effective for use once prepared from a concentrated product.

There are a number of organizations in Europe that provide lists of disinfectants for use in animal husbandry, human medicine, and the food industry. Examples are the German Veterinary Medical Society (DVG), and the Germany Society for Hygiene and Microbiology (DGHM). These lists can be consulted for commercial disinfectant preparations that can be used in certain situations and against different types of pathogens.

Under circumstances where it is critical to assure that disinfection practices are efficacious, (i.e. infectious disease outbreaks, immunosuppressed animal housing), it may be beneficial to perform a surface contamination test. There are several techniques described in the



literature that may be used for surface testing (Tamasi, 1995). The floor may be the surface of choice for such a test because it is likely to receive the most contamination of the environmental surfaces, and this surface often has irregularities that will make disinfection a challenge.

In cases of disease outbreaks, disinfection procedures may be dictated by law and close cooperation with veterinary authorities may be necessary.

Summary

Cleaning and disinfection are a major component of disease prevention and eradication in every zoological exhibition. Despite the development of antimicrobial drugs and effective vaccines, infectious diseases remain a major threat to animals kept in captivity as well as our wildlife population. The proper selection and proper use of disinfectants requires extensive knowledge not only about the effectiveness of specific disinfectant compounds, but also about the infectious agent, the conditions under which the disinfectant will be used and the side effects of each compound, including toxic, caustic and corrosive properties.

However, disinfection is only one aspect of an effective infectious disease control program in any animal or human health environment. As described in this chapter proper sanitation consists of multiple components in addition to cleaning and disinfection. Insect and vermin control, and proper food storage, handling, preparation and distribution are equally important for a healthy zoo environment. Furthermore, sanitation should be part of a preventive veterinary medicine program that would further include training of personnel, proper nutrition and feeding, routine surveillance and prophylactic medicine including vaccination, quarantine of new and sick animals, proper holding facilities, correct disposal of waste products and dead animals.

This chapter can only provide a brief overview of the guidelines of cleaning and disinfection. Before using a disinfectant, the manufacturer's label should always be studied. When dealing with a specific disease problem, further information regarding disinfection can be found in the disease specific fact sheets. Additionally, the listed references will provide more detailed information.



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Table 1: Effectiveness of classes of disinfectants against various pathogens

Infectious Agent	Class of Disinfectant									
	Alcohols	Aldehydes	Alkalis	Biguanides	Halogens		Organic and Anorganic Acids	Peroxygen Compounds	Phenols	QAC
					Chlorine Compounds	Iodine Compounds				
Bacteria	obligat intracellular									
	gram-positive									
	gram-negative									
	bacilli									
	endospores									
Fungi	including spores									
Viruses	enveloped									
	non-enveloped									
Protozoa	including oozysts									
Prions										

 highly effective
  effective
  limited effectiveness
  not effective

This table provides only a general overview of the effectiveness of various classes of disinfectants. The effectiveness of a specific compound in each of these classes may vary. For more detailed information please review the information provided in the text. Always consult the manufacturers label for correct use and concentration of each compound.

The table is based on the following sources: Boothe, 1998; Bruins and Dyer, 1995; Jaroll, 1999; Jeffrey, 1995, Lemarie and Hosgood, 1995; Maillard and Russell, 1997; McDonnell and Russell, 1999; Quinn and Markey, 1999 and 2000; Russell, 1996, 1998 and 1999; Sattar and Springthorpe, 1999; Springthorpe and Sattar, 1990; Taylor, 2000; Widmer and Frei, 1999; Williams, 1997.