United Journal of Veterinary Science and Technology

Surveillance of Pig Infectious Diseases and Increase of Animal Welfare in the Specific Pathogen-Free Pig Farms in Taiwan

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1. Abstract

Volume 1 Issue 1 - 2020 Received Date: 05 Apr 2020 Accepted Date: 24 Apr 2020 Published Date: 02 May 2020

2. Keywords

Animal Welfare; Pig infectiouds diseases; Specific pathogen-free pigs; Surveillance; Taiwan In the recent years, the demand is increasing for the medium and large size specific pathogen-free (SPF) animals in biotechnology and pharmaceutical industries. SPF animals means a group of animals that are clean and free of specific pathogens and have not been vaccinated. The Agricultural Technology Research Institute (ATRI), Taiwan, has accumulated more than 20 years of experience in cultivating SPF pigs. The objective of ATRI SPF high quality management program was to improve the environment for the pig health and pig disease surveil-lance to wish that are declared free of 10 kinds of pig diseases as foot-and-mouth disease, classical swine fever, pseudo rabies, porcine circovirus type 2 (PCV2) disease, swine enzootic pneumonia, Actinobacillus pleuro-pneumonia, atrophic rhinitis, swine dysentery, mange infestation, and toxoplasmosis. Except of PCV2 disease, 9 kinds of pig diseases has been eliminated between 2013-2019 in the SPF pig farms, ATRI, Taiwan according to the ATRI SPF high quality management program. In the future, ATRI will continuously cultivate SPF pigs to fit the demand of medium and large size laboratory animals in biotechnology and pharmaceutical industries.

3. Importance of the Experimental Pigs in the Biomedical Application

Pigs are one of the important experimental animals. In order to obtain the accuracy of the experimental data and meet the basic quality requirements of biological experiment materials, the quality of pigs for the biological experiments should reach the level of specific pathogen-free (SPF). Since SPF pigs cannot be imported from abroad in large quantities, they can only be produced on local [1-3].

SPF pigs are high quality experimental animals for the need of biomedical research. It is not feasible to import large numbers of SPF pigs from abroad, instead, supply of SPF pigs through domestic production is necessary. SPF production platform in ATRI is a crucial platform technology to produce the high qualified pigs. These SPF pigs are applied only to meet the demand for the biomedical research but also can be used to provide for research and development of swine vaccines. In order to maintain the supply chain of SPF pigs, the surveillance of pig infectious diseases is very important. Furthermore, as animal welfare has gradually attracted attention in the recent years, the reduced animal pain and quantity, and increase of the experimental refinement are the important issues in the 3R (replacement, reduction, and refinement) of animal welfare. High level of animal welfare for these SPF pigs is also need [4-7].

Animal testing is an important verification stage before the listing of biomedical products. Because of the high similarity between the physiological and anatomical characteristics of pigs and humans, there are many advantages in the use of laboratory pigs. Base on the less disease in laboratory pigs, the less the microbial infection affects the biomedical test [8-10]. In Taiwan's ATRI, the high quality management program for SPF pigs was

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established in order to avoid harmful pathogens encountered in pig herds, and continuing disease surveillance for SPF pigs with declaring free of 9 kinds of pig diseases in herds. At present, these SPF pigs in ATRI, Taiwan are provided to the biomedical research in Taiwan. Therefore, the maintain of upstream SPF pig breeding productive farms for the production of SPF pigs to provide the high quality experimental pigs is very important.

4. Animal Care and Species

ATRI, Taiwan was founded in the establishment of a platform for the commercialization and industrialization of agricultural research results. In terms of animals, the ATRI's application platform covers veterinary and animal science, biotechnology and biomedical science, and interdisciplinary studies, including a minimum disease laboratory animal production system, animal welfare promotion, the establishment of safety meat supply system, and the development of biomedical and biotechnology product testing technology. Projects that involve animal care and use, such as the development of functional feed and feed additives, animal vaccine development and promotion, animal vaccine and biotech product efficacy assessments, epidemiological surveillance and inspection, bio safety assessment and verification, assay reagent and medical material development, and laboratory animal production are managed by ATRI Institutional Animal Care and Use Committee (IACUC) and Veterinarian Care Team (VCT). IACUC uses the Guide for the Care and Use of Laboratory Animals (NRC 2011) as the primary standard for laboratory animal study, and the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS 2010) for agricultural animals. The animal care and use program is also designed to be in compliance with the Taiwan Ani-

Author Contributions: Hung YC, Chen CC, These authors have contributed equally to this article.

Citation:Shao-Wen Hung. Surveillance of Pig Infectious Diseases and Increase of Animal Welfare in the Specific Pathogen-Free Pig Farms in Taiwan. United Journal of Veterinary Science and Technology, 2020; 1(1): 1-3.

mal Protection Act (2016/05/18 amended) and the Animal Industry Act (2010/11/24 amended).

SPF pigs involve two breeds as Lee-Sun miniature pigs and domestic pigs (Landrace \times Yorkshire; LY breed) they will be separated and raised in different pens of animal facilities. The clinical signs or abnormal behaviors are observed every day, the disease pigs will be moved to the isolated viewing area in the animal facilities.

5. SPF Pig Farms in ATRI

At present, there are three SPF pig facilities in ATRI, Taiwan. These pig facilities include the primary SPF Lab (PSL), the secondary SPF Herd-I (SSH-I), and the secondary SPF Herd-II (SSH-II). Certifications in the SPF swine farms include AAALAC International certification (Unit #1695) ISO 9001: 2015 (certificate TW14/10771) and ATRI SPF certification.

PSL belongs to an HVAC (heating, ventilation, and air condition) barrier system. The air condition is a systematic operation on 100% fresh air without return air with a standard ventilation rate of 100 times per hour. The environmental conditions of each animal room are controlled and monitored according to different growing stages by a central control system. SSH-I and SSH-II belong to the nature-ventilation systems. The daily temperature and humidity of each animal room is recorded by an electronic recorder.

SPF piglets are obtained by the pregnant sows via caesarean section (Csection) in PSL, which individually reared in breeding cages in order to ensure that every piglet gets enough artificial milk replacers and to avoid biting. Since SPF piglets are not received colostrum from the sow at postfarrowing to build up their disease resistance, they are easy to cause infection or death. Taken these reasons together, the individual rearing system for SPF piglets could reduce the mortality. Additionally, the surface area of each rearing cage is 0.6 square meter and the body weight of enclosed animal is not to be over 12 kg during the 6 weeks rearing period in PSL. The rearing cages are connected in line with a tempered glass front opening for piglets can see and interact with each other and also for the animal care personnel to observe their daily health condition. Some toys are provided for piglets to reduce stress.

SPF piglets are obtained by the pregnant sows via C-section in PSL, which the operating room is equipped with a pig's anesthesia machine and a waste anesthetic gas exhaust system. Surgical room staff have to inspect anesthetic delivery systems and connections for leak checks before each operation to ensure all equipment are working effectively. In SSH-I & II, SPF piglets are obtained by the pregnant sows via the natural production. All SPF animals in SSH-I & II are raised in the barrier which is free from major diseases. No animal experiment involved zoo noses is allowed in these facilities. Even though, personnel entering the animal barrier have to take shower and change into clean room to put on personal protective equipment (PPE), such as clothing, shoes, gloves, mask, and cap, or wear disposable suits, depending on the barrier level to ensure the health and well-being of animals. Personnel also have to pre-form health check and vaccination to minimize the risk of exposure to potential animal allergens and zoonotic diseases. In addition, the room temperature and humidity of 5 weeks old weaning piglets in the first week is set at 36°C and 70% in PSL, respectively. Afterwards, the temperature and humidity will be decreased in accordance to the adjusted weight and status of pigs every week. Moreover, the room temperature will be adjusted in accordance with the age and status of pigs. The relative humidity range of pigs is from 30-70% in SSH-II.

6. Surveillance of Pig Infectious Diseases

Serum and swab samples were collected (once per three months) from the SPF pig farms in ATRI and delivered into the ISO/IEC 17025-certificated laboratory (No. 1373). In the ISO/IEC 17025-certificated laboratory (No. 1373), detections of the antigens and antibodies of pig infectious pathogens were applied with PCR, IFA, latex agglutination, ELISA, culture, and microscopy (Table 1). Theses pig infectious diseases included as foot-and-mouth disease, classical swine fever, pseudo rabies, porcine circovirus type 2 (PCV2) disease, swine enzootic pneumonia, Actinobacillus pleuropneumonia, atrophic rhinitis, swine dysentery, mange infestation, and toxoplasmosis. Data were shown that except of PCV2, 9 kinds of pig diseases were eliminated between 2013-2019 in the SPF pig farms, ATRI, Taiwan (Table 1; Figure 1).

Table 1: Surveillance of ten kinds of pig infectious diseases in the specific pathogen-free pig farms in ATRI, Taiwan during 2013 to 2019

Disease name	Detect target	Test method	No. of examined	No. of positives	Percent of positive
Foot-and-mouth disease	Antibody	ELISA	390	0	0
Clinical and swine fever	Antibody	ELISA	390	0	0
Pseudorabies	gE Antibody	ELISA	390	0	0
Porcine circovirus type 2 (PCV2) disease	PCV2	PCR	390	35	9
	PCV2 Antibody	IFA/ELISA	390	235	60.3
Swine enzootic pneumonia	Mycoplasma hyopneumoniae	culture	390	0	0
Swine pleuropneumonia	Actinobacillus pleuropneumoniae	culture	390	0	0
Swine atrophic rhinitis	Bordetella bronchiseptica/ Pasteurella multocida	culture	390	0	0
Swine dysentery	Treponema hypodysenteriae	Microscopy	390	0	0
Manage infestation	Sarcoptes scabieivar, suis	Latex	390	0	0
Toxoplasmosis	Antibody	Aagglutination/ ELISA	315	0	0



Figure 1: Surveillance of antigen (Ag) and antibody (Ab) of porcine circovirus type 2 (PCV2) in the ATRI SPF pig farms during 2013 to 2019.

7. Conclusion

Except of PCV2 disease, 9 kinds of pig diseases were eliminated in the SPF pig farms, ATRI, Taiwan according to the ATRI SPF high quality management program. In the future, ATRI will continuously cultivate SPF pigs by improving the facilities and maintaining the inspection quality and energy to fit the demand of medium and large size laboratory animals in biotechnology and pharmaceutical industries.

8. Acknowledgements

All authors thank the support of Council of Agriculture in Taiwan (Executive Yuan) and also thank the ATRI co-operation teams (Dr. Zeng-Weng Chen, Dr. Chi-Yu Yang, and senior technician Wen-Der Fang) for fully supporting the surveillance of pig infectious diseases in SPF pigs and promotion of animal welfare.

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