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Experience from sentinel health monitoring in units containing rats and mice in experiments

by *Axel Kornerup Hansen & Hans-Jørgen Skovgaard-Jensen,*
Department of Experimental Medicine, University of Copenhagen and National University Hospital, Panum Institute, Blegdamsvej 3B, DK-2200 Copenhagen N, Denmark.

Introduction

Silent infections may disturb animal experiments in several ways. Therefore, health monitoring in breeding units, as well as in the units, where the experiments are performed, is of vital importance (*Hansen* 1994).

Sentinels are animals which are placed in an animal unit with the aim of catching infections for health monitoring purposes. In many experiments this method is the only one available, as the experimental animals might be immuno-compromized or in other ways be unsuitable for health monitoring, or the health monitoring might interfere unacceptably with the experiment. Sentinels must be treated in such a way that the infections are caught by these animals. Normally, the sentinels are placed randomly in the unit, and their clean bedding is mixed with contaminated bedding from the animals in the experiment (*Rehbinder* 1994). Many questions can be raised concerning these sentinels and many factors might influence the prevalence of the infection among the sentinels. The sample size in health monitoring may be calculated as

$$(1)\quad S \geq \frac{\log C}{\log [((1-N_I)p) + (1-p)]} = \frac{\log C}{\log (1-(p*N_I))}$$

where p is the expected prevalence of the infection monitored, N_I is the nosographic sensitivity of the method used and C is *the confidence limit*, i.e. the risk of a false negative result (*Hansen* 1993). This means, that the higher the percentage of sentinels seroconverting, the lower the number of sentinels to be used, e.g. in a study with *Clostridium piliforme* (previously *Bacillus piliformis* (*Duncan et al.* 1993)) in rat colonies, the number of sentinels infected increased with the age of the sentinels at introduction, indicating that fewer sentinels will be needed if older animals are used for detection of *C. piliforme* (*Hansen et al.* 1994). The infective route of the agent is of major importance for the ability of sentinels to catch the agent if present in the experimental animals. E.g. in mice it is known that the unstable Sendai Virus does not rapidly infect sentinels through dirty bedding (*Artwohl et al.* 1994), while on the other hand *Mouse Hepatitis Virus* (*MHV*), although also rather unstable, easily infects sentinels (*Homberger & Thomann* 1994).

As illustrated by these examples optimization of sentinel programmes must be based on practical experience. As little so far has been published about sentinel health monitoring, especially concerning the use of sentinels for non-viral infections, some experiences are published in this paper to give an indication on the suitability and limitations of this method.

Materials and methods

Experimental units

At the Department of Experimental Medicine at the Panum Institute in Copenhagen rodents for experiments are kept in four different units (I, II, III and IV), of which three are protected by a barrier, and one is operated without any protective procedures. The introduction of non-screened biological products is banned in all units. The temperature and humidity is kept at 22 ± 2° and 65–80 %, respectively.

The barrier-protected units. Unit I, which is used for long-term experiments with rats and mice, has a total capacity of approximately

720 rat or mouse cages in six separate rooms. Normal occupancy is about 70 %. About 70 % of the animals are mice. Equipment, diet and bedding is autoclaved or otherwise sterilised before introduction into the unit, and change of clothing is mandatory for staff to enter the unit. Animals withdrawn from the unit are not allowed to reenter. Unit II and unit III work similarly but are used for mice only. Each of these units consists of two separate rooms, and each have a total capacity of approximately 240 cages. Normal occupancy is about 50–70 %. In unit II a limited breeding is performed from which the animals are used for shorter studies. New animals are only introduced with a low frequency. In unit III there is a mixture of long-term and short-term studies. *C. piliforme* is found in some of the rats purchased for unit I. Mice infected with *P. pneumotropica* are occasionally purchased for both unit II and III. Furthermore, in unit III an active version of *Lymphocytic choriomeningitis Virus* (*LCM*) is used for experimental infection. Except for this no infections to be declared according to the FELASA guidelines (*Kraft et al.* 1994) are present in animals purchased for unit I, II or III.

The conventional unit. Unit IV, which is used for short-term experiments, has a total capacity of approximately 720 rat or mouse cages in six separate rooms. Normally about 60 % of the animals are mice. Additionally it houses a limited number of hamsters and gerbils, five to ten cats and 100–200 rabbits in separate rooms. Normal occupancy is about 70 %. New animals are introdced every week. Animals brought to laboratory facilities elsewhere at the university are allowed to reenter. From the rodent breeders delivering to this unit *C. piliforme, Kilham Rat Virus (KRV), Pasteurella pneumotropica* and *Tritrichomonas spp.* are declared present in some of the rats, *P. pneumotropica* is declared present in some of the mice, and *C. piliforme* and *Pneumoniavirus of mice* (*PVM*) is declared present in some of the gerbils. In the rabbits *Bordetella bronchiseptica, Pasteurella haemolytica,* coccidia spp. and *Trichophyton spp.* are declared present. No other infections to be declared according to the FELASA guidelines have been found in the rodents and rabbits purchased for this unit. In the cats *B. bronchiseptica* are declared present. No protective measures are used for preventing the introduction of infections into this unit.

Examination procedures

Sentinels. The four experimental units were monitored by placing rats and mice in each unit for approximately 3 months, after which these were replaced by new sentinels. In each room inside the unit 3 to 6 sentinels were placed in one macrolon type III cage or, if more than 3 sentinels were used, two cages. The total number of sentinels placed in one unit per quarter did not exceed 10. These sentinels were 10–15 weeks old Pan:NMRI mice or Pan:WIST rats from barrier-protected breeding colonies (Panum Institute, DK-2000 Copenhagen N) monitored and found free of infections according to FELASA guidelines. They were given Altromin 1314 or 1324 diet (Altromin Denmark, DK-2820 Gentofte) as well as acidified water ad libitum. Their bedding (Tapvei, SF-73600 Kaavi) was changed every week with clean bedding mixed with approximately 20 % dirty bedding from the other animals in the unit. This paper contains observations from five periods of three months, 15 months in all. The same sentinels were used for all types of examinations, but in some serological assays the number of animals tested was reduced. The exact numbers of sentinels used are given i Table 1.

Laboratory methods. At sampling each sentinel was euthanized by intraperitoneal injection of 20 % pentobarbital with ethanol. All sentinels were inspected clinically and by macroscopic examination of the organs mentioned in FELASA guidelines (*Kraft et al.* 1994) and hereafter examined by bacteriological, parasitological and serological means with the aim of revealing those organisms shown in Table 1. All examinations were

Table 1. Infections observed in sentinel rats and mice in four experimental animal units during a 15 months period. All positive results are further described in Table 2.

	METHOD	Unit I				Unit II		Unit III		Unit IV			
		Purchased[1]		Sentinel		Purch.[1]	Sent.	Purch.[1]	Sent.	Purchased[1]		Sentinel	
		rats	mice	rats	mice	mice	mice	mice	mice	rats	mice	rats	mice
VIRAL INFECTIONS													
Ectromelia Virus	IFA	NT	Neg	NT	0/32	Neg	0/21	Neg	0/37	NT	Neg	NT	0/46
Kilham Rat Virus	IFA/ELISA/HAI	Neg	NT	9/37	NT	NT	NT	NT	NT	Pos	NT	6/41	NT
Lymphocytic Choriomeningitis V.	IFA/ELISA	NT	Neg	NT	0/26	Neg	0/18	Neg[3]	0/35	NT	Neg	NT	0/44
Minute Virus of Mice	IFA/ELISA	NT	Neg	NT	0/35	Neg	0/27	Neg	0/36	NT	Neg	NT	0/44
Mouse Hepatitis Virus	IFA/ELISA	NT	Neg	NT	2/44	Neg	0/35	Neg	0/33	NT	Neg	NT	0/44
Pneumoniavirus of Mice	IFA/ELISA	Neg	Neg	0/31	0/35	Neg	0/29	Neg	0/33	Neg[3]	Neg[3]	2/43	0/36
Rat Coronavirus	IFA/ELISA	Neg	NT	0/34	NT	NT	NT	NT	NT	Neg	NT	10/33	NT
Reovirus type 3	IFA/ELISA	Neg	Neg	0/31	0/35	Neg	0/26	Neg	0/33	Neg	Neg	0/41	0/44
Sendai Virus	IFA/ELISA	Neg	Neg	0/31	0/35	Neg	0/26	Neg	0/33	Neg	Neg	0/41	0/44
Theiler's Encephalomyelitis Virus	IFA/ELISA	Neg	Neg	0/31	0/35	Neg	0/26	Neg	0/33	Neg	Neg	0/41	0/44
Toolan's H1 Virus	IFA/ELISA/HAI	Neg	NT	0/44	NT	NT	NT	NT	NT	Pos	NT	0/39	NT
BACTERIAL INFECTIONS													
C. piliforme	IFA	Pos	Neg	2/18	0/23	Neg	0/25	Neg	0/26	Pos	Neg	6/25	0/20
Bordetella bronchiseptica	Culture	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Citrobacter freundii (4280)	Culture	NT	Neg	NT	0/46	Neg	0/35	Neg	0/41	NT	Neg	NT	0/46
Corynebacterium kutscheri	Culture	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Mycoplasma spp	ELISA	Neg	Neg	0/24	0/24	Neg	0/7	Neg	0/22	Neg	Neg	0/27	0/30
P. pneumotropica	Culture	Neg	Neg	0/37	0/44	Pos	1/38	Pos	0/41	Pos	Pos	0/36	10/49
P. pneumotropica	ELISA	Neg	Neg	24/28	17/27	Pos	6/15	Pos	7/30	Neg	Pos	26/27	4/15
Salmonellae	Culture	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
β-haemolytic streptococci	Culture	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Streptococcus pneumoniae	Culture	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
PARASITE INFECTIONS													
Arthropods	Inspection	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Syphacia spp	Flotation	Neg	Neg	0/37	0/46	Neg	0/38	Neg	0/41	Neg	Neg	2/36	0/46
Other helminths	Flotation	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Eimeria spp	Flotation	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Giardia spp	Microscopy	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Spironucleus spp	Microscopy	Neg	Neg	0/37	0/46	Neg	0/35	Neg	0/41	Neg	Neg	0/41	0/46
Tritrichomonas spp	Microscopy	Neg	Neg	0/37	0/46	Neg	0/38	Neg	0/41	Pos	Pos	0/36	0/46

Purch. Purchased Sent. Sentinel Pos Positive Neg Negative NT Not tested/not reported

[1] Based on the declarations in the health status reports of the delivering producers of animals.

[2] LCM was used for inoculation experiments within unit III.

[3] All purchased mice and rats were declared negative for PVM. However, gerbils purchased for unit IV were positive for PVM, which was confirmed by own investigations.

performed on individual, non-pooled samples. Bacteriological examinations were performed as previously described (*Hansen* 1992). Parasitology was performed using Fecalyzers® (Kruuse, DK-5290 Marslev) for flotation, and smears from ileum and caecum for direct microscopy according to *Kunstyr* (1989). The pelt was examined under a stereo microscope. Serology was performed by three methods: Immunofluorescence Assay (IFA), Haemagglutination-Inhibition Assay (HAI) and Enzyme-Linked Immuno-Sorbent Assay (ELISA). For IFA all antigens, *C. piliforme* and coronaviruses excepted, were purchased from Harlan Olag Ltd. (GB-OX60TP Oxon). Antigens for *C. piliforme* and coronaviruses were purchased from Bomtest (DK-8680 Ry). IFA and HAI were performed as previously described (*Hansen et al.* 1992, 1993) using a cut-off value of 1:20. Antigen for HAI was purchased from Charles River Ltd. (MA-01887 Wilmington, USA). Antigen-coated microtiter plates for all ELISA-tests, *P. pneumotropica* excepted, were purchased from Charles River Wiga (D-97633 Sulzfeld). ELISA-antigen for *P. pneumotropica* were produced by incubating the bacterium on blood agar for 24 hours at 37°, whereafter the agar was flushed with sterile PBS. The bacteria was further washed twice in PBS. $8 * 10^9$

cells/ml in carbonate buffer (pH 9.6) were used for coating at 4° for 24 hours in 48 of the wells of a Maxisorp ELISA-plate (Teknunc, DK-4000 Roskilde), while the remaining wells were filled with the buffer only. Hereafter the plate was washed five times with PBS-Tween 20 and blocked with PBS with 5 % bovine serum albumin (Sigma, USA-MO-63178 St. Louis) for 30 minutes at room temperature followed by washing five times in PBS. All ELISA-tests were run by incubating the plates for 2 hours at room temperature with test sera diluted 1:100 with 0.1 % phosphate-buffered saline (PBS)-Tween 20 followed by washing 6 times with 0.1 % PBS-Tween 20 with extra NaCl (15 g/l). Hereafter the plates were incubated with horse radish peroxidase conjugated rabbit anti-mouse or anti-rat immunoglobulin diluted 1:2000 (Dako Ltd., DK-2600 Glostrup) for 2 hours at room temperature followed by washing as above. As subtrate was used O-phenyldiamin (OPD), which was inactivated by 2N sulphuric acid. The cut-off values for ELISA were calculated by taking the mean plus 3 times the standard deviation of all negative results achieved in the laboratory. All sera were initially screened by IFA or ELISA and positive results were hereafter confirmed by the alternative test, except for *KRV* and *Toolan's H1* (*H1*), which were retested by HAI. All serological assays included a positive and a negative control serum. In other investigations at the laboratory the use of these laboratory methods for sentinels and animals from breeding colonies has revealed infections with *KRV, LCM, MHV, PVM, Reovirus type 3, Sendai virus, Theiler's Encephalomyelitis Virus, H1, B. bronchiseptica, C. freundii, C. piliforme, P. pneumotropica, Syphacia spp, Eimeria spp,* and *Tritrichomonas spp.* The other agents tested for in this paper have never been found in any animals tested in our laboratory. The laboratory participates in two quality assurance systems, one for bacteriology and one for serology.

Health status of purchased animals. Our department is involved in health monitoring in some of the breeding colonies delivering animals for the units I, II, III and IV, and the presence of the infections declared in the delivering breeding colonies has been confirmed.

Results

The results of all investigations are given in Table 1. All positive results are specified in Table 2. None of the sentinels sampled showed any signs of remarkable disease.

Unit I

Infection with *MHV* was found in the first sampling of sentinel mice. The infection was terminated by euthananizing all mice in the unit, cleaning the rooms used for mice and starting new experiments with non-infected mice. The rats were left in the unit. Although no animals infected with *P. pneumotropica* had been introduced, and although it was not possible to cultivate this organism from neither rat nor mouse sentinels from the unit, antibodies were found in sentinels of both species. The infection with *KRV* observed in the rat sentinels was probably caused by the illegal introduction of some contaminated cages about one month prior to the sampling of the sentinels.

Unit II and III

The only infection found was *P. pneumotropica,* and in unit III this was only observed by serology. *LCM* used for experiments in unit III was not found in any of the sentinel mice.

Unit IV

Infection with *Rat Coronaviruses* detected in the sentinel rats was terminated in the same way as terminating *MHV* in the mice of unit I, by emptying the unit for rats, cleaning the rooms and restarting experiments with non-infected rats without any precautions taken for the other species within the unit. Antibodies to *KRV, PVM* and *C. piliforme* were refound in the sentinel rats, as it has been found in the purchased animals. Additionally, eggs of *Syphacia spp.* were found.

Table 2. Results of all five independent samplings concerning those infections discovered in sentinels from experimental units for rats and mice monitored over a total period of 15 months for the infections described in Table 2.

Agent	Methods	Unit	Species	Sampling No 1	2	3	4	5
VIRAL INFECTIONS								
Kilham Rat Virus	Serology	I	Rats	0/3	0/6	0/9	0/10	9/9
Kilham Rat Virus	Serology	IV	Rats	0/10	4/8	2/10	0/6	0/7
Mouse Hepatitis virus	Serology	I	Mice	2/6[1]	0/16	0/5	0/9	0/8
Pneumoniavirus of Mice	Serology	IV	Rats	0/8	0/8	0/10	2/10	0/7
Rat Coronaviruses	Serology	IV	Rats	0/8	0/8	10/10[2]	0/7	0/7
BACTERIAL INFECTIONS								
C. piliforme	Serology	I	Rats	NT	NT	0/9	NT	2/9
C. piliforme	Serology	IV	Rats	NT	NT	1/10	3/5	2/10
P. pneumotropica	Serology	I	Mice	NT	NT	1/8	0/9	6/10
P. pneumotropica	Serology	I	Rats	NT	NT	9/9	6/10	9/9
P. pneumotropica	Culture	II	Mice	0/3	0/10	1/5	0/10	0/10
P. pneumotropica	Serology	II	Mice	NT	NT	1/5	5/10	NT
P. pneumotropica	Serology	III	Mice	NT	0/4	0 /6	5/10	2/10
P. pneumotropica	Culture	IV	Mice	0/10	9/9	1/10	0/10	0/10
P. pneumotropica	Serology	IV	Mice	NT	NT	NT	1/5	3/10
P. pneumotropica	Serology	IV	Rats	NT	NT	9/10	9/9	8/8
PARASITE INFECTIONS								
Syphacia spp.	Flotation	IV	Rats	1/10	1/8	0/10	0/9	0/8

[1] These results were confirmed by sampling sera from 26 mice inoculated with a cell culture, which due to a mistake had not been screened by a Mouse Antibody Production Test. 22 were positive to *MHV*. Hereafter all mice in the unit were euthanized, before the purchase of new mice.
[2] After this sampling all rats in the unit were euthanized, before the purchase of new rats.

P. pneumotropica was cultivated from sentinel mice and not from sentinel rats although introduced with purchased rats. However, both rats and mice were found positive to *P. pneumotropica* by serology. Except for this no infections were found in the sentinel mice, not even *PVM* or *C. piliforme,* which had been introduced with purchased gerbils and rats and had been refound in the sentinel rats.

Discussion

When *MHV* was found in the sentinel mice of unit I, only two of ten sera were positive. If this was a typical prevalence, it would indicate a sample size of 14 according to formula 1. In another sentinel study 100 % of the sentinels had seroconverted after 30 days (*Homberger & Thomann* 1994), and generally *MHV* is known to spread rapidly within few days resulting in prevalences close to 100 % (*Barthold* 1986). However, the sentinels were sampled from unit 1 rather shortly after the inoculation of the contaminated cell culture into mice within the unit. Probably, a higher number of sentinels would have seroconverted if given more time, so a lower number of sentinels would also be sufficient for the detection of *MHV*. Due to the short duration of active coronavirus infection followed by the full elimination of the agent from the organism (*Barthold & Smith* 1990), as well as the species specificity of rat and mouse coronaviruses, both coronavirus infections discovered were easily eradicated by simply euthanizing all animals of the susceptible species. The same principles are obeyed when successfully using a six week break in breeding for elimination of coronaviruses from breeding colonies (*Charles River* 1983, *Weir et al.* 1987). As coronaviruses generally are known to reach high prevalences in infected units (*Jacoby* 1986), the

sampling from unit IV resulting in ten positive out of ten sera tested for *Rat Coronaviruses* indicated that the infection had not previuosly been present in the unit, and that it with present easily infects sentinels.

KRV was only found in two of the five samplings from unit IV, although infected rats were currently brought into the unit. In another study no spread of *KRV* within a rat unit was observed (*Detmer et al.* 1990). This is, however, not that surprising, as the excretion of *KRV* varies between different strains of the virus and fecal excretion ceases after 12 days (*Novotny & Hetrick* 1970, *Lipton et al.* 1973). Also the mouse parvovirus, *Minute Virus of Mice* (*MVM*), is known to spread inefficiently by airborne transmission (*Parker et al.* 1970). Probably, the high number of rat sentinels responding serologically in unit I was due to a high propagation of the virus in the naive animals kept within that unit.

PVM, although present in the gerbils purchased for unit IV, did not spread to the mouse sentinels at all, and only to two of the 34 rat sentinels. Normally there is a great variation in the prevalences of *PVM* observed in infected rodent colonies (*Richter* 1986). Probably, *PVM,* a paramyxovirus as *Sendai Virus,* spreads as inefficiently through contaminated bedding as *Sendai Virus* (*Artwohl et al.* 1994).

The lack of spread of *LCM* to the sentinels in unit III is to be expected, as this virus neither spread by aerosols nor survive on dry surfaces. Usually, prevalences less than 10 % are observed within infected mouse colonies (*Allen & Nomura* 1986). If the sentinels had become infected it is likely that mortality among them would have been high, as this is the case when *LCM* infects adult mice (*Lehmann-Grube* 1982).

C. piliforme was refound in rat sentinels in both unit I and unit IV. However, the prevalences among the sentinels were much lower than those observed in breeding colonies (*Hansen et al.* 1990). Infection in rats occur early in life (*Hansen et al.* 1992), and spores of this agent are mostly shed and spread by contaminated bedding within 2 weeks post infection (*Motzel & Riley* 1992). Also, the number of animals per square metre is lower in experimental facilities than in a breeding facility. So, there probably was only a little number of spores present within the units. Keeping rat sentinels in the same cages as the infected animals raises the number of sentinels seroconverting to *C. piliforme* (*Hansen et al.* 1994). The absence of this infection in the sentinel mice, although introduced with rats and gerbils, is due to the strain specificity of strains of *C. piliforme* (*Fujiwara et al.* 1971a, 1971b, 1973).

Absence of positive results might have been due to a low sensitivity of the methods applied, and not only the ability of the sentinels to become infected as illustrated by *P. pneumotropica.* This agent was rather difficult to isolate from the sentinels, although it was known to be present in at least three of the four units. In contrast to this, serology indicated that both rats and mice in all four units were infected. This is a problem of either low specificity of serology or low sensitivity of cultivation or a combination of both. False positives in serology might have been caused by crossreactions with e.g. *Haemophilus spp,* which are rather common in rat and mouse colonies (*Nicklas* 1989, *Nicklas et al.* 1990). One sampling from unit IV resulted in nine out of nine positive for *P. pneumotropica* by culture (Table 2). This higher prevalence could have been due to the sentinels being in an early stage of infection, and this agent being most easily cultivated shortly after infection of the sentinels. Three months after the introduction into the unit the sentinels have developed an efficient immune response making isolation of the organism difficult. Furthermore, non-germ-free sentinels already have a mucosal flora to compete with new bacteria to be introduced.

The endoparasites *Tritrichomonas* and *Syphacia* were present in unit IV. *Syphacia* was not introduced with purchased animals,

but the eggs are highly resistant (*Miyaii et al.* 1988), and therefore it is unlikely that it should have been eliminated after the first samplings. The presence of this infestation in unit IV is obviously connected with the absence of entrance regulations. *Tritrichomonas* was not found in one single of 36 rat and 46 mouse sentinels, although currently introduced with both rats and mice. This is probably due to the fact, that parasites normally are difficult to detect in adult animals (*Kunstyr* 1989). In this sentinel study the immune system of the sentinels was given three months to develop an efficient response to the parasite infestation making it difficult to find them by the method applied. Prevalence of some of the serologically detected infections, *KRV, MHV, Rat Coronaviruses* and *P. pneumotropica,* indicates according to formula 1, that the use of e.g. 10 sentinels per unit will be sufficient. For detection of *C. piliforme* in rats a higher number of sentinels is preferable, e.g. 14, if expecting seroconversion in 20 % of the sentinels. Negative results for *KRV* and *C. piliforme,* should be interpreted as the lack of propagation and spread of these agents within the unit, rather than the total absence of these agents. This and other studies (*Artwohl et al.* 1994) shows problems of the dirty bedding technique for transferring paramyxoviruses and *LCM* to sentinels. When planning a health monitoring program for an experimental unit the possibility of caging the sentinels with or – even better – becoming serum samples directly from the animals of the experiments should be considered. Sentinel health monitoring as described here does not seem usable for the detection of parasites. However, achieving faeces directly from the animals in experiments for flotation tests is normally not a problem. Using two groups of sentinels might be an improvement: One, which is kept in the unit for only a short period, e.g. 10 days, and one, which is kept in the unit for three months. The first group is used for bacteriological and parasitological investigations, while the second group is used for serological investigations. Another improvement may be to increase the amount of dirty bedding and to add fresh bedding more frequently.

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Summary
The paper contains the results of 15 months of sentinel health monitoring in four different animal units, of which three were protected by a barrier, and one was operated without any protective procedures. Rat and mouse sentinels were placed in the units on contaminated bedding for three months and hereafter tested for the following infections: *Ectromelia Virus, Kilham Rat Virus (KRV), Lymphocytic Choriomeningitis Virus (LCM), Minute Virus of Mice (MVM), Mouse Hepatitis Virus (MHV), Pneumoniavirus of Mice (PVM), Rat Coronavirus, Reovirus type 3, Sendai Virus, Theiler's Encephalomyelitis Virus (TMEV), Toolan's H1 Virus, Bordetella bronchiseptica, Citrobacter freundii, Clostridium piliforme, Corynebacterium kutscheri, Mycoplasma spp, Pasteurella pneumotropica, Salmonellae,* β-haemolytic Streptococci, *Streptococcus pneumoniae,* arthropods, helminths, *Eimeria spp* and flagellates. The outcome of the sentinel investigations were compared with the knowledge on the health status of those animals, which were purchased for experiments in the four units. In the non-protected unit antibodies to *KRV, PVM* and *C. piliforme* were refound in the sentinel rats, as it had been found in the purchased animals. Additionally, eggs of *Syphacia spp.* were found in the non-protected unit. *P. pneumotropica,* which was also introduced into three of the units with purchased rats and mice, was cultivated from sentinel mice, only, and not from sentinel rats. Both rat and mouse sentinels were found positive to *P. pneumotropica* by serology. *PVM* and *C. piliforme,* which had been introduced with purchased gerbils and rats and were refound in the sentinel rats, were not found in the sentinel mice. *MHV* and *Rat Coronaviruses* were, independently, detected in two of the units. Both coronaviruses were easily eradicated by simply euthanizing all animals of the susceptible species with no precautions taken against animals of other species within the unit. *LCM* did not spread from experimentally infected mice to the sentinels. Concerning the use of serology for coronaviruses, parvoviruses, *C. piliforme* and *P. pneumotropica* it is concluded that the dirty bedding technique pro-

ved efficient. For bacteriological and parasitological investigations in general, as well as for serological investigations for *LCM* and *PVM*, the method seemed less efficient.

Sammendrag

Artiklen gengiver resultater af 15 måneders sentinel-baseret sundhedskontrol i fire forsøgsdyrsenheder, hvoraf de tre var beskyttet af en barriere, og én kørte uden nogle særlige beskyttelsesforanstaltninger. Rotte- og musesentineller blev anbragt på kontamineret strøelse i 3 måneder, hvorefter de blev undersøgt for: *Ectromeli Virus, Kilham Rat Virus (KRV), Lymphocytær Choriomeningitis Virus (LCM), Minute Virus of Mice (MVM), Musehepatitis Virus (MHV), Pneumoniavirus of Mice (PVM), Rotte Coronavirus, Reovirus type 3, Sendai Virus, Theilers Encephalomyelitis Virus (TMEV), Toolans H1 Virus, Bordetella bronchiseptica, Clostridium piliforme, Citrobacter freundii, Corynebacterium kutscheri, Mycoplasma spp, Pasteurella pneumotropica, Salmonellae,* β-hæmolytiske Streptococcer, *Streptococcus pneumoniae,* arthropoder, helminther, *Eimeria spp* og flagellater. Resultaterne af sentinel-undersøgelserne blev sammenholdt med den viden, der var omkring dyr, der blev indkøbt til eksperimenter i de tre enheder. I den ubeskyttede enhed blev der genfundet antistoffer imod *KRV, PVM* og *C. piliforme* i rotte-sentinellerne, ligesom disse fandtes i de dyr, der blev indkøbt til eksperimenterne. Ydermere blev der konstateret infektion med *Syphacia spp.* i den ubeskyttede enhed. *P. pneumotropica,* som også fandtes i musene, der blev indkøbt til de tre af enhederne, kunne kun dyrkes fra muse-sentineller og ikke fra rotte-sentineller. Derimod var både rotte- og musesentineller positive for *P. pneumotropica* ved serologisk undersøgelse. *PVM* og *C. piliforme,* som var blevet ført ind i den ubeskyttede enhed med indkøbte gerbils og rotter, kunne kun genfindes i sentinel-rotter og ikke i sentinel-mus. *MHV* og *Rotte Coronavirus* blev uafhængigt af hinanden konstateret i to af enhederne. Begge infektioner kunne let bekæmpes ved blot at aflive samtlige dyr af den modtagelige art uden yderligere indgreb overfor andre dyrearter. *LCM* spredte sig ikke fra nogle eksperimentelt inficerede mus til sentinel-mus. Det konkluderes, at ved brug af serologi til påvisning af coronavirus, parvovirus, *C. piliforme* og *P. pneumotropica* fungerer metoden med anvendelse af kontamineret strøelse tilfredsstillende. Med hensyn til bakteriologisk og parasitologisk undersøgelse i almindelighed, samt serologisk påvisning af *LCM* og *PVM,* opnås der derimod mindre tilfredsstillende resultater.

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