GV-SOLAS
Gesellschaft für Versuchstierkunde

Specialist information

from the Committee for Hygiene (GV-SOLAS)

Prophylactic and therapeutic measures in selected infections of laboratory rodents and rabbits

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Contents

Introduction

The use of prophylactic hygiene measures, such as controlled procurement, barrier systems, rederivation, sterilization and disinfection, is the preferred approach to maintain the health of laboratory animals. If such measures are applied according to the current level of knowledge in laboratory animal science, invasion of infectious pathogens is also largely prevented, making treatment with antibiotics or chemotherapeutic agents redundant.

Should an infection nevertheless occur in an animal population, the most reliable approach is always to isolate (and if possible, kill) the animals affected and, to preserve the strain, perform rederivation by means of embryo transfer or hysterectomy. Therapeutic measures are usually useless. However, some alternatives to the treatment of laboratory animals, i.e. without the aid of classical rederivation techniques such as embryo transfer, are known. But these are very specific and not applicable to every pathogen. One approach that has proved successful, for example, is to break the chains of infection by ensuring that no susceptible host animals (such as young animals, new imported animals) are available in an infected population any longer (use of this approach e.g. with murine hepatitis virus, murine rotavirus, Sendai virus or *Encephalitozoon cuniculi*). Vaccination, suppression of pathogens by other, less pathogenic microorganisms or crossing in of resistant host strains may also be used in exceptional cases. A complete elimination of pathogens is more likely with the use of such methods than with the therapeutic or prophylactic use of drugs. **Important factors militate against the use of drugs in laboratory animal facilities:**

1. It is generally **not possible to eliminate infectious pathogens from the animal population completely** by means of medical treatment. Even if the animals are cured of disease symptoms and appear to be free from disease, there may still be autochthonic flora in the animal or the environment that have survived treatment. Any such microorganisms may retain their infectiousness and pathogenicity and be capable of infecting further animals or, after a reproduction phase, also cause disease in the animal originally treated.
2. Shortly after medical therapy it is often impossible to detect the specific pathogens. This leads to a "**masking of the infection**". In hygiene monitoring of laboratory animal populations and also in diagnostic tests on clinically diseased animals, false-negative microbiological findings are a serious problem.
3. Moreover, **antibiotic treatment can disturb the natural balance of the microorganisms present**. Antagonistic microbial behaviour can favour particular microorganisms. Examples are known in which the antibiotic treatment of *Pasteurellaceae* species has favoured the growth of *Klebsiella*.
4. In addition, the **risk of developing antibiotic resistance** is to be expected in all animals receiving antibiotic treatment.
5. Finally, it must be borne in mind that medical therapy can also have **negative side effects on both the animal and the experiment**. Ivermectin, for example, can cross the blood-brain barrier in newborn and young animals or predisposed transgenic mice and lead to fatalities. Serious disturbances of the natural bacterial ecosystem in the gut after antibiotic treatment have been described; lethal side effects of penicillin use in guinea pigs are a classical example of this. Pretreated animals are often of no use,

especially for pharmacological studies, because pharmacotherapy could alter the effect of drugs administered later for months as a result of interactions

So why is there still a paper by GV-SOLAS on prophylactic and therapeutic measures?

In individual cases, it can certainly make sense to administer therapeutic measures in laboratory animals:

1. Therapeutic treatments may be undertaken in breeding animals if **rare laboratory animal strains (e.g. transgenic animals) with a small number of animals are infected** which puts strain preservation at a risk. Strict separation of the animals to be treated from healthy animals is essential.

   The use of antibiotics in breeding animals may also be useful in order to reduce the infection pressure and increase the probability of successful **rederivation especially in immunodeficient animals**.

2. A further reason for medical treatments is **to prevent pain, suffering and distress resulting from infections in laboratory animals**. In the case of procedures with an increased risk of postoperative infection, for example, prophylactic use of antibiotics may also be indicated in addition to the need for sterile working conditions.

3. In the case of **individual animals** in an experiment, therapeutic treatment e.g. with antibiotics is sometimes reasonable if the burden of the experiment has led to **an infection with ubiquitous microorganisms** and the animals should remain in the experiment (e.g. skin infections with *Staphylococcus aureus* or streptococci in stressed animals). Needless to say, it must be established beforehand whether or not the use of antibiotics might influence the result of the experiment.

There are several rules that generally need to be followed when administering prophylactic and therapeutic measures:

1. For technical reasons alone, the administration of therapeutics is confined **to individual animals or small numbers of animals**, unless it is possible to administer them via drinking water or feed. However, if antibiotics are administered via the drinking water/feed, it must always borne in mind that this could mean that the animals receive subtherapeutic doses and that the concentrations of active substance achieved are thus insufficient. The administration of subtherapeutic doses also facilitates the growth of resistant bacteria, which in turn carries the risk that resistant bacteria could be transmitted to humans. For this reason, preference should be given to the parenteral administration of antibiotics in therapeutically effective doses. Besides the sufficiently high dosage and long duration of therapy, it is urgently recommended to generate an antibiogram before any use of antibiotics in order to avoid unnecessary and repeated use of different antibiotics.

2. **All prophylactic and therapeutic measures must be fully documented** with an explanation of the reason for these measures, the medicines used along with the batch numbers, dose and route of administration and the time and duration of the intervention. This requirement for documentation not only applies to experiments conducted under GLP conditions but is also part of the duty of care incumbent on every animal facility management and all animal project leaders with regard to such measures in breeding and also before and during an animal experiment.

3. Potential interactions of the drugs used with test substances or any direct influence on the study parameters measured must be carefully assessed.

On the following pages, examples of the prophylactic and therapeutic use of drugs in infections with parasites, fungi and bacterial pathogens in small laboratory animals are listed and methods to combat virus infections (breeding cessation, vaccination etc.) are described. This description is no substitute for studying the cited and also the more recent literature. While the details regarding dosing and types of administration were researched with great care, the authors cannot guarantee that the data provided are correct. Further literature / databases:

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11. VETIDATA (Veterinärmedizinischer Informationsdienst für Arzneimittelanwendung, Toxikologie und Arzneimittelrecht). http://www.vetidata.de/

Examples of use

Parasites

Special note concerning antiparasitic agents

Numerous antiparasitic drugs – levamisole, tiabendazole, fenbendazole, oxfendazole, and ivermectin, among others – influence the function of various immunologically active cells. They can stimulate or also suppress these cells and hence the immune response.

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Pinworms in mice and rats (*Syphacia muris/obvelata, Aspiculuris tetraptera*)

Fenbendazole

Fenbendazole has ovicidal, larvicidal and adulticidal properties. Animal experiments (e.g. tests on motor behaviour and tumour studies) can be influenced by treatment with fenbendazole (1-6). Treatment can also lead to a reduced litter size in rats (7).

Product: fenbendazole (Panacur®, Coglazol®)

Dose: it is customary to have the product mixed with mouse and rat feed in a concentration of 100-150 ppm by a compound feed producer with a manufacturing licence.

Administration, duration: oral administration as medicated feed, duration of use at least 3 months, ideally 6 months (or longer)

Result: relatively good with prolonged treatment, provided adequate hygiene measures are taken in support of treatment (see p. 7) and there are no re-infections (8-11).

Avermectins (ivermectin, selamectin)

Important general information on avermectins:

1. Avermectins paralyse and kill adult and most larval stages of gastrointestinal nematodes as well as burrowing and blood-sucking ectoparasites. They are not effective against nematode eggs.
2. In hypersensitive animals (e.g. MDR 1 defect mutants) and in young animals during the suckling period, signs of central nervous intoxication and deaths can occur as a result

of treatment with ivermectin (10, 12-14). In mice (CF-1), maternal toxicity (minimum effect level: 0.2 mg/kg b.w.) and teratogenic effects (minimum effect level: 0.4 mg/kg b.w.) have been observed well below the therapeutic doses published by many authors (15). Selamectin appears to be better tolerated than ivermectin. With the topical application of ivermectin, skin irritation at the application site may occur as a transient side effect (16). There is also evidence to suggest that ivermectin has an influence on certain behavioural tests (10, 17-19) and may affect experiments on Cre/loxP-mediated mutagenesis in the mouse (20).

3. Ivermectin is also rapidly absorbed through the skin. It accumulates in the liver and the fatty tissue. For this reason, appropriate personal protection equipment (respiratory protection, nitrile gloves) must be used when administering in the form of a spray or drop solution.
4. Ivermectin is not water-soluble; but due to its fat solubility it can be dissolved in propylene glycol and subsequently produced an emulsion with water. In this application form, it remains stable for up to 72 hours at room temperature when sealed and protected from light (21). Care must be taken to ensure that the mixture is kept in emulsion.
5. With avermectins, the supporting hygiene measures below are necessary for all routes of administration.

<u>Ivermectin</u>

Product: ivermectin (Ivomec®)

Administration: oral

Dose (mouse): 8 mg/L drinking water – at a bodyweight of 20 g and daily water intake of 4 mL this corresponds to a dose of 1.6 mg/kg b.w.

Dose (rat): 25 mg/L drinking water – at a bodyweight of 250 g and daily water intake of 15 mL this corresponds to a dose of 1.5 mg/kg b.w.

Duration: 4 days, repeating treatment 3-4 times every 3 days

Result: eradication of *Syphacia* spp. in mice and rats (test 32 weeks after end of treatment) (22). Pinworms were still detected after only 1-2 repeat treatments.

Ivermectin has also been administered in the feed (mixed by feed producer in a concentration of 2 mg/kg feed) in laboratory animal facilities; but the method has not yet been published with regard to the effect on endoparasites.

Spray solution: 1 part ivermectin (1%) and 10 parts water

Administration: spray mice in fresh cage (incl. bedding and interior wall) with 1-2 mL solution (equivalent to 0.9-1.8 mg ivermectin)

Duration: 3 treatments at weekly intervals (23)

Caution: exact dosing is not possible.

Drop solution: Ivermectin 1% undiluted

Administration, dose: drop application between shoulder blades of mice (2 mg/kg b.w., equivalent to 1 µL/5 g b.w.)

Duration: 2 treatments at an interval of 10 days (24)

Result: with both percutaneous methods of treatment, the mice remained free of pinworm over the observation period of 6 months (23, 24).

Selamectin

Product: selamectin (Stronghold®)

Administration, dose: single dose of 10 mg/kg b.w. as spot-on application between shoulder blades of mice

Limited success: 37% success rate against *S. obvelata* and 49% against *A. tetraptera* (test 3 weeks after application) (25).

Supporting hygiene measures

Pinworm eggs are highly resistant and can survive in ambient air, air-conditioning ducts, drinking devices and so on. Aside from treating the stock, therefore, supporting hygiene measures (cleaning, disinfection) are also very important. Mechanical cleaning already brings about a substantial reduction in the contamination of equipment and facilities, ensuring that subsequent disinfection measures are more efficient. Disinfectants in normal use are unable to kill pinworm eggs. This is only possible using special disinfectants (e.g. disinfectants based on cresols). Dry heat (100°C) for 30 min., autoclaving and ethylene oxide completely kills all eggs. Treatment with formaldehyde gas and chlorine dioxide kills 94-96% of eggs. There is no sufficient data as yet on the effectiveness of gaseous hydrogen peroxide (10, 26).

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Ectoparasites

Note: The precautions and warnings regarding the use of ivermectin in pinworm infestation (see “Important general information on avermectins”, p. 6-7) must also be borne in mind when using this product to combat ectoparasites!

Mites in rabbits (*Psoroptes cuniculi*, *Sarcoptes scabiei*)

Product: Ivermectin (Ivomec®)

Dose: 0.2-0.4 mg/kg b.w.

Administration, duration: subcutaneous, twice at an interval of 1-3 weeks (1)

Peculiarities: the massive release of antigens when ectoparasites die off can lead to an increased immune response (2).

Product: selamectin (Stronghold®)

Dose, administration: single dose of 6-18 mg/kg b.w. as spot-on application on the skin of the neck (3, 4).

Product: imidacloprid/moxidectin (Advocate®)

Dose: 10 mg imidacloprid and 1 mg moxidectin per kg b.w.

Administration, duration: percutaneous (in the neck), 3 times at an interval 4 weeks each time (5).

The treatment methods listed are promising if all animals in the stock are treated. A disinfection of the environment is also absolutely essential.

Mites in guinea pigs (*Chirodiscoides caviae*, *Trixacarus caviae*)

<u>Ivermectin</u>

Product: ivermectin (Ivomec®)

Administration, dose: subcutaneous (0.5 mg/kg b.w.)

Duration: 2 treatments at an interval of 7 days

Result: clinical cure of infestation with *T. caviae* (observation period: 8 months) (6).

However, the subcutaneous administration of ivermectin (0.5-1.5 mg/animal twice at an interval of 2 weeks) was unsuccessful in the case of *C. caviae* (7).

Combination: ivermectin spray and drop solution

Spray solution: 1 part ivermectin 1% and 49 parts mixture of propylene glycol and water (1:1)

Drop solution: ivermectin 1% undiluted

Administration, dose:

- Spray solution (1st treatment): spray adult animals with 2.5 mL solution (equivalent to 0.5 mg ivermectin) and young animals (older than 1 week) with 1.2 mL solution on back and flanks
- Drop solution (repeat treatment): in the case of adult animals apply 11 drops (equivalent to 4.4 mg ivermectin) to the trunk and flanks, in young animals (up to 6 weeks) 7 drops and in newborns (less than 1 week) 4 drops

Duration: spray solution once daily over 5 days, followed by a 14-day pause, then drop solution once daily over 5 days

Result: lasting eradication of *C. caviae* (7).

Mites in mice and rats (*Myobia musculi, Myocoptes musculinus, Radfordia affinis, Radfordia ensifera*)

Ivermectin

Product: Ivermectin (Ivomec®)

Administration, dose: subcutaneous (0.2 mg/kg b.w.) in mice

Duration: 2 treatments at an interval of 7 days

Result: eradication of M. musculinus and M. musculi (test 5 weeks after end of treatment) (8).

Product: Ivermectin (Ivomec®)

Administration, dose: oral (32 mg/L drinking water) in mice

Duration: 10 days, repeat treatment twice at an interval of 7 days in each case

Result: clinical cure in mice infested with *M. musculinus* (observation period: 9 months) (9).

Administration, dose: oral (1.3 mg/kg b.w.; i.e. 12 ppm in ground feed, compounded by the feed producer and then irradiated) in mice

Duration: 8 weeks

Result: mites (*Myobia musculi* or *Myocoptes musculinus*) could not be detected after 1 week, but the authors considered an 8-week course of treatment to be more reliable. Some deaths occurred in an experiment in which tumour cells were administered intracranially to newborns from the 1st day after birth (10-12).

Spray solution: 1 part ivermectin 1% and 99 parts mixture of propylene glycol and water (1:1)

Administration: spray mice in a clean cage with 1.1 mL solution (equivalent to 0.11 mg ivermectin) from a distance of 0.5 m

Duration: 3 treatments at intervals of 7 days

Result: no mites were detected 18 weeks after the end of treatment, but mite eggs were still found (13).

Drop solution: ivermectin 1% undiluted

Administration, dose: drops between the shoulder blades of mice and rats (2 mg/kg b.w., equivalent to 1 µL/5 g b.w.)

Duration: 2 treatments at an interval of 10 days (mouse) and 3 treatments at intervals of 14 days (rats from weaning age)

Result: eradication of *M. musculi* and *R. affinis* in mice (test 24 weeks after the end of treatment) (11) and *R. ensifera* in rats (test 18 weeks after the end of treatment) (14, 15).

Combination: cross-fostering and ivermectin in mice

Mice aged 0-36 hours are placed with foster mothers (ideally hairless mice, i.e. mice that are not susceptible to ectoparasites) and are raised by these surrogates.

Administration: topical treatment of foster mothers with ivermectin immediately before the transfer; in addition, one or more topical treatments of the raised young (immediately after weaning, repeat treatment at intervals of 8-10 days if necessary)

Dose: 2 mg/kg b.w.

Result: lasting eradication of *M. musculi* and *M. musculinus*; no damage to young animals (16).

<u>Selamectin</u>

Product: selamectin (Stronghold®)

Dose, administration: single dose of 10-12.4 mg/kg b.w. as spot-on application between shoulder blades of mice

Result: eradication of *M. musculi*, *M. musculinus* and *R. ensifera* (test 3 weeks after application) (17).

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Demodex mites in hamsters (*Demodex aurati*, *Demodex criceti*)

The infection is widespread and can become clinically active as a result of stress, vitamin deficiency or hypervitaminosis A (multifactorial disease).

A reliable therapy is not known.

Demodex mites in mice (*Demodex musculi*)

Administration of ivermectin in the diet (12 ppm) for 8 weeks as described for the treatment of fur mites (Arbona et al. 2010) did not eliminate demodex mites. They were detectable 4 weeks after termination of the treatment. Elimination is possible by hysterectomy and embryo transfer

Protozoa

Flagellates in mice, rats, hamsters and guinea pigs

Treatments with various drugs have proved unsuccessful: after treatment was discontinued, protozoa could still be detected. On treatment with metronidazole, guinea pigs showed a markedly retarded weight gain.

Literature:

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Coccidia in rabbits (*Eimeria* spp.)

Product: toltrazuril (Baycox®) 2.5%

Administration: orally via the drinking water; not to be used in pregnant animals

Dose, duration: for prophylaxis, administer 10-15 mg/L drinking water continuously; for therapy administer 25 mg/L drinking water on 2 days, followed by 2-day repeat therapy after 5 days

Result: marked reduction in oocyst elimination and clinical signs of disease (1, 2).

Product: diclazuril (Clinacox® 0.5%)

Dose, administration: 1 ppm in feed

Duration: at least 4 weeks

Result: marked reduction in oocyst excretion and clinical signs of disease (3-5).

Note: in animals that already show signs of diarrhoea, treatment with anticoccidial drugs alone might not be sufficient. Elimination of the pathogen is not possible.

Literature:

1. Beck W, Arnold S, Hansen O, Pfister K. 2004. Bekämpfung der Eimeria- und Passalurus ambiguus-Infektion beim Kaninchen mit Toltrazuril (Baycox®) und einer Wirkstoffkombination aus Praziquantel, Pyrantelemboat und Febantel (Drontal®- Plus). Kleintierpraxis 49:283-288. (GERMAN)
2. Peeters JE, Geeroms R. 1986. Efficacy of toltrazuril against intestinal and hepatic coccidiosis in rabbits. Vet Parasitol 22:21-35.

3. Kintzel P, Hasslinger M. 1995. Wirksamkeit und Verträglichkeit von Robenidine und Diclazuril bei der Bekämpfung der Kokzidien des Kaninchens. Prakt Tierarzt 76:250-256, 1995. (GERMAN)
4. Vanparijs O, Desplenter L, Marsboom R. 1989. Efficacy of diclazuril in the control of intestinal coccidiosis in rabbits. Vet Parasitol 34:185-190.
5. Vanparijs O, Hermans L, van der Flaes L, Marsboom R. 1989. Efficacy of diclazuril in the prevention and cure of intestinal and hepatic coccidiosis in rabbits. Vet Parasitol 32:109-117.

Encephalitozoon cuniculi in rabbits

E. cuniculi is excreted in the animal's urine and faeces. Infectious spores may be ingested orally and nasally. In the infected animal, the parasites disseminates haematogenously in almost all organs, so diaplacental transmission is also possible. Attention must be paid to these transmission pathways when the animal population is decontaminated. Particular attention must be paid to the supervisory staff as possible vectors in order to avoid smear infections through contact with faeces and urine.

The incidence of *E. cuniculi* in breeding stocks can be minimized by systematically removing serologically positive animals (1, 2). The complete successive elimination of the pathogen that may be achieved in this way must be monitored by continuous serological screening.

Encephalitozoonosis usually is subclinical. There are no known treatments for clinically sick animals, and the chances of a cure drop as the intensity of symptoms increases. Examples of therapy in the case of clinical symptoms can be found in the overview by Ewringmann and Göbel (3).

Prophylactic and therapeutic management:

Product: fenbendazole (Panacur®, Coglazol®)

Dose: 20 mg/kg b.w.

Administration, duration: orally, 28 days

Result: prevention of experimental infections with E. cuniculi, no pathogens detected in the brain of naturally infected, seropositive animals (4).

Literature:

1. Bywater JE, Kellett BS. 1978. The eradication of *Encephalitozoon cuniculi* from a specific pathogen-free rabbit colony. Lab Anim Sci 28:402-404.
2. Cox JC, Gallichio HA, Pye D, Walden NB. 1977. Application of immunofluorescence to the establishment of an Encephalitozoon cuniculi-free rabbit colony. Lab Anim Sci 27:204-209.
3. Ewringmann A, Göbel T. 1999. Untersuchungen zur Klinik und Therapie der Encephalitozoonose beim Heimtierkaninchen. Kleintierpraxis 44:313-400. (GERMAN)
4. Suter C, Müller-Doblies UU, Hatt JM, Deplazes P. 2001. Prevention and treatment of Encephalitozoon cuniculi infection in rabbits with fenbendazole. Vet Rec 148:478-480.

Fungi

Pneumocystis spp. in immunodeficient mice and rats

Product: co-trimoxazole (Cotrim K/E ratiopharm® syrup)

1 mL Cotrim K contains 8 mg trimethoprim and 40 mg sulfamethoxazole

1 mL Cotrim E contains 16 mg trimethoprim and 80 mg sulfamethoxazole

Administration: orally via the drinking water

Dose: 5 ml Cotrim K syrup or 2.5 ml Cotrim E syrup / L drinking water – this is approximately equivalent to a dose of trimethoprim 6 mg/kg b.w. and sulfamethoxazole 30 mg/kg b.w. in a rat or a dose of trimethoprim 8 mg/kg b.w. and sulfamethoxazole 40 mg/kg b.w. in a mouse.

Duration: 2 weeks treatment, 1 week pause, 2 weeks treatment (1). Drinking bottles changed 3 times a week. All animals in the room are treated, not only the immunodeficient animals.

Result: good. With this treatment, pneumocystosis in groups of immunodeficient mice (pathogen: *P. murina*) and rats (pathogen: *P. carinii* or *P. wakefieldi*) can be favourably managed (regression of clinical disease for some months; no deaths) (2, 3). Afterwards, cases of disease relapse (pathogen not eliminated).

The use of simple filter-top cages has proved a successful means to combat the spread of infection with *Pneumocystis*.

Literature:

1. Mossmann H, Nicklas W, Hedrich HJ. 2002. Management of immunocompromised and infected animals. *In:* Kaufmann HE, Kabelitz D (eds), Methods in microbiology: immunology of infection, Vol. 32, pp. 183-231, Academic Press, London.
2. Macy JD, Weir EC, Compton SR, Shlomchik MJ, Brownstein DG. 2000. Dual infection with *Pneumocystis carinii* and *Pasteurella pneumotropica* in B cell-deficient mice: diagnosis and therapy. Lab Anim Sci 50:49-55.
3. Weisbroth SH. 2006. Pneumocystis: newer knowledge about the biology of this group of organisms in laboratory rats and mice. Lab Anim Europe 6(9):39-46.

Bacteria

Clostridium piliforme (Tyzzer's disease)

C. piliforme is an extremely tenacious spore forming bacterium. Peracetic acid (1.0%) and sodium hypochlorite (0.3%) have proved to be sporicidal, whereas formaldehyde shows only limited sporicidal activity at high concentrations and with long reaction times (1,2). Peracetic acid and a combination of glutaraldehyde with peracetic acid have been successfully used for room disinfection. Gassing of animal rooms with formalin according to the customary procedure did not reliably kill off the spores, which resulted in animal populations that had undergone hygienic sanitation by means of embryo transfer or hysterectomy becoming reinfected some time after being introduced into the animal rooms (3).

Susceptibility to *C. piliforme* may possibly be associated with genetic factors in the host (4-6). Bacterial isolates of various origin, however, also show antigenic heterogeneity which results in host specificity. It is therefore suspected that there are different strains of *C. piliforme* (7, 8).

In some breeding facilities it has been found that a change of animal species when restocking animal rooms can help to eliminate an infection. Reinfection can be prevented if decontaminated animals are no longer housed for months to years in rooms where previously infected animals of the same species were accommodated (3).

A promising therapy is not known. Treatment with tetracycline via the drinking water may be attempted in order to reduce disease and deaths (9, 10).

Literature:

1. Ganaway JG. 1980. Effect of heat and selected chemical disinfectants upon infectivity of spores of *Bacillus piliformis* (Tyzzer´s disease). Lab Anim Sci 30:192-196.
2. Boivin GP, Hook RR, Riley LK. 1993. Antigenetic diversity in flagellar epitops among *Bacillus piliformis* isolates. J Med Microbiology 38:177-182.
3. Hansen AK, Skoovgard-Jensen HJ, Thomsen P, Svendson O, Dagnaes-Hansen F, Mollegard-Hansen KE. 1992. Rederivation of rat colonies seropositive for *Bacillus piliformis* and the subsequent screening for antibodies. Lab Anim Sci 42:444-448.
4. Hansen AK, Svendson O, Mollegard-Hansen KE. 1990. Epidemiological studies of *Bacillus piliformis* infection and Tyzzer's disease in laboratory rats. Z. Versuchstierk. 33:163-169.
5. Livingston RS, Franklin CL, Besch-Williford CL, Hook RR Jr, Riley LK. 1996. A novel presentation of *Clostridium piliforme* infection (Tyzzer's disease) in nude mice. Lab Anim Sci 46:21-25.
6. Waggie KS, Hansen CT, Ganaway JR, Spencer TS. 1981. A study of mouse strain susceptibility to *Bacillus piliformis* (Tyzzer's disease): the association of B-cell function and resistance. Lab Anim Sci 31:139-142.
7. Boivin GP, Hook RR, Riley LK. 1993. Antigenetic diversity in flagellar epitops among *Bacillus piliformis* isolates. J Med Microbiology 38:177-182.
8. Franklin CL, Motzel SL, Besch-Williford CL, Hook RR, Riley LK. 1994. Tyzzer's infection: host specificity of *Clostridium piliforme* isolates. Lab Anim Sci 44:568-572.
9. Hunter B. 1971. Eradication of Tyzzer's disease in a colony of barrier-maintained mice. Lab Anim 5:271-276.
10. Yokoiyama S, Fujiwara K. 1971. Effect of antibiotics on Tyzzer's disease. Jpn J Exp Med 41:49-57.

Helicobacter spp. in mice, rats and gerbils

Helicobacter infections can be reliably eradicated by embryo transfer (1) and hysterectomy (2). It has further been shown that eradication of *H. hepaticus* (and probably also other *Helicobacter* species) can be achieved in the mouse by transferring the neonates of infected mothers to foster mothers free from *Helicobacter* (3, 4). The prospects of success are greatest here if the neonates are transferred on the first day following their birth (neonatal transfer). As

a supporting measure, antibiotic-containing feed (triple therapy, see below) may be administered to the pregnant dams as well as the foster mothers and the offspring (5). Similar strategies have proved successful in the sanitation of rats (3, 6).

Antibiotics (amoxicillin or combinations with amoxicillin) have been successfully used in therapy and prophylaxis for *Helicobacter*-associated diseases (diarrhoea, hepatitis and typhlitis) in immunodeficient mice (7, 8). In the literature, however, differing results are cited regarding the success rates of antibiotics administered for the eradication of *Helicobacter* infections (6, 8-11). To date, the most promising approach has proved to be the administration of amoxicillin, clarithromycin, metronidazole and omeprazole (a proton pump inhibitor) in the feed (quadruple therapy, see below) (6, 11).

Caution: deaths have been observed in the gerbil as a result of *Clostridium difficile*-associated enterotoxaemia following triple therapy (12).

Examples of treatment:

Combination: cross-fostering and triple therapy (amoxicillin, metronidazole and bismuth)

Mice aged 0-24 hours are placed with Helicobacter-free foster mothers and raised by them.

Administration, duration: with the feed; treatment of mothers from the 2nd week of gestation until neonatal transfer; in addition, 5 weeks of therapy in foster mothers and young animals

Dose: 3 mg amoxicillin, 0.69 mg metronidazole and 0.185 mg bismuth in 5 g of feed

Result: *H. bilis* and *H. hepaticus* are not detectable in the decontaminated mouse colony (28 months after end of treatment) (5).

Quadruple therapy (rat): amoxicillin, clarithromycin, metronidazole and omeprazole

Administration: with the feed

Dose: 6.7 mg amoxicillin, 1.7 mg clarithromycin, 3.3 mg metronidazole and 0.07 mg omeprazole in 5 g of feed

Duration: 2 weeks, 3 times at intervals of 2 weeks

Result: *H. bilis*, *H. rodentium* and *H. typhlonius* are not detectable (8 months after end of treatment) (6).

Quadruple therapy (mouse): amoxicillin, clarithromycin, metronidazole and omeprazole

Administration: with the feed

Dose: 3 mg amoxicillin, 0.5 mg clarithromycin, 1 mg metronidazole and 0.02 mg omeprazole in 5 g of feed

Duration: 8 weeks

Result: *H. bilis* and *H. hepaticus* are not detectable (19 months after end of treatment) (11).

Literature:

1. Van Keuren ML, Saunders TL. 2004. Rederivation of transgenic and gene-targeted mice by embryo transfer. Transgenic Res 13:363-371.

2. Glage S, Dorsch M, Hedrich HJ, Bleich A. 2007. Rederivation of Helicobacter hepaticus-infected Mongolian gerbils by Caesarean section and cross-fostering to rats and mice. Lab Anim 41:103-110.
3. Singletary KB, Kloster CA, Baker DG. 2003. Optimal age at fostering for derivation of Helicobacter hepaticus-free mice. Comp Med 53:259-264.
4. Truett GE, Walker JA, Baker DG 2000. Eradication of infection with Helicobacter spp. by use of neonatal transfer. Comp Med 50:444-451.
5. Kerton A, Warden P. 2006. Review of successful treatment for Helicobacter species in laboratory mice. Lab Anim 40:115-122.
6. Jury J, Gee LC, Delaney KH, Perdue MH, Bonner RA. 2005. Eradication of Helicobacter spp. from a rat breeding colony. Contemp Top Lab Anim Sci 44:8-11.
7. Russell RJ, Haines DC, Anver MR, Battles JK, Gorelick PL, Blumenauer LL, Gonda MA, Ward JM.1995. Use of antibiotics to prevent hepatitis and typhlitis in male scid mice spontaneously infected with Helicobacter hepaticus. Lab Anim Sci 45:373-378.
8. Shomer NH, Dangler CA, Marini RP, Fox JG. 1998. *Helicobacter bilis/Helicobacter rodentium* co-infection associated with diarrhea in a colony of scid mice. Lab Anim Sci 48:455-459.
9. Foltz CJ, Fox JG, Yan L, Shames B. 1995. Evaluation of antibiotic therapies for eradication of Helicobacter hepaticus. Antimicrob Agents Chemother 39:1292-1294.
10. Foltz CJ, Fox JG, Yan L, Shames B. 1996. Evaluation of various oral antimicrobial formulations for eradication of Helicobacter hepaticus. Lab Anim Sci 46:193-197.
11. Kostomitsopoulos N, Donnelly H, Kostavasili I, Paronis E, Alexakos P, Karayannacos P. 2007. Eradication of *Helicobacter bilis* and *H. hepaticus* from infected mice by using a medicated diet. Lab Anim Europe 7(6):17-22.
12. Bergin IL, Taylor NS, Nambiar PR, Fox JG. 2005. Eradication of enteric Helicobacters in Mongolian gerbils is complicated by the occurrence of *Clostridium difficile* enterotoxemia. Comp Med 55:265-268.

Mycoplasmas in rats

Treatment with various drugs (e.g. tetracycline or enrofloxacin) leads to a reduction in clinical symptoms, but after the withdrawal of these medications, the continued presence of the pathogens can be expected. Since mycoplasmas have an especially close association with the host cells, particularly binding tightly to their membranes, it is not possible to eliminate the pathogen from the host.

Examples of treatment:

Product: tetracycline (Tetraseptin®)

Dose: 5 mg/mL drinking water

Administration: orally via the drinking water

Duration: at least 5 days

Peculiarities: solution every 3 days with the addition of potassium sorbate (1.35 mg/mL drinking water) to prevent the growth of yeast or prepare afresh each day.

Product: enrofloxacin (Baytril®)

Dose: 10 mg/kg b.w.

Administration: orally (5 mL 2.5% Baytril solution in 1 L drinking water)

Duration: at least 5 days.

Literature:

1. Harkness JE, Wagner JE. 1995. Specific diseases and conditions. *In:* Harkness JE, Wagner JE (eds), The biology and medicine of rabbits and rodents, 4th edition, pp. 171-321. Williams & Wilkins, Baltimore.

***Pasteurellaceae* in mice, rats and rabbits**

Pasteurellaceae show good *in vitro* susceptibility to many active substances. Nevertheless, antibiotics should only be used to treat *Pasteurellaceae* after testing for sensitivity, because many strains are resistant to certain treatments. It has been repeatedly reported that the pathogen could not be eliminated *in vivo*, although the bacteria have shown good *in vitro* sensitivity to the antibiotics used.

Positive effects of antibiotics used to treat *Pasteurellaceae* infections in various organ systems have repeatedly described. Treatments with various active substances (e.g. ampicillin, chloramphenicol and tetracycline) led to the regression of clinical symptoms in mouse and rat (1-3), but even after the administration of several antibiotics *P. pneumotropica* (now *Rodentibacter* sp.) was still detected for some time after the end of treatment.

Examples of treatment:

Pasteurella pneumotropica (now *Rodentibacter* sp.) in mice

Product: enrofloxacin (Baytril®)

Dose: 25.5 or 85 mg/kg b.w.

Administration, duration: orally (170 mg or 570 mg/L drinking water) for 2 weeks

Result: with both doses, *P. pneumotropica* could no longer be detected (30 days after the end of treatment) (4). The subcutaneous administration of enrofloxacin at the above doses (twice daily for 2 weeks) was likewise successful. However, our own experiences make us suspicious and call for verification and reproduction of these results.

Pasteurella multocida in rabbits

Product: enrofloxacin (Baytril®)

Dose: 5 mg/kg b.w.

Administration, duration: subcutaneous, twice daily for 10 days

Result: clinical improvement; elimination of the pathogen is not possible (5).

Literature:

1. Ackermann JI, Fox JG. 1981. Isolation of *Pasteurella ureae* from reproductive tracts of congenic mice. J Clin Microbiol 13:1049-1053.
2. Moore GJ. 1979. Conjunctivitis in the nude rat (*rnu/rnu*). Lab Anim 13:35.
3. Moore GJ, Aldred P. 1978. Treatment of *Pasteurella pneumotropica* abscesses in nude mice (*nu/nu*). Lab Anim 12:227-228.
4. Goelz MF, Thigpen JE, Mahler J, Rogers WP, Locklear J, Weigler BJ, Forsythe DB. 1996. Efficacy of various therapeutic regimens in eliminating *Pasteurella pneumotropica* from the mouse. Lab Anim Sci 46:280-285.
5. Mähler M, Stünkel S, Ziegowski C, Kunstyr I. 1994. Inefficacy of enrofloxacin in the elimination of *Pasteurella multocida* in rabbits. Lab Anim 29:192-199.

Viruses

Besides the tried-and-tested methods of embryo transfer and hysterectomy, it is also possible in certain virus infections to interrupt breeding, to transfer neonates to specific-pathogen-free foster mothers and to vaccinate in order to decontaminate infected stocks.

Interruption of breeding

The aim of this method is to break the chain of infection. It presupposes that the virus spreads rapidly in a population and that the immune response of the host eliminates the virus within a few weeks (transient infection) and protects against reinfection. A strategy of breeding cessation together with an import stop leads to the result that the infection runs its course and dies out. As a consequence of breeding cessation pups that are susceptible to the infection are not born. In addition, susceptible animals must not be introduced from outside (e.g. through import) for a period of at least 6-8 weeks. This strategy has proved successful especially in infections with coronaviruses in the mouse (1) and rat (2). But it has to be assumed that breeding cessation can also be successfully used in some other transient viral infections (e.g. murine rotavirus and Sendai virus). The practical procedure is explained in more detail in the section on murine hepatitis virus (pp. 19-20).

No success is to be expected in immunodeficient mice, because these animals are predisposed to persistent infections with sustained excretion of the virus! Scepticism in this respect is also advisable in genetically modified mice.

Literature:

1. Weir EC, Bhatt PN, Barthold SW, Cameron GA, Simack PA. 1987. Elimination of mouse hepatitis virus from a breeding colony by temporary cessation of breeding. Lab Anim Sci 37:455-458.
2. Brammer DW, Dysko RC, Spilman SC, Oskar PA. 1993. Elimination of sialodacryoadentitis virus from a rat production colony by using seropositive breeding animals. Lab Anim Sci 43:633-634.

Neonatal Transfer

It has been shown that eradication of certain virus infections in the mouse (murine norovirus [MNV], murine hepatitis virus [MHV], Theiler's murine encephalomyelitis virus [TMEV] and murine rotavirus) can be achieved by transferring newborn mice of infected and/or seropositive mothers to specific-pathogen-free foster mothers (1-3). A prerequisite for the success of this method is that no infection has yet occurred in the neonates. Therefore, the transfer should take place within 24-48 hours after birth. Furthermore, the neonates should be dipped in a tissue-conserving disinfectant solution (e.g. iodophor solution) for a few seconds before being transferred to wash off or kill any pathogens adhering to the surface of the skin.

The prospects of success are greatest in immunocompetent animals (formation of maternal antibodies and low probability of transmission across the placenta) and in the case of pathogens that are only excreted for a short period (e.g. MHV, murine rotavirus) and/or are transmitted primarily by the faecal-oral route (e.g. MNV, TMEV, murine rotavirus).

Literature:

1. Artwohl JE, Purcell JE, Chrusciel K, Lang M, Fortman J. 2007. Assessment of cross- foster rederivation in the elimination of mouse norovirus and *Helicobacter* (Abstract). J Am Assoc Lab Anim Sci 46:84.
2. Lipman NS, Newcomer CE, Fox JG. 1987. Rederivation of MHV and MEV antibody positive mice by cross-fostering and use of the microisolator caging system. Lab Anim Sci 37:195-199.
3. Watson J, Thompson KN, Feldman SH. 2005. Successful rederivation of contaminated immunocompetent mice using neonatal transfer with iodine immersion. Comp Med 55:465-469.

Vaccination

In principle, it is possible to provide effective protection of small laboratory animals against the outbreak of acute virus infections through the use of appropriate vaccines. Vaccines considered suitable are mainly so-called inactivated (or killed) vaccines, i.e. vaccines that contain virus that is no longer capable of reproducing and an immunostimulant. However, vaccines based on apathogenic virus capable of reproducing (live vaccines) appear to be unsuitable for laboratory animals in particular because there is a risk of test materials (transplantation tumours, virus, bacterium or parasite strains, cell cultures and so on) becoming contaminated.

When the pros and cons of immunization are weighed, the arguments in favour are clearly outweighed by the counterarguments, the most important of which are the following:

1. Almost all known virus infections run a predominantly subclinical course. Acute diseases are therefore the exception. To this extent there is little distinction between the stress of a vaccination and that of a spontaneous infection.
2. Acute viral diseases occur almost exclusively in young animals of suckling age or shortly after weaning, i.e. at a time when a useful vaccination cannot yet be considered in view of a lack of immunocompetence.

3. A stress-free immunization with inactivated vaccines can only be achieved with a basic vaccination followed by a repeat vaccination. Further booster vaccinations may be necessary at 5-month intervals depending on the length of the housing period. Since inactivated vaccines have to be administered by the parenteral route, every animal in the stock has to undergo at least two injections.
4. To ensure that the lasting elimination of a pathogenic field virus is achieved, the 100% immunization of an animal group must be maintained for years - if not decades - (recall, for example, the decades-long smallpox immunization of humans).
5. Serological monitoring of an animal populations` health status cannot differentiate between vaccination and field virus antibodies with the inactivated vaccines customarily used to date.

To summarize, therefore, it remains to be established that the considerable amount of work and hence also cost associated with vaccination bears no relation to the anticipated benefit of such a measure. It is easier, quicker, and cheaper to replace an infected colony by purchasing virus-free animals, which no longer presents a problem with the current levels of hygiene of professional breeders. Before building up a new population with animals from commercial breeding facilities, however, it is advisable to assure oneself of the desired status by performing one's own tests. With the above-mentioned species, the hygienic sanitation of rare animal strains by embryo transfer or hysterectomy is also likely to be a quicker and cheaper way of obtaining virus-free animals whose hygienic status can also be easily and reliably monitored by means of regular serology.

Examples of the sanitation of virus-infected mouse stocks

Murine hepatitis virus: breeding cessation

Since MHV in immunocompetent animals results in only a transient infection lasting 2-4 weeks, a constant supply of susceptible animals (young animals, new purchases) is necessary for the infection to persist in an animal population. If this supply is interrupted, the infection dies out, because previously infected animals have already eliminated the virus and are now immune to this particular strain of the virus.

The practical approach is as follows: the infected mouse population is quarantined for about 2 months, all breeding animals are separated and young animals eliminated (1). During this period, no new animals are introduced to the population. The elimination is checked using virus-free sentinel animals. This method is especially suitable for small colonies (the smaller the colony, the greater the chances of success). Since MHV is a rapidly mutating virus, large mouse populations frequently succumb to new mutated strains of the virus that differ sufficiently from the original strain to undermine the strain-specific immune protection of the first infection. The mice are infected again, and elimination by interruption of breeding is prevented.

Large breeding populations are ideally compartmentalized for sanitation. A few seropositive breeding pairs are selected and kept in isolation in an isolator, in IVC or filter-top cages or behind a barrier for about 2 months. They are then mated, and the offspring is serologically monitored. Attention must be paid, however, to possible interference by maternal antibodies; these may be detected up to 7 weeks after weaning (2, 3). The success of elimination is checked using sentinel animals.

If IVC or filter-top cages are used, it must be borne in mind that several separate populations are formed as a result. This may disrupt the procedure described if the cages are not correctly handled and contamination occurs between the cages. This could then lead to constant new infections because the entire population is not equally contaminated and thus also not equally immunized.

Caution: no success is to be expected in immunodeficient and genetically modified mice, because these animals are predisposed to persistent infections with constant excretion of the virus (2, 4, 5).

Literature:

1. Weir EC, Bhatt PN, Barthold SW, Cameron GA, Simack PA. 1987. Elimination of mouse hepatitis virus from a breeding colony by temporary cessation of breeding. Lab Anim Sci 37:455-458.
2. Dimigen J. 1996. MHV-Sanierung mit Individually Ventilated Cages (IVC-Rack): Eine Alternative zur Hysterektomie und Embryotransfer. Der Tierschutzbeauftragte 177-180. (GERMAN)
3. Homberger FR. 1992. Maternally-derived passive immunity to enterotropic mouse hepatitis virus. Arch Virol 122:133-141.
4. Barthold SW, Smith AL, Povar ML. 1985. Enterotropic mouse hepatitis virus infection in nude mice. Lab Anim Sci 35:613-618.
5. Rehg JE, Blackman MA, Toth LA. 2001. Persistent transmission of mouse hepatitis virus by transgenic mice. Comp Med51:369- 374.

Murine hepatitis virus: vaccination

Various attempts to protect mouse populations against infection with MHV by means of vaccinations have been described. Attenuated virus strains and a recombinant adenovirus expressing MHV-A59 structural proteins were used (1). However, the resulting protection was only specific to the strain of virus. Added to which, an attenuated vaccine strain causes a subclinical infection that is accompanied by many of the disorders of a natural infection. This means that the vaccination produces precisely the situation that should actually be prevented.

A monoclonal antibody targeting an MHV receptor on host cells was also used (2). This led to a marked reduction of the virus titre (homologous strain) in infected tissue, but replication was not prevented.

Literature:

1. Wesseling JG, Godeke GJ, Schijns VE, Prevec L, Graham FL, Horzinek MC, Rottier PJ. 1993. Mouse hepatitis virus spike and nucleocapsid proteins expressed by adenovirus vectors protect mice against a lethal infection. J Gen Virol 74:2061-2069.
2. Smith AL, Cardellichio CB, Winograd DF, de Souza MS, Barthold SW, Holmes KV. 1991. Monoclonal antibody to the receptor for murine coronavirus MHV-A59 inhibits viral replication *in vivo*. J Infect Dis 163:879-882.

Sendai virus

In the literature, it has been reported that Sendai virus infections in mice can be successfully eliminated by vaccination. Since immunocompetent mice eliminate the virus within 1-2 weeks, it can be assumed that the method of breeding cessation described for MHV can likewise be successfully used for sanitation.

Literature:

1. Eaton GJ, Lerro A, Custer RP, Crane AR. 1982. Eradication of Sendai pneumonitis from a conventional mouse colony. Lab Anim Sci 32:384-386.

Ectromelia virus: vaccination

Vaccines based on the heterologous vaccinia virus have been used for the active immunization of mouse populations (1-3). As a rule, immunization protects mice against serious illness, but not against transmission of the pathogen in an animal population, so this vaccination cannot be recommended.

Literature:

1. Bhatt PN, Jacoby RO. 1987. Effect of vaccination on the clinical response, pathogenesis and transmission of mousepox. Lab Anim Sci 37:610-614.
2. Buller RML, Wallace GD. 1985. Reexamination of the efficacy of vaccination against mousepox. Lab. Anim. Sci. 35:473-476.
3. Mahnel H. 2012 Vaccination against mousepox. Tierärztl Prax 13:403-407.

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