Implication for research of bacterial infections in laboratory animals

by *Kosaku Fujiwara*
Nihon University School of Veterinary Medicine, Fujisawa, Japan.

The laboratory animal is a biological entity that may have various kinds of bacterial diseases causing not only serious damage in animal production but also erroneous evaluation of data in animal experimentation. In addition, there may be hazardous problems of bacterial zoonoses transmitted to man from laboratory animals. For supplying quality animals from breeding colonies to sites of experimentation, the health assessment as well as environment control are indispensable (*Fujiwara* 1980). Freedom from infections with specified pathogenic bacteria including opportunistic or potential pathogens should be routinely monitored within breeding colonies and sites of experimentation.

Implication of bacterial infections in breeder and research colonies of laboratory animals

Any pathogenic bacteria being introduced into laboratory animal colonies may cause a considerable loss in production and can unfavourably modify the quality of animals as well as their response to experimental treatment.

Neonates and weanlings which are much more susceptible to bacterial diseases than adults usually constitute a large population of breeding colonies. If infections do occur, they may cause serious problems and damage. In case of infection in breeder colonies, their customers' research colonies may run high risks of involvement. The occurrence of classical bacterial infections such as salmonellosis and septicaemic pasteurellosis has been minimized, since most large scale breeding colonies today are barrier-protected.

On the other hand the risks of infection in research or test animal facilities are still high since animals in such colonies are indispensably and repeatedly exposed to various treatments and other stress factors, which may provide opportunity for inapparent infections to be turned into clinical and fatal illness. The occurrence of such types of opportunistic infection is common during animal experimentation especially when the animals are treated with immunosuppressive drugs (*Yamada et al.* 1969) or irradiated (*Flynn* 1963) or put into chronic toxicity tests. Without elimination of pathogenic organisms including potential or opportunistic pathogens from the research or test failities, observations and data might be difficult to interprete.

Monitoring for bacterial infections in breeder and research colonies of laboratory animals

The barrier system for preventing infections has been widely and successfully adopted in rodent breeder and test colonies. However, their microbiologically clean status should be routinely checked, because pathogens may invade at any time into the barriered colony, in spite of strict barrier control.

The monitoring may be carried out bacteriologically, pathologically or serologically. Microorganisms can be detected in appropriate test samples. Drinking water for Pseudomonas (*Maejima et al.* 1972), feces for salmonella spp. (*Habermann & Williams* 1958), and nasal mucosa swabs for *Bordetella bronchiseptica* (*Nakagawa et al.* 1969a) are recommended as material to be examined.

In overt disease pathological lesions may appear, but in cases of latent infection pathological changes or presence of bacteria are often overlooked. Such inapparent infec-

tions are of great importance in laboratory animal medicine. Consequently, immunological i.e. serological monitoring is often a first step to take, since it is effective in detection of inapparent infection (*Fujiwara* 1971, *Fujiwara et al.* 1981). Moreover, it is immunologically specific, technically simple and also less expensive as compared to bacteriological or pathological monitoring. Serological testing can be made with a large number of samples without risk of infection, if all antigens are inactivated. The serum antibody is comparatively stable and test samples are easy to transport without decrease of antibody titers. On the other hand, it should be born in mind that serology gives only information about antigens used. In cases where sensitivity is a problem, certain additional technical devices may be required (*Fujiwara* 1971).

Implication and monitoring of common bacterial infections in animal experiments

A number of bacterial infections may interfere with biomedical research using laboratory animals, and effective monitoring for these infections is necessary in safe animal experimentation. The monitoring should be based upon sufficient information on epizootiology, symptomatology, microbiology and pathology characteristic of each specific infection. Implication and monitoring of most common bacterial infections will be briefly described in alphabetical order.

Bordetellosis

Respiratory disorders due to *Bordetella bronchiseptica* may occur in guinea pigs, rats, and other species of laboratory animals. The cases in guinea pigs are most important. Fatality is high especially in young guinea pigs reared for complement supply. Adult animals may also be affected in cases of complex infection with Sendai virus or *Mycoplasma pulmonis* (*Ganaway et al.* 1965). In rats and rabbits infection is usually subclinical but may constitute a source of infection for guinea pigs. Transmission occurs by the nasal route and both apparently or inapparently infected animals may carry the organism in the nasal mucosa.

For diagnosis organisms can be isolated from the nasal mucosa and affected tissues on McConkey or DHL media. Serum agglutinin is detectable using formalin-treated phase I organisms (*Nakagawa et al.* 1969a) as well as by complement fixation test and enzyme-linked immunosorbent assay (ELISA) (*Mikazuki et al.* 1992). Pathological changes are characterized by catarrhal rhinitis and bronchopneumonia.

Brucellosis

Canine abortion (*Carmichael & Kenny* 1968) a zoonotic disease (*Jones & Emerson* 1984) caused by *Brucella canis* is of the same importance in laboratory animal medicine as is classic brucellosis due to *B. abortus, B. suis* and *B. melitensis* in veterinary medicine. The infection causes serious damage in dog breeding colonies as well as in research laboratories (*Jones & Emerson* 1984). Researchers and caretakers should take great care to avoid catching the infection. Galloping abortion in female breeders and orchitis with markedly enlarged testicles in males may occur (*Carmichael & Kenny* 1968), especially in beagle dogs (*Yamauchi et al.* 1974) being markedly susceptible to the infection. Oral transmission may occur with urine, vaginal discharge, or aborted fetuses (*Serikawa et al.* 1981) as well as along coital and transplacental routes. Inapparent infection is seen in its turn resulting in overt disease and followed by recurrent bacteremia.

The causative organisms can be isolated from blood and affected organs of infected animals. The diagnose can also be obtained by plate or tube serum agglutination. Examination at quarantine is of importance for elimination of carrier animals, while vaccination is effective for prevention of the disease. Autopsy reveals exudation in the uterus, enlarged or atrophied testicles and enlarged lymph nodes. Inflammatory oedema

is commonly present in the scrotal skin. In severe cases the spleen and liver are enlarged. By microscopy an acute diffus metritis and necrotic orchitis and epididymitis are characteristic.

Cilla-associated respiratory (CAR)-bacillosis

The CAR-bacillus is the agent causing chronic respiratory disease in rats and also weight loss and decreased food consumption during chronic toxicity tests (*Ganaway et al.* 1985, *Van Zwieten et al.* 1980). Nasal transmission ccurs between cage-mates. Infected animals have a noisy respiration and dyspnea and oedema under the mandibule (*Itoh et al.* 1987). There is no recovery after the onset. Wild rodents and rabbits are also susceptible.

The organism can be isolated by inoculating the chorio-allantoic membrane of embryonated eggs (*Ganaway et al.* 1985) with the affected tissue. The causative bacterium, being a Gram-negative, non-motile, filamentous, slender bacillus forming no spores, still remains unclassified. Serum antibody is detectable in infected animals by ELISA (*Ganaway et al.* 1985). The presence or absence of *Mycoplasma pulmonis* (*Nelson* 1963) should be confirmed. At autopsy there is a large amount of transparent or turbid exudate in the trachea with bronchiectasia and atelectasis. Microscopically, there is peribronchial infiltration of neutrophils and lymphocytes. In Fontana or Warthin-Starry stained smear preparations or sections of affected tissues, filamentous bacilli will be seen between bronchial cilia and within the bronchial lumen.

Colibacillosis

Enteric infection with *Escherichia coli,* characterized by diarrhea, is encountered in colonies of SPF animals having only a very limited enteric flora, as well as in research colonies using young mice (*Muto et al.* 1969). Although germ free mice exhibit no clinical disease after infection, severe fatal disease can be produced in adult mice immediately after their conventionalization (*Itoh et al.* 1979, 1980). The incidence is highest in mice aged 2 or 3 weeks. They show remarkable weight loss and dirty hairs stained with waterly yellow feces. Death occurs 2 to 5 days after the onsett.

Isolation of the causative bacterium on DHL and other media and its identification by specific antisera is needed for the final diagnosis. Being different from other organisms of the same genera, the causative *E. coli* has no flagella, is not producing indole, and show a serotype of O115a,c; K(B) (*Nakagawa et al.* 1969b). At necropsy of weanlings the wall of the small as well as large intestines is remarkably thickened. In those older than 4 weeks of age, only the large intestine, especially the colon, is affected. In cases with rectum involvement, rectal and anal prolapse may occur. In suckling mice the liver shows small yellow necrotic spots. Microscopically, there is a remarkable proliferation of enterocytes in the crypts, and goblet cells are activated and markedly increased in number. In the large intestine the ring muscle layer is thickened, whereas no inflammatory changes are visible in the submucosa.

Corynebacteriosis

Suppurative infection with *Corynebacterium kutscheri* is common in conventional colonies of rats and mice (*Weisbroth & Scher* 1968a). In most cases the infection is present in an inapparent form which may be converted to overt disease by stress imposed by transport, drug administration, or irradiation (*LeMaistre, Schechmeister & Adler* 1953). Severely diseased animals are inactive showing roughed hairs and dyspnea. Cutaneous abscesses and suppurative arthritis may be seen in some cases. Infected animals may in feces discharge organisms which are transmissible by oral as well as nasal routes. Sometimes infection occurs via cutaneous injuries.

Diagnosis can be readily made by detecting

Gram-positive bacteria on impression preparations or sections of affected tissue as well as by isolation of organisms on culture media such as blood agar. Serum agglutinin is detectable in infected mice and rats (*Fujiwara* 1971, *Weisbroth & Scher* 1968b, *Yokoiyama et al.* 1977). Cortisone provocation may be effective in detecting inapparent infection (*Takagaki et al.* 1967, *Utsumi et al.* 1969). Grossly various-sized white or yellow colored nodules containing a caseous mass are seen in the lungs, heart, kidney and lymph nodes. This is why the disease has been called murine pseudotuberculosis. In the intestinal type of infection, hemorrhagic ulcers and abscesses are formed in the gut mucosa, and the mesenteric lymph nodes are enlarged. Microscopically pseudotuberculous nodules, produced in the parechymatous organs, are characterized by massive necrosis, neutrophil infiltration and fibrous encapsulation, the thickness of which depends on the chronicity of the disease.

Pasteurellosis

Pasteurella pneumotropica which has been proposed to be an actinobacillus spp., is a causative organism for either chronic respiratory disease or systemic disorders in mice and rats. The infection is often inapparent (*Hoag et al.* 1962, *Saito et al.* 1981) while some cases show purulent pneumonia, conjunctivitis and subcutaneous abscesses (*Van der Schaaf et al.* 1970). *P. pneumotropica* is so widely differing from other species of pasteurella in pathogenesis as well as sugar fermentation that it has been proposed to be classified into other genera.

Pasteurella multocida is a serious problem in rabbits used for pyrogen test and other toxicologic studies, producing severe clinical disease resulting in failure of entire studies. Infected rabbits exhibit a considerable amount of suppurative discharge from the nostrils and the symptom has been called "snuffles" (*Hagen* 1958). Conjunctivitis and subcutaneous cellulitis are also produced. Animals with otitis media show head inclination and rolling movement (*Fox et al.* 1971).

Necropsy and microscopy reveal purulent inflammation and abscess formation in the lungs, uterus, mammary gland, testicles and middle ear. Fibrinous pleuritis and peritonitis may be present. Although clinical signs of "snuffles" are indicative of rabbit pasteurellosis it is necessary to isolate and identify the causative organisms from nasal exudate, affected tissues or septicemic blood on selective media (*Garlinghouse et al.* 1981, *Mikazuki et al.* 1987).

Pseudomoniasis

Pseudomonas aeruginosa is widely distributed in soil and water in nature. It may cause septicemic deaths in mice, rats and rabbits having been irradiated (*Flynn* 1963) or treated with antibiotics (*Halkett* 1968, *Verder & Rosenthal* 1961, *Urano & Maejima* 1978). It may live in the lower digestive tract, which provides a favourable intestinal microenvironment for *P. aeruginosa.* The elimination of *P. aeruginosa* from the environment is indispensable for experiments concerning immunosuppression. Diseased mice and rats may show suppurative otitis media, pyelonephritis and pneumonitis leading to septicemia in the moribund stage. Animals with otitis media may keep their heads askew and show circling movements. Dermatitis and endocarditis may be seen in rabbits and dogs. Local lesions are characterized by necrosis with infiltration of neutrophils.

Routine bacteriological examination of the water in drinking bottles as well as swab samples from the oral mucosa, using selective NAC media, is helpful in detecting inapparent infections in animal colonies. Organisms can also be isolated from feces or lesions. Identification is readily made by identification of the specific green pigment, pyocyanin, produced by the organism. Clorination or adding gentamycin to drinking water (*Urano et al.* 1977) is effective for the prevention of infection.

Salmonellosis

While rare in barrier colonies, enteric and systemic infection with salmonella spp., may be a serious problem in conventional laboratory animals (*Habermann & Williams* 1958) followed by possible infection in caretakers and researchers. The mouse (*Margard & Litchfield* 1963, *Simpson & Simmons* 1981) and the guinea pig (*Olfert et al.* 1976, *Onyekaba* 1983) are most susceptible to salmonellae, followed by the hamster and the rabbit. After oral transmission, by ingesting soiled feed, two types of disease may appear; an acute epizootic type with high mortality or a chronic or inapparent type with low mortality. In the case of acute disease septicemic deaths occur during several days after infection. Chronically infected animals may show no clinical signs, and like inapparently infected ones shed infective organisms in their feces for long time.

Routine monitoring for salmonellosis is necessary for maintaining an acceptable hygienic state of animal colonies. Monitoring and diagnosis can be made by isolation of organisms from feces, blood or affected tissues using selective media such as McConkey and DHL. The most frequent isolates from laboratory animals are *Salmonella typhimurium* and *S. enteritidis,* but even other species may be pathogenic. Autopsy reveals splenomegaly, multiple necrotic foci in the liver and enlarged mesenteric lymph nodes. By microscopy the liver and lymphoid organs have disseminated necrotized lesions with neutrophil infiltration and accumulations of macrophages.

Shigellosis

Digestive tract infection with *Shigella, dysenteriae, S. flexneri, S. sonnei* and other *Shigella spp.* is common in non-human primates for laboratory use. Since no natural infection has been recognized in the wildlife (*Carpenter & Cooke* 1965, *Takasaka et al.* 1964), infection of monkeys is believed to occur after capture and contact with man. Hygienic precaution is indispensable in handling and treating laboratory monkeys as shigella spp. readily infects human. A few days after oral infection animals show vomiting and watery or bloody diarrhea, sometimes resulting in 70 % or higher mortality within a week.

For diagnosis the causative organisms can be isolated on DHL or McConkey medium. The most frequent isolates are those of *S. flexneri* causing dysentery also in man. At autopsy the mucosa of large intestines is thickened with oedema, erosions and fibrin deposits. Sometimes ulcerations are seen. Microscopy reveals catarrhal or diphteric enteritis.

Streptococcosis

The guinea pig is highly sensitive to infection with *Streptococcus zooepidemicus,* a Gram-positive coccus showing beta hemolysis in culture. A large number of animals are acutely and severely diseased showing considerable purulent discharge from eyes and nostrils. Most animals may die within a few days. Sporadically chronic cases occur showing gradual weight loss, conjunctivitis, arthritis and otitis media with remarkable enlargement of superficial lymph nodes. In more chronic cases suppurative pleuritis and peritonitis as well as abscess formation in many organs may be seen. Since the organism is widely distributed among many species of animals causing no illness, attention should be paid to isolation of susceptible guinea pigs from other animal species. The rat, mouse and guinea pig have high susceptibility also to *S. pneumoniae* (*Ford* 1965, *Saito et al.* 1983).

The swelling of superficial lymph nodes and nasal and eye discharge are indicative of the infection. Diagnosis is obtained by means of bacterial culture from affected tissues. Pathology reveals necroses with a considerable accumulation of neutrophil leukocytes, which are surrounded by epithelioid cells and fibrous tissue.

Tuberculosis

Acute or chronic disease due to *Mycobacterium tuberculosis* or *M. bovis* is frequently encountered in simian species captured or bred for experimental use. The organisms are also highly infective for guinea pigs, rabbits and man. Dogs and cats are less sen sitive but occasionally they may get acutely and fatally infected. Isolation or prompt sacrifice of infected animals is recommended for prevention against infection to man and other susceptible animals. Transmission occurs mostly by respiratory and sometimes by oral routes from affected animals.

For diagnosis tuberculin test is useful in case of non-human primates but it should be noticed that there is an anergic phase in severely affected cases, for which X-ray examination, other clinical findings or bacterial culture (*Tribe & Welburm* 1976) may be helpful. At autopsy tubercle formation may be seen in the liver, spleen, kidney adrenal and lung. In the lung, cavernous lesions can be produced after discharge of massively necrotized tissue. The regional lymph nodes are always affected. Histopathologically tuberculous lesions consist of central caseous necrosis, proliferative epithelioid cells and Langhans giant cells, accumulation of lymphoid cells and surrounding fibrosis. Organisms can be detected within necrotized tissue and epithelioid cells by acid-fast stain.

Tyzzer's disease

The disease is characterized by enterohepatitis and myocarditis caused by a strictly intracellular bacterium, which was named "*Bacilus piliformis*" by *Tyzzer* (1917). The causative organism is a Gram-negative slender rod with peritrichous flagella forming endospores, growing on cultured murine hepatocytes (*Kawamura et al.* 1989) as well as in chick embryos (*Fujiwara et al.* 1975). It remains unable to grow on artificial media. Natural disease is encountered in the rat, mouse, hamster, guinea pig, Mongolian gerbil, dog, cat and rhesus monkey (*Fujiwara* 1978). Mongolian gerbils and hamsters are more susceptible to oral infection than rats and mice (*Waggie et al.* 1987, *Yokomori et al.* 1989). Although acute illness with hemorrhagic diarrhea may occur, most apparent cases are seen in connection with stress such as pregnancy or immunosuppressive treatment (*Yamada et al.* 1969). The serum transaminase level is elevated in parallel with the progress of necrotized lesions in the liver, followed by bacteremia at moribund stage. A genetic background has been suggested behind apparent illness of rats (*Hansen et al.* 1990). Epizootics of the disease seem to be rare (*Itoh & Kagiyama* 1985).

At autopsy the liver shows multiple focal necroses in subacute or chronic cases while diffuse and confluent necroses are seen in acute fatal cases. Intestinal lesions characterized by oedema and hemorrhage mostly appear in the ileocecal areas (*Yokomori et al.* 1989). Intact or degenerated hepatocytes, surrounding necrotic foci in the liver, contain slender bacilli detectable by Giemsa, PAS or Grocott stain. The same organisms are present within enterocytes of affected intestines and in the myocardium (*Allen et al.* 1956, *Fries & Svendsen* 1978). Serum antibody is detectable by immunofluorescence, complement fixation test or ELISA (*Toriumi et al.* 1986, *Fujiwara et al.* 1981), while some antigenic differences are noted among organisms from different origins (*Fujiwara et al.* 1985). Natural infection may be established by ingestion of spores.

References

Allen AM, JR Ganaway, ID Moore & RF Kinard: Tyzzer's disease syndrome in laboratory rabbits. Am. J. Pathol. 1965, *46,* 859–882.

Carmichael LE & RM Kenny: Canine abortion caused by *Brucella canis.* J. Amer. Vet. Med. Assoc. 1968, *152,* 605–616.

Carpenter KP & ERN Cooke: An attempt to find *Shigellae* in wild primates. J. Comp. Pathol. 1965, *75,* 201–204.

Flynn RJ: Pseudomonas aeruginosa infection and radiobiological research at Argonne National Laboratory: Effect, diagnosis, epizootiology, control. Lab. Anim. Care. 1963, *13* (Part II), 25–35.

Ford TM: An outbreak of pneumonia in laboratory rats associated with *Diplococcus pneumoniae* type 8. Lab. Anim. Care. 1965, *15,* 448–451.

Fox RR, RF Norberg & DD Meyers: The relationship of *Pasteurella multocida* to otitis media in the domestic rabbit (*Oryctolagus cuniculus*). Lab. Anim. Care. 1971, *21,* 45–48.

Fries AS & O Svendsen: Studies on Tyzzer's disease in rats. Lab. Anim. 1978, *12,* 1–4.

Fujiwara K: Problems in checking inapparent infections in laboratory animals: An attempt at serological checking by anamnestic response. Proc. the 4th Internat. Symp. Lab. Anim. 1971, pp. 77–92. Nat. Acad. Sci. Washington, D.C.

Fujiwara K: Tyzzer's disease. Jpn. J. Exp. Med. 1978, *48,* 467–480.

Fujiwara K: Health assessement for laboratory rodent production colonies. Lab. Anim. Sci. 1980, *30,* 298–303.

Fujiwara K, S Takahashi, S Takenaka, M Nakayama & T Tamura: Hepatotropisme de bacilles de Tyzzer chez l'embryen de poulet experimentalement infectes. C. R. Soc. Biol. 1975, *169,* 1108–1113.

Fujiwara K, M Nakayama & K Takahashi: Serologic detection of inapparent Tyzzer's disease in rats. Jpn. J. Exp. Med. 1981, *51,* 197–200.

Fujiwara K, M Nakayama, H Nakayama, W Toriumi, S Ogihara & A Thunert: Antigenic relatedness of *"Bacillus piliformis"* from Tyzzer's disease occurring in Japan and other regions. Jpn. J. Vet. Sci. 1985, *47,* 9–16.

Ganaway JR, AM Allen & CW McPherson: Prevention of acute *Bordetella bronchiseptica* pneumonia in a guinea pig colony. Lab. Anim. Care. 1965, *15,* 156–162.

Ganaway JR, TH Spencer, TP Moore & AM Allen: Isolation, propagation and characterization of a newly recognized pathogen, cilia-associated respiratory bacillus of rats, an etiological agent of chronic respiratory disease. Infect. Immun. 1985, *47,* 472–479.

Garlinghouse Jr LE, RF DiGiacomo, GL Van Hoosier Jr & J Gordon: Selective media for *Pasteurella pneumotropica* and *Bordetella bronchiseptica.* Lab. Anim. Sci. 1981, *31,* 39–42.

Habermann RT & FP Williams: Salmonellosis in laboratory animals. J. Nat. Cancer Inst. 1958, *20,* 933–947.

Hagen KW: Enzootic pasteurellosis in domestic rabbits I. Pathology and bacteriology. J. Amer. Vet. Med. Assoc. 1958, *133,* 77–80.

Halkett JAE: The effect of cold stress and *Pseudomonas aeruginosa* lavage on the survival of three-week-old Swiss mice. Lab. Anim. Care. 1968, *18,* 94–96.

Hansen AK, O Svendsen & KE Møllegaard-Hansen: Epidemiological studies of *Bacillus piliformis* infection and Tyzzer's disease in laboratory rats. Z. Versuchstierk. 1990, *33,* 163–169.

Hoag WG, PW Wetmore, J. Rogers & H Meier: A study of latent *Pasteurella* infection in a mouse colony. J. Infect. Dis. 1962, *111,* 135–140.

Itoh K, K Maejima, K Ueda & K Fujiwara: Difference in susceptibility of mice raised under barrier-sustained (SPF) or conventional conditions to infectious megaenteron. Microbiol. Immunol. 1979, *23,* 909–913.

Itoh K, K Ueda & K Fujiwara: Susceptibility of germ-free mice to infectious megaenteron. Microbiol. Immunol. 1980, *24,* 281–290.

Itoh T & N Kagiyama: An epizootic form of Tyzzer's disease in a rat colony. Exp. Anim. 1985, *34,* 85–88.

Itoh JT, K Kohyama, A Takakura, T Takenouchi & N Kagiyama: Naturally occurring CAR bacillus infection in a laboratory rat colony and epizootiological observation. Exp. Anim. 1987, *36,* 387–393.

Jones RL & JK Emerson: Canine brucellosis in a commercial breeding kennel. J. Amer. Vet. Med. Assoc. 1984, *184,* 834–835.

Kawamura S, T Mitsuoka & K Fujiwara: Initial phase of infection with Tyzzer's organism in cultured hepatocytes. J. Gen. Microbiol. 1989, *135,* 2099–2106.

LeMaistre C & R Tompsett: The emergence of pseudotuberculosis in rats given cortisone. J. Exp. Med. 1952, *95,* 393–408.

Maejima K, T Urano, K Fujiwara & Y Homma Jr: Bacteriological and clinical observations of experimental infection with *Pseudomonas aeruginosa* in mice. Jpn. J. Exp. Med. 1972, *42,* 569–574.

Margard WL & H Litchfield: Occurrance of unusual Salmonellae in laboratory mice. J. Bact. 1963, *85,* 1451–1452.

Mikazuki K, K Takahashi, K Nanba & Y Hayashi: Selective media for *Pasteurella pneumotropica.* Exp. Anim. 1987, *36,* 229–237.

Mikazuki K, T Hirasawa, Y Sakai, S Ohhara, H Nenui & K Takahashi: Evaluation of enzyme-linked immunosorbent-assay for serodiagnosis of *Bordetella bronchiseptica* infection in guinea pigs. Exp. Anim. 1992, *41,* 357–363.

Muto T, M Nakagawa, Y Isobe, M Saito, T Nakano & K Imaizumi: Infectious megaenteron of mice. I. Manifestation and pathological observation. Jpn. J. Med. Sci. Biol. 1969, *22,* 363–374.

Nakagawa M, T Muto, T Nakano, H Yoda, K Ando & K Imaizumi: Some observations on diagnosis of *Bordetella bronchiseptica* infection in guinea pig. Exp. Anim. 1969a, *18,* 105–116.

Nakagawa M, R Sakazaki, T Muto, M Saito, T Hagiwara & K Imaizumi: Infectious megaenteron of mice II. Detection of coliform organisms of an unusual biotype as the primary cause. Jpn. J. Med. Sci. Biol. 1969b,*22,* 375–382.

Nelson JB: Chronic respiratory disease in mice and rats. Lab. Anim. Care. 1963, *13,* 137–143.

Olfert ED, GE Ward & D Stevenson: Salmonella typhimurium infection in guinea pigs: observation on monitoring and control. Lab. Anim. Sci. 1976, *26,* 78–80.

Onyekaba CO: Clinical salmonellosis in a guinea pig colony caused by a new salmonella serotype, *Salmonella ochiogu.* 1983, *17,* 213–216.

Saito M, K Kojima, J Sano, K Nakayama & M Nakagawa: Carrier state of *Pasteurella pneumotropica* in mice and rats. Exp. Anim. 1981, *30,* 313–316.

Saito M, T Muto, S Haruzono, M Nakagawa & M Sato: An epizootic of pneumococcal infection occurring in inbred guinea pig colonies. Exp. Anim. 1983, *32,* 29–37.

Schechmeister IL & FL Adler: Activation of pseudotuberculosis in mice exposed to sublethal total body radiation. J. Infect. Dis. 1953, *92,* 228–239.

Serikawa T, T Muraguchi, J Yamada & H Takada: Long-term observation of canine brucellosis. Excretion of *Brucella canis* into urine of infected male dog. Exp. Anim. 1981, *30,* 7–14.

Simpson W & DJC Simmons: Salmonella livingstone salmonellosis in laboratory mice. Successful containment and treatment of the disease. Lab. Anim. 1981, *15,* 261–262.

Takagaki Y, M Naiki, M Ito, G Noguchi & K Fujiwara: Checking of infections due to Corynebacterium and Tyzzer's organism among mouse breeding colonies by cortisone injection. Exp. Anim. 1967, *16,* 12–19.

Toriumi W, S Kawamura & K Fujiwara: Application of enzyme-linked immunosorbent assay (ELISA) to detection of antibodies against Tyzzer's organism (*Bacillus piliformis*) in mice. Jpn. J. Vet. Sci. 1986, *48,* 1241–1244.

Takasaka M, S Honjo, T Fujiwara, T Hagiwara, H Ogawa & K Imaizumi: Shigellosis in cynomologus monkeys (*Macaca irus*). I. Epidemiological surveys on shigella infection rate. Jpn. J. Med. Sci. Biol. 1964, *17,* 259–265.

Tribe GW & AE Welburm: Value of combining the erythrocyte sedimentation rate test with tuberculin testing in the control of tuberculosis in baboons. Lab. Anim. 1976, *10,* 39–46.

Urano T, K Maejima, O Okadama, S Takashina, S Shumiya & H Tamura: Control of *Pseudomonas aeruginosa* infection in laboratory mice with gentamycin. Exp. Anim. 1977, *26,* 259–262.

Urano T & K Maejima: Provocation of pseudomoniasis with cyclophosphamide in mice. Lab. Anim. 1978, *12,* 159–161.

Utsumi K, Y Matsui, R Ishikawa, S Fukagawa, H Tatsumi, Y Fujimoto & K Fujiwara: Checking of corynebacterial infection in rats by cortisone treatment. Exp. Anim. 1969, *18,* 59–67.

Van der Schaaf A, JW Mullink, RJ Nikkels & J Goudswaard: Pasteurella pneumotropica as a causal microorganism of multiple subcutaneous abscesses in a colony of Wistar rats. Z. Versuchstierk. 1970, *12,* 356–362.

Van Zwieten MJ, HA Solleveld, JR Lindsey, FG de Groot, C Zurcher & CF Hollander: Respiratory disease in rats associated with a filamentous bacterium: a preliminary report. Lab. Anim. Sci. 1980, *30,* 215–221.

Verder E & SM Rosenthal: Role of infection in delayed deaths of mice following extensive burn injury. Proc. Soc. Biol. Med. 1961, *108,* 501–505.

Waggie KS, LP Thornburg, KJ Grove & JE Wagner: Lesions of experimentally induced Tyzzer's disease in Syrian hamsters, guinea pigs, mice and rats. Lab. Anim. 1987, *21,* 155–160.

Weisbroth SH & S Scher: Corynebacterium kutscheri infection in the mouse. I. Report of an outbreak, bacteriology and pathology of spontaneous infections. Lab. Anim. Care. 1968a, *18,* 451–458.

Weisbroth SH & S Scher: Corynebacterium kutscheri infection in mice II. Diagnostic serology. Lab. Anim. Care. 1968b, *18,* 459–468.

Yamada A, Y Osada, S Takyama, T Akimoto, H Ogawa, Y Oshima & K Fujiwara: Tyzzer's disease syndrome in laboratory rats treated with adrenocorticotropic hormone. Jpn. J. Exp. Med. 1969, *39,* 505–518.

Yamauchi C, T Suzuki, T Nomura, Y Kukita, T Iwaki, Y Kazuni & A Gohda: Canine brucellosis in a beagle colony. Jpn. J. Vet. Sci. 1974, *36,* 175–182.

Yokoiyama S, K Mizuni & K Fujiwara: Antigenic heterogenecity of *Corynebacterium kutscheri* from mice and rats. Exp. Anim. 1977, *16,* 263–266.

Yokomori K, N Okada, Y Murai, N Gatao & K Fujiwara: Enterohepatitis in Mongolian gerbils (*Meriones unguiculatus*) inoculated perorally with Tyzzer's organism (*Bacillus piliformis*). Lab. Anim. Sci. 1989, *39,* 16–20.