

## New infections to be considered in health monitoring of laboratory rodents

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### Introduction

In 1994 the *Federation of European Laboratory Animal Science Associations (FELASA)* issued a set of guidelines for health monitoring in breeding colonies of rodents and rabbits (Kraft *et al*, 1994). These guidelines recommended specific agents to be included in health monitoring as well as specific test methods and sampling sites to be applied (Table 1). During the six years past these guidelines have had an essential impact on health monitoring principles in Europe and today most commercial breeders perform health monitoring, which as a minimum fulfils the standards of the FELASA guidelines.

Laboratory animal science and microbiology are both rapidly developing fields and it is impossible to set up a set of guidelines, which in an unchanged version can be valid for decades. At present, a working group under FELASA is revising these guidelines (FELASA, 2000). It is the intention of the paper to describe infections, which need consideration in a revised set of guidelines. Some of these agents such as *Cilia associated respiratory (CAR) Bacillus* were mentioned but not recommended for mandatory screening in the 1994 version of the guidelines, some such as adenovirus in guinea pigs have not been as easily accepted as part of health monitoring schedules as other agents, while some agents such as Orphan Parvovirus were not included in the guidelines in 1994.

### Bacterial infections

#### *Helicobacter spp.*

The murine *Helicobacter* spp. are of interest primarily due to their probable undesirable influence on biomedical research in rodents in which evidence is emerging that these bacteria may cause or contribute to a wide range of disease conditions such as hepatitis (Ward *et al*, 1994; Fox *et al*, 1995), inflammatory bowel disease (Shomer *et al*, 1997; Haines *et al*, 1998; Fox *et al*, 1999) and enterocolitis (Franklin *et al*, 1999). Furthermore, the presence of *Helicobacter hepaticus* in mice may increase hepatic carcinogenesis (Diwan *et al*, 1997) and modify hepatic metabolism (Fox *et al*, 1996). *H. hepaticus* was first recognised in a long-term carcinogenesis study in which a probable association with hepatic carcinogenesis was demonstrated (Fox *et al*, 1994). More recently it has been established as the causative agent in inflammatory bowel disease in certain immunodeficient and/or transgenic models (Ward *et al*, 1996). In hamsters *H. cholecystus* has been associated with cholangiofibrosis and pancreatitis (Franklin *et al*, 1996). *H. bilis* occurs as a natural infection in rats and mice and has been associated with inflammatory bowel disease in immunodeficient animals of both species (Haines *et al*, 1998; Shomer *et al*, 1997). In mice it has also been associated with hepatitis, typhlitis and colitis in immunodeficient strains (Franklin *et*

	Mice	Rats	Hamsters	Guinea pigs
<i>Viral infections</i>				
Minute virus of mice	+	+	-	-
Mouse hepatitis virus	+	-	-	-
Pneumonia virus of mice	+	+	+	+
Reovirus type 3	+	+	+	+
Sendai virus	+	+	+	+
Theiler's murine encephalomyelitis virus	+	+	-	-
Hantaan virus	-	+	-	-
Kilham rat virus	-	+	-	-
Toolan's H1 virus	-	+	-	-
Guinea pig adenovirus	-	-	-	+
Lymphocytic choriomeningitis virus	-	-	+	-
Simian virus type 5	-	-	+	+
<i>Bacterial infections</i>				
Bordetella bronchiseptica	+	+	+	+
Citrobacter rodentium	+	-	-	-
Clostridium piliforme	+	+	+	+
Corynebacterium kutscheri	+	+	-	-
Leptospira spp.	+	+	-	-
Mycoplasma spp.	+	+	-	-
Pasteurella spp.	+	+	+	+
Salmonella	+	+	+	+
Streptobacillus moniliformis	+	+	-	+
$\beta$ -hemolytic streptococci	+	+	-	+
Streptococcus pneumoniae	+	+	-	+
Yersinia pseudotuberculosis	-	-	-	+

Table 1. Bacterial and viral infections recommended by FELASA guidelines as part of a quarterly mandatory screening of rodents (*Kraft et al, 1994*).

al, 1998). Recently a novel species "*H. typhlonicus*" has been associated also with inflammatory bowel disease (Fox et al, 1999; Franklin et al, 1999). *H. muridarum*, *H. rodentium* and "*F. rappini*" and *H. trogontum*, has not yet been directly associated with any disease conditions in rodents. *H. rodentium*, however, has been described in cases of diarrhea associated with typhlitis, colitis and proctitis ranging from mild watery to fatal hemorrhagic diarrhea. This was only found in coinfection with *H. bilis* (Shomer et al, 1998).

*H. hepaticus* may be isolated from the entire gastro-intestinal tract and liver, but it is most easily isolated from the cecum (Shomer et al, 1997; Kullberg et al, 1998; Zenner et al, 1999; Moura et al, 1998; Fox et al, 1994; Phillips et al, 1983). *H. hepaticus* and *H. cholecystus* (Franklin et al, 1996) are those two *Helicobacter* spp. which may grow anaerobically (Fox et al, 1994). The *Helicobacter* spp. are very fastidious and grow only on specific nutrient enriched solid media. Normally medias containing 5% or 10% defibrinated blood are well suited for culture of these organisms (Wang et al, 1997; Goto et al, 1998; Monteiro et al, 1997). Growth in primary culture is usually detectable after 3-5 days but for slower growing species several weeks may be necessary (Shomer et al, 1997). Most murine species grow as a clear spreading film on the surface of the media and can be nearly invisible. Weak growth can be detected by carefully scraping the surface of the agar. In primary cultures addition of antibiotics to growth media to inhibit growth of unwanted bacteria is widely used. A combination of 5 µg Trimethoprim, 10 ng Vancomycin, 2,5 µg Polymyxin per ml (TVP) seems to be the most common combination (Fox et al, 1999; Haines et al, 1998; Fox et al, 1996) sometimes with addition also of Amphotericin B (2 mg/l) (Lee et al, 1992; Paster et al, 1991). Alternatively Vancomycin (6 mg/l), Nalidixic acid (20 mg/l) and Amphotericin B (2 mg/l) (Queiroz et al, 1992), or other antibiotic supplements (Phillips et al, 1983) are added to the medium. The antibiotics used in selective media may, however, hamper the growth of many of the murine species and a technique based on other mechanisms for

avoiding overgrowth with normal gut flora on non-selective media is therefore desirable. For isolation of murine *Helicobacter* spp. filtration of the homogenised tissue sample or intestinal content through a 0.45 µm pore size nylon filter has proved useful for isolation of *H. hepaticus* (Kullberg et al, 1998; Li et al, 1998; Russell et al, 1995; Fox et al, 1996) and *H. rodentium* (Shen et al, 1997). Some researchers have used a pre-filter with a larger pore size (Fox et al, 1999; Haines et al, 1998). For isolation of *H. bilis* (Franklin et al, 1998) and "*H. typhlonicus*" (Franklin et al, 1999) a filter with a pore size of 0.8 µm has been used to allow passage of these larger organisms. A procedure in which a few drops of a PBS suspension of cecal contents are applied to a 0.8 µm nitrocellulose filter placed directly on 10% Horse blood agar plates yields good results and high sensitivity (authors unpublished data). The use of filters reduces the importance of using selective media, and therefore increases the sensitivity of cultivation as a detection method. *Helicobacter* spp. do not grow under aerobic conditions, and must be cultured in a microaerophilic atmosphere (Fox et al, 1995; Hanninen et al, 1996; Franklin et al, 1996; Paster et al, 1991; Andersen et al, 1999; Fox et al, 1994; Lee et al, 1992; Schauer et al, 1993; Shen et al, 1997; Mendes et al, 1996; Franklin et al, 1999; Kiehlbauch et al, 1995). Gas mixtures containing 5% CO<sub>2</sub>, 7% O<sub>2</sub> and 90% N<sub>2</sub> has frequently been used in Brewer gas jars or anaerobic jars with good results (Phillips et al, 1983) or 5% CO<sub>2</sub>, 5% H<sub>2</sub> and 90% N<sub>2</sub> (Shen et al, 1997; Fox et al, 1995; Fox et al, 1994) or 5% O<sub>2</sub>, 7% CO<sub>2</sub>, 7% H<sub>2</sub> and 81% N<sub>2</sub> (Bronsdon et al, 1991). The simplest but also most expensive solution is to use a microaerobic incubator which automatically measures and adjusts O<sub>2</sub> and CO<sub>2</sub> to the desired levels. Few species (*H. hepaticus*, *H. cholecystus*) can grow under anaerobic conditions (Fox et al, 1994; Franklin et al, 1996). Hours of exposure to air are not tolerated (Andersen et al, 1997). Cultivation in broth is possible in BHI broth supplemented with 10% fetal calf or sheep serum, but for all broth culture systems it is prerequisite that a water bath shaker is used to agitate the broth culture. Broth cultures require long incubation

times of 1 week or more (Morgan et al, 1987). The risk of contamination is, however, considerable and the resulting suspension should always be controlled for contaminating bacteria before use or analysis. During attempts to isolate *Helicobacter* spp. from clinical samples, it is recommended that plates should be incubated and evaluated for up to 3 weeks before making a determination of no growth (Shomer et al, 1997). Cultivation of *Helicobacter* spp. is difficult and requires specially equipped laboratories.

Serology would be most useful in health monitoring and a both sensitive and specific procedure for detection of *H. hepaticus* has been described (Livingston et al, 1997). For this procedure a purified membrane protein from cultivated bacteria has been used for analysis. Production of bacterial material for serological assays such as this is, however, very laborous due the low output resulting from culture. To overcome this a recombinant *H. hepaticus* membrane protein has been produced (Livingston et al, 1999). However, the sensitivity was significantly lower using this recombinant antigen in the ELISA, so development of a good serological test still needs to be done. For the detection by PCR of *Helicobacter hepaticus* in archival formalin preserved paraffin embedded liver specimens a protocol has been published using a semi-nested PCR (Malarkey et al, 1997). A PCR protocol with an internal radiolabelled probe directed at a *H. hepaticus* specific sequence within the amplified product to be used on any type of material has been proposed (Battles et al, 1995). A PCR-RFLP procedure that distinguishes between *H. hepaticus*, *H. muridarum*, *H. bilis* and "*Flexispira rappini*" has been described (Riley et al, 1996). The restriction enzyme analysis with *MboI*, *HhaI* and *MaeI* could not differentiate *H. bilis* from "*F. rappini*" and a PCR amplification step was added using a *H. bilis* specific reverse primer to separate the two. This two step PCR can cause problems with contamination with amplified fragments leading to false positive results. The PCR technique has the immediate advantage for detection that it can be performed on unculturable organisms as it has been done on the '*candidatus H. suis*' (De Groot et al, 1999a) and '*candidatus*

*H. bovis*' (De Groot et al, 1999b) from swine and cattle, respectively. It can be used on faeces if the inhibitors, typically complex polysaccharides from the diet, are avoided as with the QIAmp method (Monteiro et al, 1997) or with the procedure described by Shames et al. (Shames et al, 1995). Both methods give good results in mice (Beckwith et al, 1997; Shames et al, 1995). For detection of *H. hepaticus* by PCR on faeces a specific protocol has been described. The sensitivity of this protocol has been evaluated by testing clinical material from known positive colonies. The specificity was evaluated by testing controls of *H. muridarum*, "*F. rappini*" and *H. bilis*, *H. pylori*, *H. mustelae*, *H. felis* and more distantly related *Campylobacter* spp. without cross reactions (Shames et al, 1995). In a recent study a PCR protocol detecting the murine *H. muridarum*, *H. hepaticus*, *H. bilis* and "*F. rappini*" was presented (Mahler et al, 1998). Only *H. bilis* is immediately distinguishable from the other species in this assay by the size of the amplified fragment. Further differentiation of *H. hepaticus* from the others is done by the procedure described by Battles et al (1995). This method can be used as a screening procedure to detect murine *Helicobacter* spp. Positive findings will however need other analyses for definitive species identification. Many breeders now routinely monitor their colonies using fecal PCR for detection of members of the *Helicobacter* genus. At present this seems to be the method of choice (Charles River Laboratories 2000; Taconic, 2000; Harlan, 1999). The sensitivity of the murine *Helicobacter* genus assays (Mahler et al, 1998; Beckwith et al, 1997; Riley et al, 1996) has not been validated for diagnosis of the newer species of the genus and the results should be interpreted with caution since a negative result in this assay does not necessarily exclude infection with the newer *Helicobacter* spp.

In general US breeders of laboratory rodents do not accept the presence of *H. hepaticus*, whereas *H. bilis* and other species are tolerated to different extents (Table 2). Charles River US immediately stamps out colonies where *H. hepaticus* is found, whereas discovery of other *Helicobacter* spp. puts up the colony for future sanitation. Diagnosis is done by genus specific PCR. The findings of these

Breeder	Helicobacter						
	hepaticus	bilis	muridarum	trogontum	rappini	rodentium	typhlonicus
Ch. River,US	E	T+	T	T,Q	T	T	T,Q
Taconic	E	E	E	E,Q	E	T,+	E,Q
Harlan US	T,+	T,+	T	T,Q	T	T	T

Table 2. US breeder policies on murine *Helicobacter spp.* (Charles River Laboratories, 2000; Harlan US, 1999; Taconic, 2000)

E - not tolerated immediate eradication,

Q - questionable detection

T-Tolerated temporarily, customers are informed

+ infected colonies officially present at time of publication.

organisms are published in the health reports (*Charles River, 2000*). European colonies are not monitored for the presence of *Helicobacter spp.* Harlan US accepts *H. hepaticus* and other *Helicobacter spp.* but lists their presence in the health monitoring reports. Harlan UK, Harlan France and Harlan Italy normally tests mice colonies for *H. hepaticus* and *H. bilis* by *Helicobacter* species PCR but the method is not detailed further and only presence of *H. hepaticus* and *H. bilis* are declared (*Harlan US, 1999*). Except for the Horst barrier at Harlan rats from Harlan Netherlands are not tested for *Helicobacter spp.* Taconic US does not accept any *Helicobacter spp.* in rats or mice in their barrier units and claim to be free of all *Helicobacter spp.* including *H. hepaticus* and *H. bilis* but except *H. rodentium* which has been found in two barriers (*Taconic 1999*). They employ a genus specific PCR assay described by Riley et al 1996 (*Riley et al, 1996*) that combines PCR and restriction enzyme analysis for detection and identification. Species diagnosis is confirmed by a species specific PCR (*Geistfeld 1999, Weisbroth 1999*). In Europe only Harlan has marketed *Helicobacter* free animals. RCC, Switzerland, M&B, Denmark and B&K, Sweden/Norway do not include *Helicobacter spp.* in their screening programs (RCC Ltd, M&B).

#### *Campylobacters*

*Campylobacter jejuni* and *coli* are sporadically isolated from rodents and rabbits. The prevalence in an infected colony is normally below 5% (*Hansen, 1999*). Detection by cultivation requires use of selective media, filtration techniques as described above for the *Helicobacter spp.* PCR has not been used for detection of *Campylobacter spp.* in laboratory rodents.

These bacteria have hitherto not been associated with disease in rodents or rabbits, but are well-known disease agents in humans and a range of farm animals. Most commercial breeders do not include *Campylobacter spp.* in their screening programmes.

#### *Pasteurellaceae*

*Pasteurellaceae* are Gram-negative bacteria varying from coccobacilli to more rod-like cells, so-called pasteurellaforms. Three genera are known: *Pasteurella*, *Actinobacillus* and *Haemophilus*. "*Pasteurella*" *pneumotropica* was first described by Jawetz in 1948. Before that time all *Pasteurellaceae* in rodents were characterized as *Pasteurella multocida*. However, improved characterization methods have spread types infecting different species on different genera, and today *P. multocida* is in principle only to be considered an infection of rabbits, although it may

in theory infect rodents, also. However, in rodents infection is normally caused by members of one of the genera *Actinobacillus* and *Haemophilus*.

"*Pasteurella*" *pneumotropica*, which has for long been recognized as an important rodent bacterium, has in 1985 been reclassified as *Actinobacillus* (*Mutters et al*, 1985). Another *Actinobacillus*, *A. muris*, is a seldom isolated bacterium of rats and mice, which may cause infertility due to abortion, metritis, and stillbirths in mice (*Ackerman & Fox*, 1981). The infection is seldom nowadays, and when present it is mostly latent. It has formerly been identified as *Pasteurella ureae* or *Actinobacillus urea*, which, however, seems to be a specific human agent (*Mutters et al*, 1984). Characterization has previously been described (*Hansen*, 1999). Some performers of health monitoring also report isolation of "*Pasteurella haemolytica*" from rodents. However, this agent is primarily found in farm animals, and closer examination of so-called *P. haemolytica* from rodents normally identifies these as *P. pneumotropica*.

*Haemophilus spp.* is a common finding in colonies of rats and guinea pigs and occasionally rabbits and mice (*Nicklas et al*, 1993), even if these are barrier-bred. One species from mice, *H. influenzae murium*, is clearly defined, while species isolated from rats are less characterized (*Hansen*, 1999). Infection in rats has been associated with mild inflammatory cell infiltration and a light diffuse hyperaemia in the lungs, but in general, no severe symptoms should be expected as a result of infection with *Haemophilus spp.* Prevalences in infected colonies are less than 20 % (*Hansen*, 1999). If testing for cytochrome oxidase is performed as described by Kovács (1956) *Haemophilus spp.* are oxidase positive, while other tests may give negative results. Growth depends on either X-factor (protoporphyrin or protohemin), V-factor (nicotine amide dinucleotide, NAD) or both. Growth is most simply achieved by inoculation on chocolate agar at 37° aerobic or microaerophilic for 24 - 48 hours. Lincomycin (5 µg/ml) or clindamycin (2 µg/ml) may be added to prevent growth of Gram-positive bacteria. The main sampling sites are within the respiratory system and from the vagina

or the prepuce. Characterization profiles have previously been given (*Hansen*, 1999). For the test of biochemical characteristics the X- and V-factor must be added to the media. Identification tests may be carried out using the commercial kit API NH (Bio Merieux, France). The API computer software does not give clear results for rodent species, but API NH profiles for rodent *Haemophilus spp.* have been reported by Boot et al (1997, 1999). Enzyme-linked immunosorbent assay (ELISA) and immuno-fluorescence assay (IFA) may be attempted for screening for infection in rodent colonies. Although cross reactions between different strains of *Haemophilus* and *Pasteurella pneumotropica* may occur, ELISA for *P. pneumotropica* should not be relied on as a secure tool for revealing infection with *Haemophilus*. Polymerase chain reaction (PCR) is applicable for diagnosis on samples from pharynx, trachea and lungs (*Weigler et al*, 1998).

#### *Cilia associated respiratory (CAR) Bacillus*

This Gram-negative agent was listed in the 1994 version of the FELASA guidelines, but screening was not made mandatory. It is claimed to be the cause of *chronic respiratory* disease in rats, the cause of which have previously been given as a range of other agents. Infection has also been reported in mice and rabbits, and additionally in farm animals such as pigs and goats, in Europe, USA and Japan. However, the pathogenicity is not fully clarified. In general, infected rodents may be fully asymptomatic (*Shoji et al*, 1988), but clinical signs, if present, may include weight loss, rough hair coat, wheezing and rales. *Chronic respiratory disease* is a highly contagious epizootic, slowly progressive and uncontrollable disease, in which symptomatic rats histopathologically show various degrees of pulmonary changes, such as mucopurulent exudate and severe peribronchial lymphoid cuffing (*Itoh et al*, 1987). The same clinical picture may be observed in mice (*Griffith et al*, 1998), although it should be considered that other pathogens might be involved, as well. There are no differences in the susceptibility between rat strains, but different strains of the agent are more or less virulent. Severity of symptoms during experimental infection seems to be worse if an

isolate from the infected species is used (*Shoji et al, 1988; Schoeb et al, 1999*). In rabbits no clinical signs of respiratory disease have been observed. Contact infection may play a major role in the transmission of this disease.

Isolates from rodents differ from those of rabbits and should be regarded as different bacteria that belong to distinct genera. Isolates from rats are approximately 0.2  $\mu$  and 4 - 8  $\mu$  long with a triple-layer cell wall and bulbous ends. Cells are Gram-negative, non-acid-fast, non-spore-forming, and heat labile (56° for 30 minutes). They are motile but lack structures resembling flagella, pili or axial filaments. The rabbit bacilli are smaller and forms fewer aggregates during propagation. Determination of gene sequences have shown that rodent *CAR bacillus* are related to *Flavobacterium* (*Wei et al, 1995*), while the rabbit isolates show a higher similarity with *Helicobacter* (*Cundiff et al, 1995*).

In paraffin-embedded infected lungs the bacteria may be detected on the border of the tracheal and bronchial epithelium by various silver stains, e.g. Warthin-Starry, or more specifically by using either indirect immunofluorescence or immunoperoxidase staining (*Oros et al, 1996*). Cultivation in traditional bacteriological media is difficult. Propagation by inoculation of embryonated chicken eggs via the allantoic route has been used, but the agent may also be propagated in Dulbecco's or Eagle's minimum essential medium supplemented with 10 % fetal calf serum (*Hansen, 1999*). Antibodies to *CAR Bacillus* may be detected in the early stage of the infection. The first method developed was IFA using tracheal sections of infected mice as antigen (*Matsushita et al, 1987*), but today the most common serological assay is ELISA. PCR may be applied on infected lung tissue (*Cundiff et al, 1994*) or on swabs from the oral cavity already on day 3 after exposure.

#### *Viral infections*

##### *Orphan parvovirus*

Orphan parvoviruses (OPV) are a group of rodent parvoviruses distinct from both Minute Virus of Mice (MVM), Kilham Rat Virus (KRV) and

Toolan's H1 virus (H1), which are the only parvoviruses listed in the 1994 version of the FELASA guidelines. The first OPV was isolated by McKisic et al in 1993. It was discovered by the fact, that antibodies to known rodent parvoviruses were detected by the IFA but not by haemagglutination inhibition assay (HAI) in commercial breeding colonies of rats and mice. These antibodies were considered to be a response to so-called "orphan parvovirus". Today, OPV's have been isolated from mice, rats and hamsters, and they have further been divided into Mouse parvovirus (MPV), Rat Orphan Parvovirus (ROPV) and Hamster Orphan Parvovirus (HOPV). Cross-infection between species-specific strains does not seem to occur, as when ROPV was inoculated to mice and hamsters, no evidence of viral production and antibody response was demonstrated (*Ueno et al, 1997*). Parvovirus infections in rats are common in Europe, while it is far less common in mice. Results from Japan suggest that parvovirus infection, which is found with a prevalence rate of 13-22 % in rats, is most often caused by ROPV (*Ueno et al, 1998*).

ROPV replicate mainly in lymphoid or hematopoietic tissues, and may be detected in feces, urine and oropharynx of the infected rats at one to four weeks post infection. The infective virus may also be detected in peripheral leukocytes and various tissues at an acute phase of infection. They decrease in most tissues after eight weeks of infection, but viral DNA is persistent in lymphoid tissues at least up to 24 weeks post infection (*Ueno et al, 1997*). The virus is transmissible by intraperitoneal inoculation of infected materials, and direct contact with infected rats or contaminated bedding (*Ueno et al, 1996*). In tissues of experimentally infected random-bred, inbred, and immunodeficient mice MPV has been localized by Smith et al (1993) by in situ hybridization in exocrine and endocrine pancreas, abdominal lymph nodes, mesentery, intestine, and sporadically in other organs. Transmission of OPV by mice inoculated as infants was found to be intermittent, whereas transmission by mice inoculated as weanlings was consistent during the first two weeks both by direct contact and by exposure to soiled bedding. The longest duration

of transmission was six weeks among mice inoculated as infants. Depending on host genotype and route of virus exposure urinary, fecal, and perhaps respiratory excretions of virus seem to occur (Smith et al, 1993).

OPV's do not cause essential clinical disease, but they are known to have a serious impact on the immune system. MPV may interfere with the ability of T cell clones to thrive and, therefore, has the potential to alter immune responses (McKisic et al, 1993). ROPV is known to alter cell tropism and persist in lymphoid or hematopoietic tissues in order to escape from the host immune system (Ueno et al, 1997).

ROPV is assumed to be a variant of KRV (Ueno et al, 1997). MPV resemble MVM in genome size, replication intermediates, and nonstructural (NS) proteins. Restriction analysis of the MPV genome indicate that many restriction sites in the capsid region are different from those of MVM, but most sites in the NS region of the genome are conserved. The predicted amino acid sequence of the NS proteins of MPV and MVM are nearly identical, while the predicted amino acid sequence of the capsid proteins of MPV is different from sequences of other parvoviruses, which explain the antigenic differences between MPV and MVM (Ball-Goodrich & Johnson, 1994).

A very common method for diagnosing infection with OPV's is by the combination of IFA or

ELISA with HAI (Table 3). Riley et al (1996) has constructed a recombinant baculovirus containing the NS1 gene of MVM and set up an ELISA with rNS1 as the antigen for serologic testing of laboratory rodents. This rNS1 ELISA proved to be a more sensitive method for the detection of antibodies to OPV's as well as MVM, KRV and H1 than conventional parvovirus ELISAs using whole parvovirus virions as antigen. Besselsen et al (1995) has designed a PCR primer set specific for OPV's. This PCR amplifies the expected 260 bp products only in the presence of DNA from MOPV, HOPV, or LuIII, a parvovirus of unknown species origin. The assay may detect as little as 10 pg of MPV viral DNA or 1 pg of HOPV viral DNA, and it is able to detect MOPV in tissues from naturally infected mice and HOPV in tissues from experimentally infected hamsters, while it does not amplify from tissues of uninfected mice or hamsters.

*Guinea pig cardiovirus*

It is recommended in the 1994 version of the FELASA guidelines to monitor rats and mice for the cardiovirus *Theilers murine encephalomyelitis virus* (TMEV), Guinea pigs may also harbor a cardiovirus, which may be the causative agent of the disease guinea pig lameness (GPL). GPL is a paralytic and mortal disease similar to murine poliomyelitis which is caused by TMEV and to

Species	ELISA or IFA			HAI			Diagnosis
	KRV	HI	MVM	KRV	HI	MVM	
Mouse			+			+	MVM
			+			-	MPV
Rat	+	+		+	-		KRV
	+	+		-	+		H1
	+	+		-	-		ROPV

Table 3. Diagnosis of infection with parvovirus in rodents

human poliomyelitis.

In the beginning of the 20th century, experiments showed that GPL was transmissible by a filter passing agent indicating the presence of a viral pathogen (*Petrie and O'Brien, 1910; de Gasperi and Sangiorgi, 1911; Berger, 1913; Römer, 1924; Cole and Kuttner, 1926; reviewed in Hansen 1997*). More recently, serological studies revealed the presence of antibodies against TMEV in guinea pigs suffering from lameness, thus indicating a cardioviral pathogen (*Hansen et al., 1997*).

Our group has obtained genetic material from cerebrospinal fluid of guinea pigs suffering from lameness using PCR (unpublished data). A 231bp sequence was found to be identical with the internal ribosomal entry site sequence of encephalomyocarditis virus (EMCV). So although at this point, the virus has not been isolated, strong indications exist of a cardiovirus closely related to EMCV as the causative agent of GPL.

Vitamin C plays an unclarified role in the etiology of GPL (*Clausen et al., 2000*). Symptoms may be induced by a diet with low content of vitamin C (unpublished data) and the disease is successfully treated by vitamin C (*Hansen et al., 1997*). The pathogenesis may be similar to murine and human poliomyelitis in which the virus initially replicates in the alimentary tract and from there infects the central nerve system inducing demyelinating action (*Muir and Loon, 1997; Lipton and Jelachich, 1997*). Deficiency of vitamin C may be a predisposing factor allowing spread to the central nervous system, and, therefore, in colonies outbreaks of GPL are likely to occur if for accidental reasons the content of vitamin C in the diet is low.

#### *Guinea pig adenovirus*

Guinea pig adenovirus is a mandatory screening agent in the 1994 version of the FELASA guidelines. The recommendation is, however, not followed by all commercial breeders, although in guinea pigs involved in research projects a spontaneous necrotising bronchoalveolitis is regularly observed. Our group has since 1995 been involved with five cases, four in Denmark and one in Germany. Large basophilic intranuclear

inclusion bodies in desquamated bronchial epithelial cells are very characteristic for the disease. In the scientific literature Naumann et al. (1981) first reported the disease. Two years after it was again described by Brennecke et al. (1983). They described the intranuclear inclusion bodies and viral particles by light and electron microscopy. The viral particles closely resembled adenovirus particles.

The disease has been reproduced in new-born animals, but not in adolescent guinea pigs (*Kunstyr et al., 1984*) and attempts to transfer the disease to rats and hamsters have been unsuccessful (*Kunstyr et al., 1984*). Electron microscopic evidence of adenovirus as the cause of the disease has also been reported by Kunstyr et al. (1984) and Kaup et al. (1984) after successful experimental transfer to new-born guinea pigs.

Since then a number of publications describing similar natural outbreaks of a fatal pulmonary disease with adenovirus like inclusion bodies in bronchial epithelial cells have occurred (*Brandon, 1995; Breuer et al., 1997; Crippa et al., 1997; Harris et al., 1985; Junker et al., 1988; Feldman et al., 1990*).

The virus has not yet been isolated in vitro. In a recent study, Butz et al. (1999) also failed to reproduce the disease after inoculation with contaminated material. However, with PCR the virus was identified in the upper airways on days six through fifteen after inoculation. In addition, the virus was spontaneously transmitted from an experimentally infected animal to two out of five immunenaive cage mates.

The disease has a low contagiousness. The morbidity is also low but the course is acute with a very high mortality. The disease has been observed in Europe, USA and Canada.

In most cases of the disease, no distinct clinical signs are observed, but occasionally dyspnoea is noted. The disease is discretely scattered among animals in a room and very seldom are both animals in a cage affected. This suggest a fairly low contagiousness.

In animals dying from the disease, distinct consolidation of cranial lobes of both lungs is seen. Minor areas of consolidation may also be seen in remaining lobes. In a few cases

hydrothorax and ascites can be seen. Microscopically bronchi and bronchioles are partly or completely filled with necrotic material, debris of inflammatory cells and desquamated epithelial cells (Fig. 1). In larger bronchi the necrotic changes may include the submucosa or the whole bronchial wall. Numerous desquamated bronchial epithelial cells located in the periphery often contain large intranuclear basophilic inclusion bodies (Fig. 2). The inclusion body may sometimes completely fill the nucleus.

In an animal unit where guinea pigs in one or more rooms were affected by the disease, it was proven possible to house new disease-free guinea pigs in the unit kept on strict isolation from the infected animals without carry over of the disease. As soon as the rooms housing the affected animals were emptied, cleaned and disinfected, the disease-free animals could be moved into those rooms without acquiring the disease.

Our group has in all five cases observed been able to show antibodies in guinea pigs from the affected colonies by the use of ELISA using the antigen from Charles River (Wilmington, USA). The Charles River laboratory, Anmed in Praha, Czech Republic, has confirmed the test results.

#### *Guinea pig parainfluenza virus type III*

Parainfluenzaviruses (PIV) belong to the paramyxoviruses, which are RNA-viruses causing non-persistent infection in a range of animal species. PIV's may be divided into type 1, type 2, type 3 and type 4, the latter, however, only found in humans. In the 1994 version of the FELASA guidelines it is recommended to screen guinea pigs for the parainfluenzaviruses Sendai, PVM and SV5. However, none of these viruses have ever been isolated from guinea pigs.

The most important type in laboratory animals has for long been PIV-1 known as Sendai virus infecting rats and mice. Antibodies to this virus has also been revealed in guinea pigs, e.g. in Japan in 1986 80 to 100% of animals and 11 out of the 14 colonies examined were found positive (Nakagawa, 1986). As Sendai virus has not been isolated from guinea pigs, these results may be due to infection with another PIV. In the United States complement-fixing antibodies to PIV-3 were

found in guinea pigs already in the seventies, where 47 percent were positive to PIV-3 antigen (Welch *et al*, 1977).

In the nineties a PIV-3 was isolated from guinea pigs. A comparison of the nucleotide sequence among PIV-3s revealed that this guinea pig PIV-3 is closely related to human PIV-3. A phylogenetic analysis clearly show that guinea pig PIV-3 is a lineage of human PIV-3, suggesting that guinea pig PIV-3 has probably been introduced into guinea pig colonies via infected humans (Osahwa *et al*, 1998).

Few or no studies have described the possible impact of guinea pig PIV-3 on research. However, as experimental infection with human PIV-3 is a well-known research model, a huge amount of knowledge is available concerning the effects of human PIV-3 on guinea pigs. As human PIV-3 and guinea pig PIV-3 are closely related there is no reason to believe that such lessons from infection with human PIV-3 should not also be valid for infection with guinea pig PIV-3. PIV-3 infection in guinea pigs increase airway responsiveness to various substances, such as histamine, arecoline, acetylcholine, methacholine and substance P aerosols (Folkerts & Nijkamp *FP*, 1990; Kudlacz *et al*, 1994; Riedel *et al*, 1997). Increase in the number of broncho-alveolar cells (Folkerts *et al*, 1990a), a decrease in superoxide production by broncho-alveolar cells (Folkerts *et al*, 1990a) and the release of various inflammatory mediators, e.g. the arachidonic acid metabolite thromboxane B<sub>2</sub>(TxB<sub>2</sub>), is involved in this process (Henricks *et al*, 1993). PIV-3 also enhances inhalative allergic sensitization in guinea pigs, maybe due to increased mucosal permeability (Riedel *et al*, 1996). PIV-3 damages inhibitory M<sub>2</sub> muscarinic receptors on pulmonary parasympathetic nerves in the guinea pig (Fryer & Jacoby, 1991).

Serological screening for antibodies most easily performs diagnosis. ELISA using e.g. bovine PIV-3 as antigen is at present the method of choice. In the first PIV-3 screenings in guinea pigs, in which complement fixation assay was used, positive reactors were found in all animals above eight weeks. Animals had the highest titres at 12 weeks of age, while the highest prevalence (92%) was of

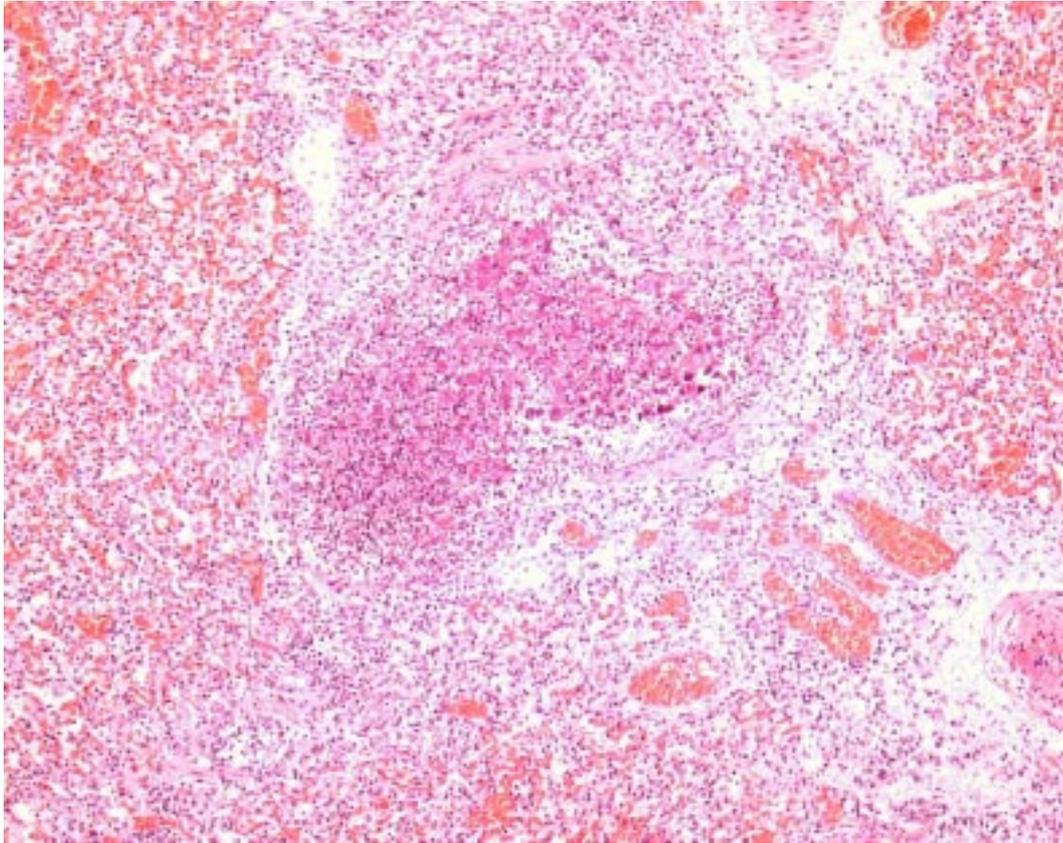


Figure 1. Overview of affected lung in guinea pig infected with adenovirus. Bronchi filled with necrotic material including debris of inflammatory cells and desquamated epithelial cells with basophilic intranuclear inclusion bodies. Objective magnification 10 X.

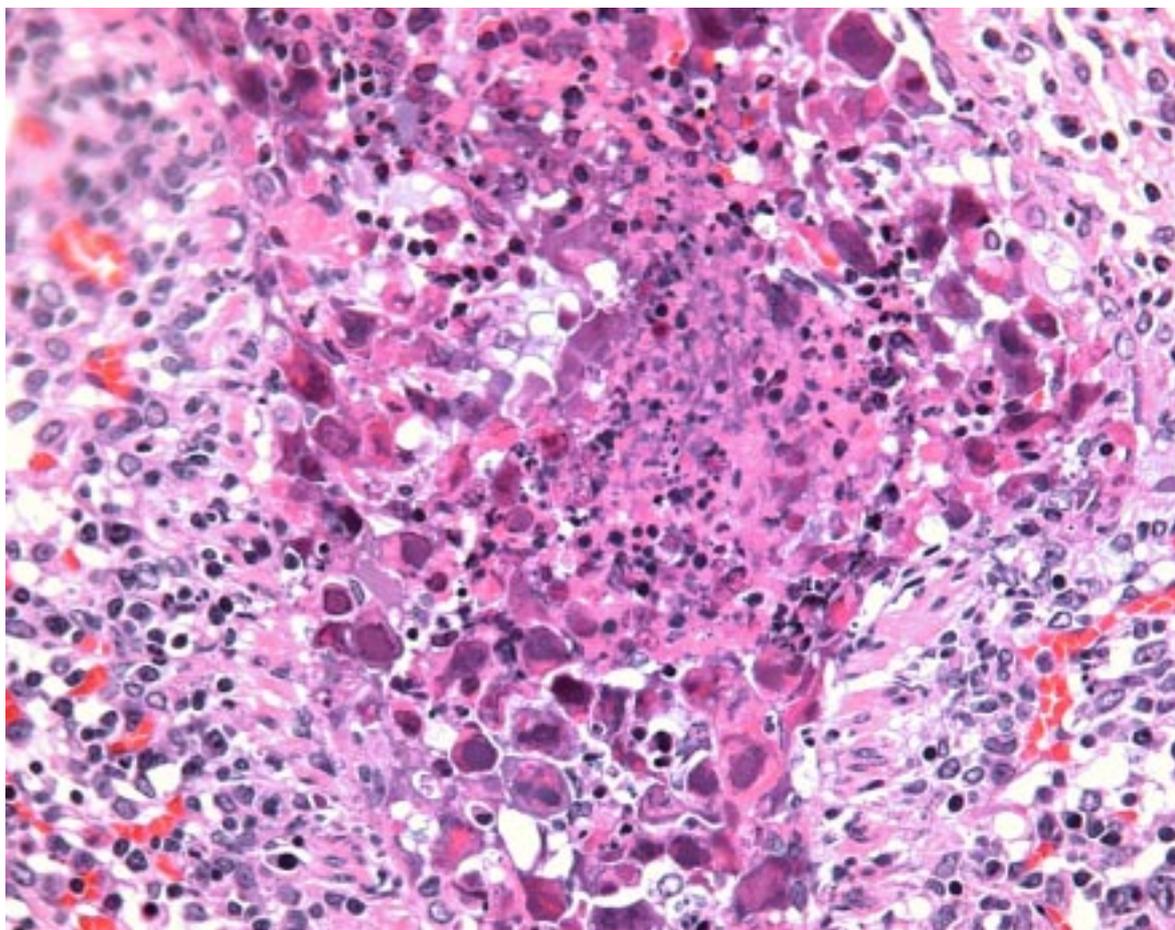


Figure 2. Close-up of affected lung in guinea pig infected with adenovirus. Desquamated bronchial epithelial cells with intranuclear inclusion bodies. Objective magnification 40 x.

among animals more than one year old (*Welch et al, 1977*). It has been shown that caesarian section may be used as a method for rederiving infected colonies (*Welch et al, 1977*).

#### Discussion

Several of the agents described in this paper need consideration in a revised set of health monitoring guidelines, simply because they were not described timely enough to be included in the 1994 FELASA guidelines. Furthermore, the transgenic technology has supplied researchers with tools to produce a great number of immunodeficient strains, which are more susceptible to a number of opportunistic infections which have hitherto not been considered of any particular importance. Intestinal disorders with an autoimmune component is now being associated with both new pathogens such as the *Helicobacter spp.* and others such as *Klebsiella spp.* which have been known for many years.

Examination of European rat and mouse colonies for clarification of *Helicobacter* status is much needed. For most European breeders little or insufficient information on *Helicobacter* status and test methods are available. In North America these agents are dealt with seriously, and researchers using rodents much less tolerate them. As research in mucosal and especially intestinal immunology advances, these subtle agents closely associated with the mucosal surfaces, receive increasing attention as causative agents of a long range of chronic inflammatory disease conditions in both animals and man. All species within *Pasteurellaceae* should be mandatory in health monitoring. In theory, the obligation to declare only "*Pasteurella spp.*" might in the worst cases be interpreted as no need to declare any findings, as the *Pasteurellaceae* in rats and mice are systematically to be regarded as either *Actinobacillus* or *Haemophilus*. CAR Bacillus should be moved to the mandatory list in the guidelines. Infection with orphan parvovirus may be the main parvoviral problem in European rats, and, therefore, they need consideration in a revised set of health monitoring guidelines.

In guinea pigs infection with parainfluenza virus, adenovirus and cardiovirus are probably more

common than some of the other virus infections listed in the 1994 FELASA guidelines (*Kraft et al, 1994*). Although adeno- and cardiovirus from guinea pigs have not yet been isolated in vitro, it is evident that they may cause clinical symptoms.

There is, therefore, all reasons to revise the guidelines which since 1994 has been the main instructions for health monitoring of laboratory rodents in Europe.

#### Summary

In 1994 the *Federation of European Laboratory Animal Science Associations* issued guidelines for health monitoring in breeding colonies of rodents and rabbits recommending specific agents, test methods and sampling sites for each species. Today most commercial breeders in Europe perform health monitoring, which as a minimum fulfils the standards of the FELASA guidelines. Laboratory animal science and microbiology are, however, rapidly developing fields, which has made a revision necessary; a process, which is going on at the moment. This paper describe rodent infections, which need consideration in a revised set of guidelines. The infections considered are *Helicobacter*, *Campylobacter*, *Haemophilus*, *Cilia associated respiratory bacillus* and orphan parvovirus in rats and mice, as well as adenovirus, cardiovirus and parainfluenzavirus in guinea pigs. Some of these agents were mentioned but not recommended for mandatory screening in the guidelines, some have not been as easily accepted as part of health monitoring schedules as other agents, while some were not, at all, included in the guidelines.

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