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Correlation between goose circovirus and goose parvovirus with gosling feather loss disease and goose broke feather disease in southern Taiwan

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ABSTRACT

Background: Goslings in several Taiwanese farms experienced gosling feather loss disease (GFL) at 21–35 days and goose broke feather disease (GBF) at 42–60 days. The prevalence ranges from a few birds to 500 cases per field. It is estimated that about 12,000 geese have been infected, the morbidity is 70–80% and the mortality is 20–30%.
Objectives: This study aims to investigate the pathogens that cause GFL and GBF. Focus on the study of the correlation between goose circovirus (GoCV) and goose parvovirus (GPV) with the goose feather loss in southern Taiwan. Furthermore, a phylogenetic tree was established to align the differences between southern and northern Taiwan and compare with virus strains from China and Europe.
Methods: Samples were collected from animal hospitals. Molecular and microscopy diagnostics were used to examine 92 geese. Specific quantitative polymerase chain reaction (Q-PCR) assays are performed to evaluate GPV and GoCV viral loads and simultaneously evaluated the feather loss conditions in geese with the scoring method.
Results: High prevalence of GoCV and GPV infection in geese showing signs of GFL and GBF. Inclusion body was detected in the feather follicles and Lieberkühn crypt epithelial cells. The Q-PCR showed the high correlation between feather loss and viruses during 3rd–5th week. However, the infection was not detected using the same test in 60 healthy geese.
Conclusions: Thus, GFL and GBF appear to be significantly closely related to GoCV and GPV. The geese feathers showed increasing recovery after being quarantined and disinfected.

Keywords: Circovirus; goose disease; parvovirus; polymerase chain reaction; Taiwan

INTRODUCTION

Goslings in several Taiwanese farms experienced gosling feather loss disease (GFL) at 21–35 days old and goose broken feather disease (GBF) at 42–60 days. There are about 40 farms of infections. The incidence ranges from a few birds to 500 cases per field, which accounts for about 1%. It is estimated that about 12,000 geese have been infected, the morbidity is 70–80%, and the mortality is 20–30%. The economic loss may be as high as 10 million. This

Conflict of Interest
The authors declare no conflicts of interest.

Author Contributions
Conceptualization:Ting CH, Lin CY, Liu SS, Peng SY, Wu HY; Data curation: Ting CH, Peng SY, Wu HY; Formal analysis: Ting CH, Lin CY, Liu SS, Peng SY, Wang CW; Funding acquisition: Wu HY; Investigation: Ting CH, Huang YC, Wu HY; Methodology: Ting CH, Lin CY, Wu HY; Project administration: Ting CH, Lin CY, Liu SS, Peng SY, Wu HY; Resources: Wu HY; Software: Ting CH; Supervision: Ting CH, Liu SS, Peng SY, Wu HY; Validation: Ting CH, Lin CY, Liu SS, Peng SY, Wu HY; Visualization: Ting CH, Liu SS, Peng SY, Wu HY; Writing - original draft: Ting CH, Lin CY, Liu SS, Peng SY, Wu HY; Writing - review & editing: Ting CH, Wu HY.

study aims to investigate the pathogens causing GFL and GBF. A total of 92 geese with these feather diseases were sent to animal hospitals by goose farmers. They came from 40 different farms in 5 counties (**Supplementary Fig. 1A and B**).

There are many factors affecting feather follicle atrophy or underdevelopment, including viral infections, bacterial, parasites infections, and endocrine disorders. Common viral infections can cause GFL and GBF such as goose circovirus (GoCV), goose parvovirus (GPV). Malabsorption of nutrients after enteritis that cause feathers disease, such as new gosling viral enteritis (NGVE) and goose haemorrhagic polyomavirus (GHPV).

GoCV was first identified in a Germany's large commercial flock of geese with a history of increased mortality and runting by Soike [1,2]. This case occurred in a one-week-old goose. The clinical symptoms included growth retardation and feather shedding lesions. Histopathology showed varying degrees of lymphocyte loss and histiocytosis lesions in the Fahrenheit bursa, spleen, and thymus. The same geese were also found to have infectious serositis and aspergillosis infection. Soike et al. [1] believed that GoCV infection would inhibit the immune system of the geese, making the geese prone to secondary infections, causing the invasion of mold and bacteria that result geese growth retardation. Spherical or coarse-grained cytoplasmic inclusions were found in Fahrenheit bursa and Lieberkühn crypt epithelial cells. When the ultramicroscopic structure of virus inclusion bodies was examined, it was confirmed that the virus particles arranged in crystals were multilayered or irregular in size 14 nm. The author speculates that the circovirus can induce immunosuppression and make geese prone to secondary infection. In 2004, the survey results in Hungary's goose farm showed that the Fahrenheit bursa of 52 flocks of geese were examined by histology, and 75% of the GoCV-positive populations and 50% of the GoCV-negative populations were observed to have lost lymphocytes in the Fahrenheit bursa. Inclusion bodies were only found in three positive geese flocks, and the three geese flocks were positive for GoCV by dot blot hybridization test (DBH) and polymerase chain reaction (PCR) [3].

GPV infection was first reported in Hungary in 1967 as a goose farm often called gosling plague, goose viral hepatitis, goose influenza, and ascites hepatitis, with high mortality, mainly caused by violations of goose fiber necrotizing enterocolitis [4]. This is an acute, highly contagious and fatal disease, mainly caused in goslings, fibrous necrotizing enteritis, also known as Derzsy's disease [5]. However, it first invaded Taiwan goose farms in Taiwan in 1982. Experts and scholars unified the disease as goose viral enteritis. This disease is currently infected in Europe, United States, Canada, Russia, Israel, China, Japan and Vietnam. The virus is different from the small virus that infects humans and other poultry [6]. The severity of the disease is greatly related to age. If birds are infected with this virus before one week of age, the mortality is as high as 100%. Infections over four weeks of age rarely have clinical features. If the yolk antibody is administered before three weeks of age, it will effectively combat the occurrence of GPV [7].

A new epidemic occurred in western France in 1989, causing 40–50% mortality of 2–4 weeks old Muscovy ducks. Although the clinical symptoms are similar to GPV infection, it is not pathogenic to geese and other strains of ducks. After virus isolation and resistance analysis, it was found that this virus and goose-origin waterfowl virus (GPV) are parvoviruses, but there are significant differences in viral nucleic acid and amino acid sequences, so this virus was named Muscovy duck parvovirus (MDPV). Woolcock et al. [8] pointed out that MDPV has been isolated in many countries such as the United States, Germany, Malaysia, Japan, Taiwan,

and Thailand. The disease causes the most serious death at the age of about five weeks ago, there will be 30–80% morbidity and 10–50% mortality, and older birds are also susceptible to infection when their immunity is weak [8].

NGVE is an infectious disease that affects goslings < 30 days old with haemorrhagic, fibrinonecrotic, hyperaemic, necrotic and exudative enteritis in the small intestine [9]. It is caused by an adenovirus called the NGVE virus (NGVEV), which was first reported by Cheng [10]. Haemorrhagic nephritis enteritis of geese is an epizootic viral disease caused by infection with GHPV [11]. Since 1969, GHPV has caused serious economic losses in European countries, including Hungary [12], Germany [13] and southern France [14,15]. Geese may also be infected with lice and red mites, which can induce feather loss and occasionally cause naked feathers known colloquially as 'ghost pull hairs.' Insufficient essential amino acids in the feed and malnutrition could become an important secondary factor after the outbreak of GFL and GBF.

MATERIALS AND METHODS

Experimental design

This study is divided into 2 parts. Part one explores the pathogens of GFL and GBF using microbiology, histopathology and molecular biology. In the second part, the infected geese are separated, treated and observed for feather recovery. Real-time quantitative polymerase chain reaction (Q-PCR) was used to detect the presence of GPV and GoCV viruses in the blood (**Supplementary Fig. 2**). All experiments within this study conform to the ethical standards issued by the National Research Council. All animal experiments have been approved by the ethics committee of the National Pingtung University of Science and Technology, and care was taken to comply with the 3R concept. In this study, the laboratory animal application form protocol number was NPUST-99-065 and approved number was NPUST-IACUC-99-065.

Birds

A total of 92 geese from 40 farms, comprising 56 showing signs of GFL and 36 with signs of GBF, were examined. Sixty healthy geese (30 goslings and 30 geese) were procured from the same farms with rigorous disinfection and management protocols; they were not affected by goose feather loss or broken feather diseases. These healthy geese were used as negative controls. The grading evaluation of feather shedding uses the feather shedding evaluation proposed in the past literature. According to the feather loss evaluation table, it is divided into back and wings (**Supplementary Figs. 3** and **4**), there are 5 levels, the lower the score the more complete the feather and the higher the score, obvious severity [16].

Histopathology

The tissues were embedded in paraffin and processed into 2- to 3-μm-thick sections that were stained with haematoxylin and eosin for histopathological examination. Tissue samples from other gross lesions observed were similarly collected and processed for histopathological assessment.

Molecular biological detection

PCR protocols and the specificity of primer pairs used are summarised (**Supplementary Table 1**). RNA and DNA were extracted from tissue using Corning Axygen AP-MN-BF-VNA-250 AxyPrep™

Body Fluid Viral DNA & RNA Purification Miniprep Kits (BIOKIT, Taiwan). Each PCR was performed in a total volume of 20 μL containing 9 μL template DNA, 10 μL Taq DNA, polymerase 2× Master Mix red (1.5 mM $MgCl_2$) and 1 μL primer.

GoCV PCR protocol was as follows: pre-denaturation 94°C for 5 min; denaturation 94°C for 45 sec; annealing 55°C for 45 sec; extension 72°C for 45 sec, performed for 35 cycles; final extension 72°C for 5 min. GPV PCR protocol was as follows [17,18]: pre-denaturation 94°C for 3 min; denaturation 94°C for 30 sec; annealing 60°C for 30 sec; extension 72°C for 30 sec, performed for 35 cycles; and final extension 72°C for 5 min. GHPV PCR protocol was as follows [19]: pre-denaturation 94°C for 3 min; denaturation 94°C for 30 sec; annealing 55°C for 30 sec; extension 72°C for 60 sec; performed for 30 cycles; and final extension 72°C for 5 min. Goose adenovirus PCR protocol was as follows: pre-denaturation 94°C for 5 min; denaturation 94°C for 40 sec; annealing 54°C for 45 sec; extension 72°C for 40 sec, performed for 30 cycles; and final extension 72°C for 10 min. PCR products were analysed by electrophoresis in a 1.5% (w/v) agarose gel.

Real-time Q-PCR in quarantined and disinfected birds

Twenty geese from a farm in Wandan Township of Pingtung were separated into 4 groups, 2 GFL and 2 GBF. The geese were treated daily with a spray of 400-times-diluted iodine solution 50–60 mL. The solution was sprayed onto the head, back, wings and abdomen of each goose, and blood samples were collected every 3 days. Q-PCR Bio-Rad CFX Manager software was used to analyse the data of real-time Q-PCR products to continuously observe the quantification of GPV and GoCV in infected geese. Viral variables in geese from 39 to 66 days of age and from 49 to 76 days of age after GPV and GoCV infection were observed. DNA extractions were performed using whole blood. Real-time PCR detection of GPV and GoCV viruses, using the National Center for Biotechnology Information (NCBI) website design provided by Primer, was as follows: GoCV F, 5′-CCAGTCCATTGTCCGAA-3′; GoCV R, 5′-GGAGGAAGACAACTATGGC-3′; GPV 3F, 5′-ACAACTTTGAGTTTACGTTTGAC-3′; and GPV 3R, 5′-ATTCCAGAGGTATTGATCC ACTA-3′. Bio-Rad Manager 3.1 Software was used to interpret PCR results. The measured OD values were entered into Excel Software for conversion into a standard curve. After that, the plastid concentration was used to convert the virus amount (plasmid copy number). When Q-PCR was completed, the Cq value was obtained.

Sequence sequencing and comparison

This experiment refers to the GPV-specific primer pair GPV (VP2)-F: CCGGGTTGCAGGAGGTAC, R: AGCTACAACAACCACATC that was used by Limn et al. in 1996. It was amplified fragment 800 bp, and the GoCV-specific primer pair GoCV (466-1014)-F: CGGAAGTACCCGACGACTTA, R: ACAATGGACTGGGCTTT CAC that was used by Lin in 2005. It was amplified fragments of 568 bp. The virus-specific fragments were sequenced and analyzed for virus sequence. The sequence analysis used DNASTAR software MegAlign Version 7.1 (DNASTAR, Inc., USA), and provided it in NCBI database. Gene sequence comparison, GoCV part is based on TW11/2001 (AF536941.1), TW10/2001 (AF536940.1), TW9/2001 (AF536939.1), TW8/2001 (AF536938.1), TW7/2001 (AF536937.1), TW6/2001 (AF536936.1), TW5/2001 (AF536935.1), TW4/2001 (AF536934.1), TW3/2001 (AF536933.1), TW2/2001 (AF536932.1), TW1 /2001 (AF536931.1) as the reference sequence; the GPV part uses GPV strain Y (China) (KC178571.1), GPV strain E (China) (KC184133.1), GPV strain GDa (China) (HQ891825.1), GPV strain SH (China) (JF333590.1), GPV strain SHFX1201 (China) (KC478066.1), GPV strain VG32-1 (Europe) (EU583392.1), GPV strain Virulent B (Hungary) (U25749.1), GPV strain 06-0329 (Taiwan) (EU583391.1), GPV strain 82-0321 (Taiwan)

(AY382884.1), Muscovy duck parvovirus (AY510603.1) as reference sequence, and use ClustalW in the system to compare and draw a phylogenetic tree. The phylogenetic tree was obtained using 1000 bootstrap replications to evaluate the supporting values for lineage grouping.

RESULTS

PCR diagnosis

PCR results are shown negative results for GHPV and NGVEV. The incidence of various pathogens in geese with GFL were found to be GoCV 94.6% (53/56), CPV 60.7% (34/56) (**Supplementary Fig. 5**), whereas geese with GBF showed the following incidence of pathogens: GoCV 83.3% (30/36), GPV 72% (26/36) (**Table 1**). The result shows that GoCV and GPV are significantly correlated with GFL and GBF ($p < 0.05$).

Gross lesions and histopathological examination

Figures showing pathological sections (**Fig. 1**) reveal the gross and histopathologic findings in cases of goose infectious disease with GFL and GBF: **Fig. 1A** shows the clinical findings for feather loss (GFL), and **Fig. 1B** shows the clinical findings for broken feather (GBF). **Fig. 1C-F** shows histopathologic findings in cases of GoCV infection by PCR assay: feather follicle necrosis with inclusion body (**Fig. 1C**); folliculitis with necropsy follicular inflammatory cell infiltration (**Fig. 1D**); degeneration and necrosis of epithelial cells on the mucous membrane and the crypts of Lieberkühn (**Fig. 1E**) and intranuclear inclusion bodies in the degenerated epithelial cells of the crypts of Lieberkühn (**Fig. 1F**).

Table 1. Prevalences of GFL and GBF by PCR diagnostic assay

Pathogen	Positive		Control (healthy geese)	
	Gosling	Goose	Gosling	Goose
GPV	60.7% (34/56)	72% (26/36)	0 (0/30)	0 (0/30)
GoCV	94.6% (53/56)	83.3% (30/36)	0 (0/30)	0 (0/30)
GPV with GoCV	44.6% (25/56)	66.7% (24/36)	0 (0/30)	0 (0/30)

GFL, gosling feather loss disease; GBF, goose broken feather disease; PCR, polymerase chain reaction; GPV, goose parvovirus; GoCV, goose circovirus.

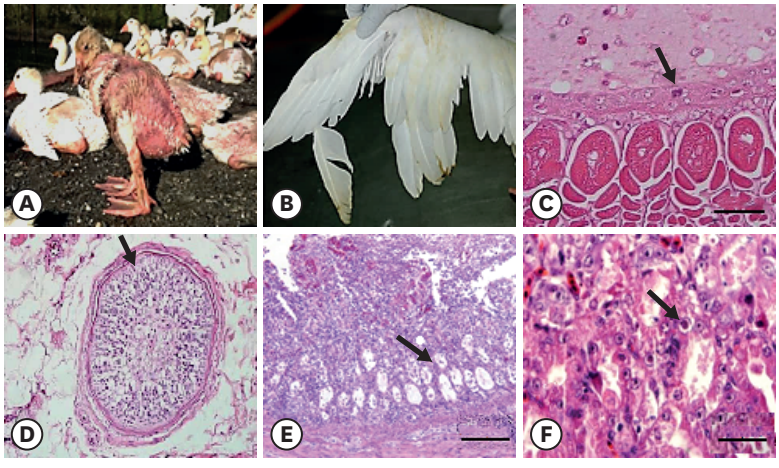

Fig. 1. Gross and histopathological lesions were observed in tissues of geese infected with various pathogens. Stained haematoxylin and eosin. (A) Feather loss. (B) Broken feather. (C) Inclusion body (arrow), feather follicle, 100× (bar = 20 μm). (D) Folliculitis (arrow), feather follicle, 400×. (E) Degeneration and necrosis of epithelial cells (arrow) on the mucous membrane and the crypts of Lieberkühn (bar = 100 μm). (F) Intranuclear inclusion bodies (arrow) in the degenerated epithelial cells of the crypts of Lieberkühn (bar = 20 μm).

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Building a phylogenetic tree from the alignment

New sequences of GoCV and GPV (569 bp and 806 bp, respectively) were generated and submitted to a public repository GenBank. The GoCV amplified sequence (VP1) was examined by PCR genetic sequence analysis software. A phylogenetic tree was built on the basis of GoCV alignment (VP1) in samples of Pingtung, Kaohsiung, Yunlin and Taiwan. This was compared with 11 GoCV sequences isolated in Taiwan. They were generally divided into two groups. For the first group, both Kaohsiung (W103-1337) and Pingtung areas (W103-1342), the GoCV similarity rate was 100%, whereas in the Taiwanese strains (TW1), (TW10) and (TW11), the similarity was 98%–99%. The other groups were amplified sequences from the Yunlin samples (W103-1368) and (W103-1346). The similarity of (W103-1368) compared with (TW9) and (TW8) was 100%. The similarity of (W103-1346) compared with (TW8) and (TW6) was 98%. The South Taiwan samples were similar to the Yunlin area samples (95%). This implies that GoCV strains differ between the south and north of Taiwan (**Fig. 2**).

In the GPV (VP2) group of amplified sequence fragments found by PCR in this study, the Taiwanese strains, 06-0329 and 82-0321, were compared with strains from China and Europe, and found to strongly resemble them with a similarity of over 99.6%, compared with Muscovy duck parvovirus in which the similarity is only 80.9% (**Fig. 3**).

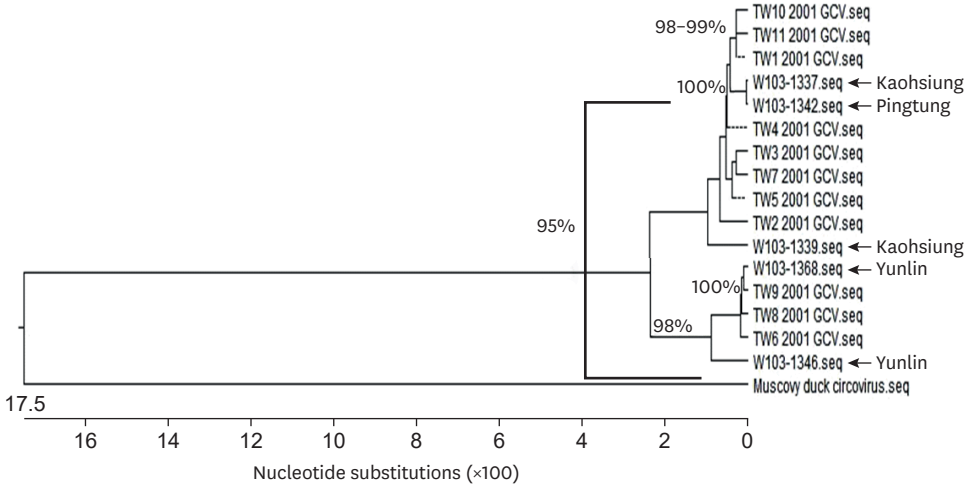

Fig. 2. The phylogenetic tree of goose circovirus.

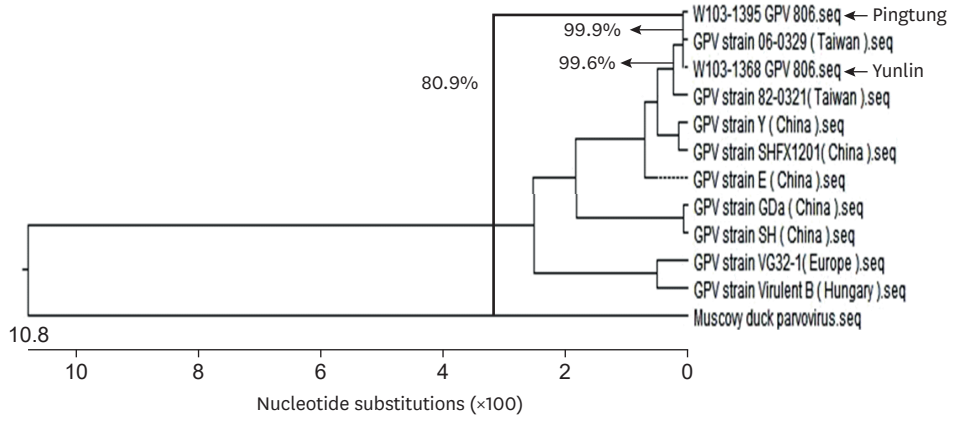

Fig. 3. The phylogenetic tree of goose parvovirus.

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Real-time Q-PCR in quarantined and disinfected birds

The viral variable was observed in the GLF birds from 39 to 66 days old. By the age of 66 days, although the feathers had all returned to normal growth, the virus had not been eliminated from the system. To reduce the effects of environmental pathogens and secondary infection by bacteria, geese were separated into the treatment group (T) and control group (C). Virus levels were compared between the 2 groups using a t-test. No significant difference in GoCV levels was found for T and C ($p = 0.082$; $p > 0.05$). GPV levels in C and T did not differ significantly ($p = 0.3$; $p > 0.05$).

Virus levels were monitored in birds from the age of 39 to 66 days and the decrease in GoCV and GPV levels over time noted. Using the self-designed primer to quantify the change curve of virus quantity (**Fig. 4A**) and the change of the feathers of the goose (**Fig. 4B**) when we conduct field monitoring two viral loads. Compared with the change trend of feather score, it can be seen that there seems to be a similar trend, so we divide the data into two sections, and we will perform the Pearson's correlation coefficient test after 35 weeks and 6 weeks of age mentioned earlier. The results of GoCV and GPV of GLB by t-test calculation indicated no significant difference in the GoCV levels in C and T ($p = 0.43$; $p > 0.05$) and no significant difference in the GPV levels in C and T ($p = 0.348$; $p > 0.05$).

However, during the treatment period, the birds between 42 and 63 days old with GFL did not have folliculitis, mobility also gradually improved, the grooming behaviour of the geese gradually became normal and the feather colour turned from yellow to white (**Fig. 5A**). Similarly, birds 49–75 days old with GBF gradually recovered. The recovery of broken feathers was maintained for about 3–4 weeks. Results indicated that if the breeding environment is properly controlled, there might be cases of early recovery (**Fig. 5B**).

Negative control

The 60 healthy geese did not show the presence of GoCV infection, GPV infection or inclusion bodies (**Table 1**).

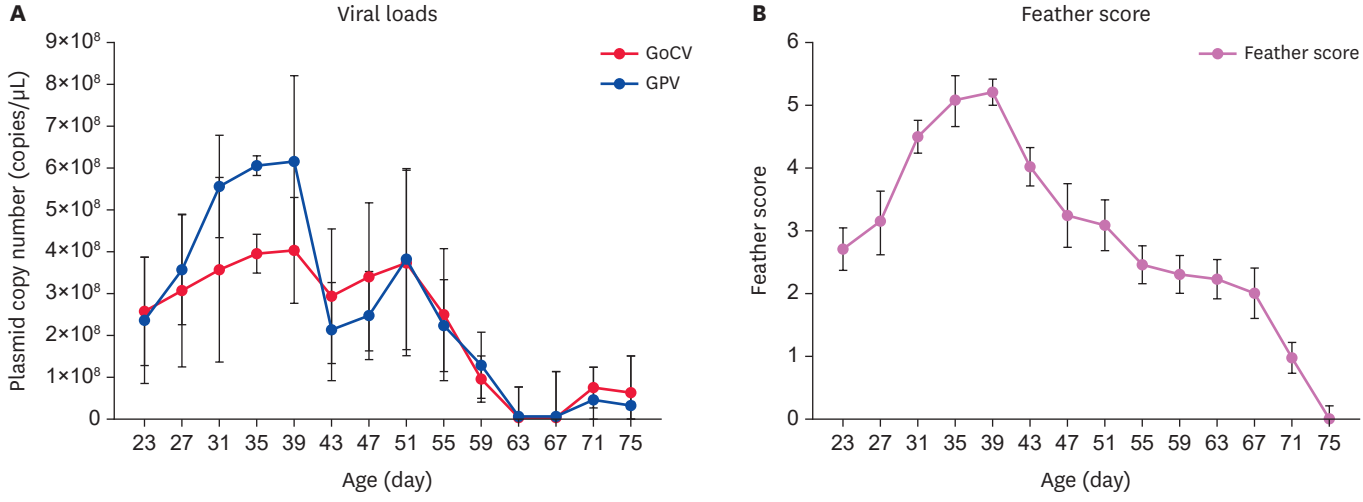

Fig. 4. The results of blood GPV and GoCV quantification and feather loss evaluation of sick goose. (A) The change curve of virus quantity. (B) The change of the feathers of the goose.
GPV, goose parvovirus; GoCV, goose circovirus.

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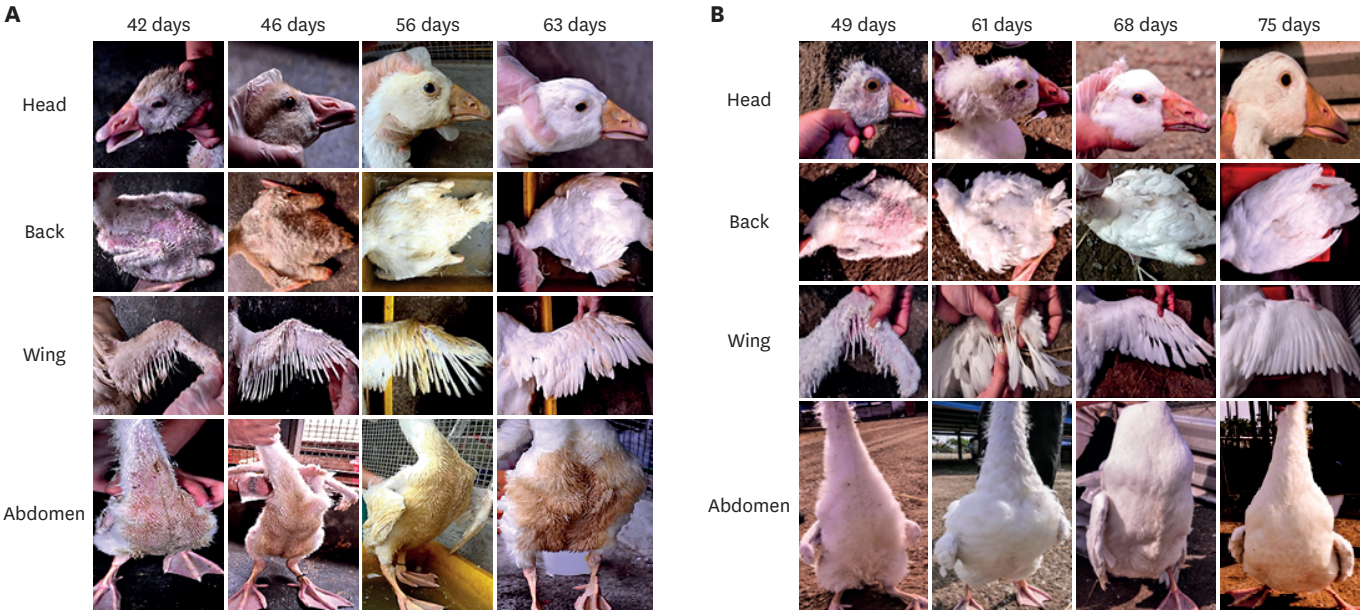

Fig. 5. Feather recovery. (A) Feather recovery from gradual decrease in the number of GPV and GoCV of birds with GFL. (B). Feather recovery from GoCV and GPV of birds with GBF.
GPV, goose parvovirus; GoCV, goose circovirus; GFL, gosling feather loss disease; GBF, goose broken feather disease.

DISCUSSION

GFL and GBF appear to be a common phenomenon in Taiwan. In this study, 2 cases of inclusion bodies were observed in the feather follicles, and viral nucleic acids were detected in cases of GoCV infection. Neither GHPV nor NGVEV was detected.

This study was similar to previous outbreak investigations in terms of the challenge and disease investigation. No inclusion bodies were observed, but the nucleic acids detected were universal [20-23]. Chen conducted an epidemiological survey of GoCV and found that the positive rate of GoCV in Taiwan is as high as 94.7% (197/208). GoCV in Taiwan has become a common virus. It is inferred that although circovirus can cause lymphocyte loss and lead to immunosuppression that could be induced many disease outbreaks [24]. Ball focused on the epidemiological investigation of GoCV, simultaneously, the parvovirus, polyomavirus, reticuloendotheliosis virus, salmonella, streptococcus and mycoplasma were detected [3]. Jing et al. [25] purified the circovirus and inoculated with 21-day-old goslings, and observed pathological changes that caused goose diarrhea, growth retardation and feather growth disorder. It can be found circovirus positive in the Fahrenheit bursa, thymus, spleen, duodenum, and liver when PCR test. The virus can be detected in the blood 14 days after vaccination. Pathological examinations have lymphopenia in the Fahrenheit bursa, spleen and liver. Inflammatory cells proliferate in the lungs and kidneys [25]. Australia has also found fractured feathers in crow with circovirus infection, which is quite similar to the situation of the goose found in this study. In the case, inclusion bodies were found in the basal layer of the feather follicle under the section, and a large number of inflammatory cells infiltrated in the feather follicle. It is the same as the nuclear inclusion bodies in the feather follicle seen in this study. Although some reports have pointed out that no inclusion bodies were seen under the slices in the investigation of the challenge and disease, but the nucleic acids detected were universal [20,21].

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Research on the etiology of GFL and GBF is becoming more and more urgent. At present, many scholars have carried out quantitative detection of viruses for GPV and GoCV, and their sensitivity can be detected up to 28 copy number variants [26,27]. Woźniakowski et al. [28] also explored the relationship between the clinical investigation of the aquatic parvovirus and the amount of virus found in the system, using quantitative GPV, combined with clinical pathology, which can be used as the main method of elucidation of epidemiology. The previous study showed that there was no comparison of virus levels between GPV and GoCV and broken feathers disease. Therefore, this study designed primers for Q-PCR testing and found that the virus levels of goose infected in the wild are usually quite unstable. The virus levels ranges from 10^5 to 10^8, and it is impossible to determine the amount of virus from the appearance. Therefore, when statistics are made, there will be thought the difference in the amount of each virus exceeds 100 or more, but the data trends shown are almost the same.

After the full-length sequencing and comparison of the GoCV strains, isolated from different regions in 2002–2003, the sequence similarity is above 97%, showing the genome sequence of the GoCV strain is very stable in Taiwan [24]. The sequence analysed in this study is VP1 (rep) nucleic acid between nt466 and nt1014. Although it is not a full-length sequence, there are still differences when compared with W103-1368 and W103-1346 from Yunlin County and W103-1339, W103-1337 and 1342 from the south of Kaohsiung. We found that the sequence from the south of Kaohsiung is significantly different from that of Yunlin. It is speculated that there may be differences in different types of viruses due to regional relationships; further research into this topic is required.

GPV infection has so far produced commercially available vaccines, and farmers usually vaccinate within 1 week of age. If the maternal antibody is administered at this time, it will last for 35 days. Immunity value continues to 20 weeks of age. Most of the infected birds will have the results in reduced growth and feather development. If the gosling is older than one month, the survivor will become the carrier for life. When the goose recovers from the parvovirus infection, a large number of antibodies will continue to remain in the body for 80 months. At this time, the maternal antibody of breeding geese will be able to transfer to the hatched goslings and will achieve a good immune effect [29]. Goose infection with parvovirus often causes hemorrhagic enteritis, which results in poor absorption efficiency, relatively slow growth and uneven feathering. The feather loss is mainly related to GPV. Although it can be seen a large number of inflammatory cell infiltration in the feather follicle, PCR diagnosis of parvovirus is positive. After that, *in situ* hybridization (ISH) or immunohistochemical staining (immunohistochemistry, IHC) is still needed to observe whether GPV causes the feathers disease.

The bacterial infection is usually a secondary infection in feather diseases. However, the prevalence of bacteria was found in this study: *Riemerella anatipestifer* and *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus* and *Pasteurella multocida*, however, no ectoparasitic infestation was found. Sporadic cases also have amyloidosis, renal tubular degeneration and deposition of calcium salt. The feeds have been found that all essential amino acids are insufficient compared with the standard value, so it will be malnutrition after the outbreak of the disease, which will lead to more serious GFL and GBF.

The GoCV and GPV in Taiwan are still quite prevalent in most goose farms. Because the circovirus is an unenveloped virus, according to a previous study, it was quite difficult to eliminate GoCV and GPV. The pathogenic microorganism was still present in the system after

being treated with common surfactants and halogen disinfectants [30,31]. It needs to be cleaned for a long time when it is empty, and it must be disinfected with formalin fumigation and phenol, which can at least remove the pathogen to reduce and improve the biological safety control of geese. The disinfectant used in this study is halogen-based iodine, and the disinfectant used in cleaning the environment is Anteweak. Although the disinfection effect tends to reduce the virus, the virus cannot be completely eradicated.

SUPPLEMENTARY MATERIALS

Supplementary Table 1
PCR protocol and primer

Click here to view

Supplementary Fig. 1
Sampling regions.

Click here to view

Supplementary Fig. 2
Q-PCR experiment flow chart.

Click here to view

Supplementary Fig. 3
Feather scoring for the back area.

Click here to view

Supplementary Fig. 4
Feather scoring for the wings.

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Supplementary Fig. 5
PCR products of feather abnormal geese.

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