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Microbiological Monitoring of Guinea Pigs Reared Conventionally at Two Breeding Facilities in Korea

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Abstract: *In this study, microbiological monitoring of guinea pigs reared conventionally in two facilities was performed twice in 2004, with a three-month-interval between surveys. This study was based on the recommendations of the FELASA Working Group, with some modifications. In serological tests in the first survey, some animals from facility A showed positive results for* Encephalitozoon cuniculi*, Sendai virus, pneumonia virus of mice (PVM), and Reovirus-3 (Reo-3); facility B showed a positive result only for* E. cuniculi. *The results of the second survey were similar to the first, except for the presence of Sendai virus; all animals from the two facilities were Sendai virus-negative in the second experiment. No pathogenic bacteria were cultured in the organs of any of the animals in the first survey. However, in the second survey,* Bordetella bronchiseptica *was cultured from the lung tissue of two 10-week-old animals from facility A. Chlamydial infection was examined by the Macchiavello method, but no animal showed positive results. Tests using fecal flotation or the KOH wet mount method showed no infection of endoparasites, protozoa, ectoparasites, or dermatophytes in any animal in both surveys. However, in the histopathological examination, an infection of protozoa-like organisms was observed in the cecum of some animals from facility A. The present study revealed that microbiological contamination was present in guinea pigs reared conventionally in two facilities in Korea, suggesting that there is a need to improve environmental conditions in order to eradicate microbial contamination.*
Key words: *contamination, environmental condition, guinea pig, microbiological monitoring*

Introduction

Rapid advances in biotechnology are leading to the increased use of laboratory animals. The scientists using laboratory animals are required to consider the animals' welfare, as it could have an effect on achieving precise scientific results. For this reason, it is important to strictly control the quality of laboratory animal

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facilities. Microbiological control is important because the use of animals that possess pathogens may affect experimental results, leading to a loss of time and money.

The guinea pig is a representative laboratory animal and has been used for more than 200 years in a broad range of fields, including anaphylaxis, asthma, delayed-type hypersensitivity, immunology, infectious diseases, and pharmacology [8]. The use of guinea pigs has gradually increased; approximately 505,000 were used for studies in the U.S. in 2000 (U.S. Department of Agriculture, Animal and Plant Health Inspection Service). In Korea, most commercial breeding facilities provide not only specific pathogen-free guinea pigs with microbiological monitoring data, but also provide conventional animals, for which the microbiological status is unidentified. Thus, there is a need to clarify the microbiological status of the conventional animals and if any problem is found, recommendations should be made to improve the environment.

The Federation of European Laboratory Animal Science Associations (FELASA) has revised its recommendations for monitoring the health of laboratory animals, including guinea pigs, in breeding and experimental units [5]. In the present study, based on the recommendations of FELASA, with some modifications, guinea pigs reared conventionally at two breeding facilities were microbiologically monitored twice in 2004, with a three-month-interval between surveys.

Materials and Methods

Animals

Two groups of ten conventionally-reared guinea pigs (which consisted of two animals that were 4-week-old, four that were 10-week-old, and four that were 24-week-old) were purchased from two breeding facilities in Korea (10 animals from each facility). Once delivered to our laboratory, the animals were anesthetized with a combination of ketamine (45 mg/kg) and xylazine (8.8 mg/kg) and blood samples were collected by cardiac puncture. The animals were sacrificed by cervical dislocation, then used for microbiological monitoring (first survey). After three months, this survey was repeated using the same conditions for animal composition and the same experimental protocols (second survey). All animal experiments were carried out based on the guidelines and regulations for the care and use of laboratory animals of Seoul National University.

Serological tests

Serological tests were performed on the 10-week-old and 24-week-old animals from both groups. After bleeding, sera were collected by centrifugation at 3,000 rpm for 10 min and stored at –20°C until use. For the detection of antibodies to *Encephalitozoon cuniculi*, Sendai virus, pneumonia virus of mice (PVM), reovirus 3 (Reo-3), and simian virus (SV-5), commercially available enzyme-linked immunosorbent assay (ELISA) kits (Charles River Laboratories, Inc, Wilmington, MA, USA) were used according to the manufacturer's direction.

Examination for ectoparasites and dermatophytes

Portions of the guinea pigs' skin that showed a gross lesion (e.g., a scab or scar) were stamped with Scotch cellophane tape. For animals not showing any gross lesions, sampling was performed randomly from dorsal or dorsolateral skin. The cellophane tape was attached to a glass slide and examined microscopically for the presence of ectoparasites. For the detection of dermatophytes, several strips of hair including the roots were pulled out using forceps. The hairs were placed on a glass slide and one drop of 10% KOH solution (potassium hydroxide 10 g, glycerin 10 ml, distilled water 80 ml) was added. The slides were incubated for 10 min at room temperature and examined microscopically.

Examination for chlamydial infection

A conjunctival swab from each animal was spread on a slide and stained using the Macchiavello method [1] with some modifications. Briefly, the slides were air-dried, immersed in basic fuchsin solution for 5 min, then washed with tap water. The slides were allowed to react in 0.5% citric acid for 1 s, then washed, and stained with 1% methylene blue for 10 s. Then, the slides were air-dried and examined microscopically.

Bacterial culture

Lung and fecal specimens were used for bacterial culture. The specimens were gently homogenized and the homogenate was smeared onto agar with 5% sheep blood for the culture of *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Pasteurella multocida*, and *Streptococcus* spp. Xylose lysine deoxycholate (XLD) and cefsulodin-irgasan-novobiocin (CIN) agar were used

for the culture of *Salmonella* spp. and *Yersinia pseudotuberculosis*, respectively. For the culture of *Streptobacillus moniformis*, brain heart infusion (BHI) agar containing 20% horse serum was used. After an incubation period of 24–48 h at 37°C (CIN agar, for *Y. pseudotuberculosis*, at 25°C), each single colony was subcultured and a VITEK system (BioMerieux, Inc., Durham, NC, USA) was used to characterize the bacterial species.

Examination for endoparasites

The contents of the large intestine were used for the detection of endoparasites and this experiment was performed according to the method used in our previous report [15].

Examination of histopathology

At necropsy, major organs were fixed in 10% buffered formalin for 24 h, processed in an alcohol-xylene series, and embedded in paraffin wax. Sections, 2 μm thick, were prepared, stained with hematoxylin and eosin, and examined microscopically.

Results

Serology

In the first survey, the results of the serological tests showed that *E. cuniculi*, Sendai virus, PVM, and Reo-3 were present in the animals from facility A, regardless of their age, and *E. cuniculi* was present in 24-week-old animals from facility B (Table 1). In the second survey, we detected the presence of antibodies against *E. cuniculi*, PVM, and Reo-3 in the animals from facility A, but no animals were seropositive for Sendai virus. Some animals from facility B were seropositive for *E. cuniculi*, but all were seronegative for Sendai virus, PVM, Reo-3, and SV-5 (Table 1).

Ectoparasites, dermatophytes, and chlamydial infection

The presence of ectoparasites, dermatophytes, or Chlamydia was not observed in any animal in both surveys.

Bacteriology

In the present study, the target microorganisms for bacterial culture included *B. bronchiseptica*, *C. kutscheri*, *P. multocida*, *Salmonella* spp., *S. moniliformis*, *Streptococcus* spp., and *Y. pseudotuberculosis*. In the first experiment, no microorganisms were isolated from the lung or fecal specimens from any animal from both facilities. However, in the second experiment, *B. bronchiseptica* was found in cultures from the lung specimens of two animals (both 10-week-old) from facility A. No other microorganisms were isolated from the rest of the animals from both facilities.

Table 1. Summary of serological tests*

Facility	Age	No. of positive samples / no. of samples tested				
		ECUN	SEND	PVM	REO-3	SV-5
A	10 weeks old	2/4 (4/4)	1/4 (0/4)	3/4 (4/4)	4/4 (4/4)	0/4 (0/4)
	24 weeks old	2/4 (2/4)	4/4 (0/4)	3/4 (0/4)	4/4 (0/4)	0/4 (0/4)
B	10 weeks old	0/4 (2/4)	0/4 (0/4)	0/4 (0/4)	0/4 (0/4)	0/4 (0/4)
	24 weeks old	2/4 (3/4)	0/4 (0/4)	0/4 (0/4)	0/4 (0/4)	0/4 (0/4)

*This survey was performed twice with a three month interval between surveys. The data in parentheses show the results of the second survey. ECUN: *E. cuniculi*, SEND: Sendai virus, PVM: Pneumonia virus of mice, REO-3: Reovirus-3, SV-5: Simian virus-5.

Endoparasites

The presence of endoparasites was examined from large intestinal contents using the fecal floatation method. No endoparasites or larvae were observed in the intestinal contents samples from the guinea pigs.

Histopathological findings

The histopathological findings are summarized in Table 2. In both the first and second surveys, various lesions such as necrosis, hyperplasia, and inflammation were observed randomly in several organs of the animals. The most remarkable pathological finding was moderate to severe interstitial pneumonia, which was observed in all animals from facility A in both surveys. Inflammation was characterized by thickness of the alveolar wall and multifocal or diffuse lymphocytic infiltration adjacent to the bronchi (Fig. 1A). Some animals showed necrotic foci with granulocytic infiltration in the lungs. This was also found in several

Table 2. Summary of histopathological findings*

Facilities	A			B		
Age	4 weeks	10 weeks	24 weeks	4 weeks	10 weeks	24 weeks
Extramedullary hematopoiesis in the spleen	2/2 (0/2)	0/4 (1/4)	0/4 (0/4)	0/2 (2/2)	0/4 (3/4)	0/4 (4/4)
Hepatic necrosis with chronic inflammation	2/2 (2/2)	2/4 (2/4)	0/4 (0/4)	0/2 (0/2)	0/4 (0/4)	0/4 (1/4)
Interstitial pneumonia	2/2 (2/2)	4/4 (2/4)	4/4 (4/4)	1/2 (1/2)	0/4 (3/4)	2/4 (4/4)
Interstitial nephritis	0/2 (0/2)	1/4 (0/4)	1/4 (0/4)	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)
Pericarditis/myocarditis	0/2 (0/2)	1/4 (0/4)	0/4 (0/4)	0/2 (1/2)	0/4 (2/4)	1/4 (0/4)
Inflammation or degeneration in the brain	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)
Conjunctivitis	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)
Papillary hyperplasia in the eyelid	1/2 (0/2)	0/4 (0/4)	0/4 (0/4)	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)
Inflammation of the gastrointestinal tract	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)
Parasitic infection in the cecum	0/2 (1/2)	0/4 (2/4)	2/4 (0/4)	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)
Inflammation in the genital system	0/2 (0/2)	0/4 (0/4)	1/4 (2/4)	0/2 (0/2)	0/4 (0/4)	0/4 (0/4)

*The data in parentheses show the results of the second survey.

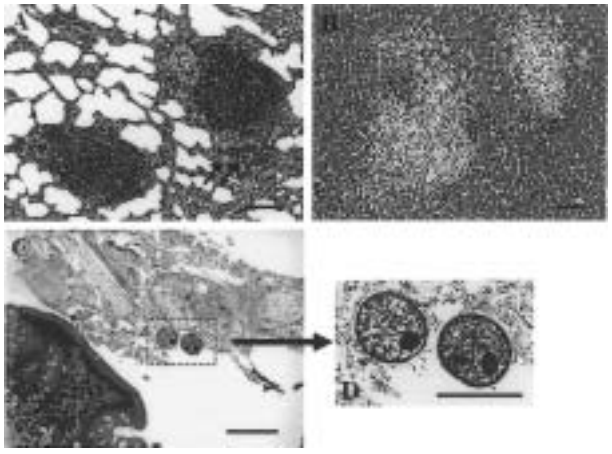

Fig. 1. Interstitial pneumonia with lymphocytic infiltration adjacent to the bronchi and alveolar wall thickness is shown in a 10-week-old animal from facility A (A). Arrows show the thickness of the alveolar wall due to infiltration of inflammatory cells (A). Hepatic necrosis with lymphocytic infiltration is shown in a 4-week-old animal from facility A (B). Protozoa-like organisms were present in the lumen of the cecum of a 24-week-old animal from facility A (C and D). H&E stain, Bar=100 μm.

animals from facility B. Focal necrosis with lymphocytic infiltration was observed in the liver of some animals (Fig. 1B). In addition, *Balantidium coli* were observed in the cecum of some animals from facility A, but this was not detected by the fecal floatation method (Figs. 1C and D). Other less common findings included: extramedullary hematopoiesis, interstitial nephritis, pericarditis/myocarditis, and inflammation of the genital system (Table 2). No conjunctivitis was observed in any animal, although papillary hyperplasia was seen in the eyelid of one animal (Table 2).

Discussion

The present study was performed to determine the microbiological status of guinea pigs bred under conventional conditions in two domestic breeding facilities in Korea. Infections of *E. cuniculi*, Sendai virus, PVM, Reo-3, SV-5 were serologically assayed. Only sera collected from the 10- and 24-week-old animals underwent serological tests because sera from 4-week-old animals may show false positive results due to the transfer of maternal immunoglobulin. The results showed evidence of severe microbiological contamination, especially at facility A, the guinea pigs of which were positive for *E. cuniculi*, Sendai virus, PVM, and Reo-3 infections. Although most of these pathogens cause subclinical infections in guinea pigs [8], some were responsible for aggravating lesions by coinfection with other microorganisms [6, 14]. Also, all of these pathogens have the ability to infect and cause various lesions in other laboratory animals including mice, rats, and rabbits. At facility B, animals were only seropositive for *E. cuniculi*. While several serological surveys have reported that *E. cuniculi* infection in guinea pigs, the clinical signs and necropsy findings have been not been well-reported [8]. *E. cuniculi* is primarily known for

its role in the etiology of encephalitis and nephritis in rabbits suffering from paralytic disease [13], although infection can occur in various mammals, including humans. This suggests that eradication of the microorganisms is necessary, regardless of whether they cause lesions in guinea pigs, since they can infect other laboratory animals bred simultaneously in the same breeding facility.

In bacteriological examinations, *B. bronchiseptica* was isolated from the lung tissue of two 10-week-old animals from facility A in the second survey. This microorganism has a broad host spectrum including mice, rats, rabbits, pigs, and primates. Although most infections are subclinical, in an immunosuppressive condition, they can adhere firmly to pulmonary epithelia with cilia and cause cilia paralysis, inflammation, and the inhibition of clearance activity, which can lead to fibrous and suppurative bronchopneumonia [8].

Recently, the family Chlamydiaceae was reclassified into two genera, *Chlamydia* and *Chlamydophila* [2]. *Chlamydophila caviae*, formerly known as the guinea pig inclusion conjunctivitis strain of *C. psittaci*, is highly specific to guinea pigs [2, 11]. This microorganism causes ocular disease in young guinea pigs, especially those 4 to 8 weeks of age. Clinical cases of infection showed mild inflammatory conjunctivitis with a slight, yellowish-white discharge, conjunctival hyperemia, and even severe conjunctivitis with profuse purulent ocular exudates [10, 12]. Diagnosis can be accomplished by observation of intracytoplasmic inclusion bodies in conjunctival epithelia with Giemsa or Macchiavello stain. In the present study, using the Macchiavello method, no animals showed chlamydial infection. Chlamydia diagnosis by staining a conjunctival swab is needed to differentiate between *Streptococci* spp., *Staphylococcus aureus*, and *Pasteurella multocida*, which can cause bacterial conjunctivitis in guinea pigs. The Macchiavello stain is helpful for viewing the organism, but definite classification is not possible with this stain. Therefore, it is better to perform a PCR assay along with staining of the conjunctival swab for definitive diagnosis [3, 4, 9]. PCR was not performed in this study.

In the histopathological examinations, the most remarkable finding was interstitial pneumonia, which was more severe in the animals from facility A than those from facility B. The lesions were thought to be due to the Sendai virus or PVM infections. However, the role of *B. bronchiseptica* in the appearance of these lesions was unclear because similar lesions were also observed in many animals from facility B, in which the bacteria was not cultured. Other findings, such as extramedullary hematopoiesis in the spleen, interstitial nephritis, pericarditis/myocarditis, papillary hyperplasia in the eyelid, and inflammation in the genital system, were observed randomly in some animals. However, the present study did not clarify the definitive cause of the lesions. In addition, protozoa-like organisms were observed in the lumen of the cecum of some animals from facility A. These organisms were morphologically considered to be *Balantidium coli*, which is a ciliated protozoan indigenous to the cecum of guinea pigs [8]. This organism was not detected by a floatation assay with saturated sucrose. Although the fecal floatation assay is well-known to be an effective diagnostic tool for the detection of intestinal parasites, it also has some disadvantages. For example, fatty stool samples are unsuitable for this procedure. Therefore, histopathological observation would be better for a more accurate diagnosis.

In conclusion, the present study determined the status of microbiological contamination in conventionally-reared guinea pigs. The environmental conditions can be improved by removing the contaminated animals and replacing them with new animals. In addition, Hansen *et al.* [7] suggested that new infections should be considered in health monitoring of laboratory rodents, especially cardiovirus, adenovirus, and parainfluenza virus type 3 in guinea pigs. We recommend that these new infectious agents be included in further microbiological monitoring.

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