Production and characterization of polyclonal antibody against Bengal tiger (Panthera tigris tigris) immunoglobulin G

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ABSTRACT
The immunoglobulin G (IgG) antibody is important for detecting and evaluating immune responses. Despite increasing opportunities for tigers to be exposed to emerging diseases due to global changes coupled with increasing interaction with humans, there is no available antibody specific to tigers. Aims of this study were to produce a rabbit anti-Bengal tiger (Panthera tigris tigris) IgG polyclonal antibody and to determine its specificity. The molecular weight of the purified Bengal tiger IgG was approximately 170 kDa. The cross-reactivity of rabbit anti-Bengal tiger IgG to IgG of other wild felid species and other domestic animals was analyzed by indirect enzyme-linked immunosorbent assay. The cross reactivity to other related wild animals ranged from 53.69% to 75.63%. There was high cross-reactivity with domestic cats (63.58%) and pigs (38.45%) and low cross-reactivity with domestic cows (10.46%), goats (8.60%), sheep (7.25%), and chickens (6.91%). To confirm cross-reactivity with domestic animals’ IgG, western blotting was performed. These results indicated that the purified rabbit anti-Bengal tiger IgG polyclonal antibody strongly reacted with species in the family Felidae. The polyclonal antibody generated in this study has the potential to aid in the development of useful tools for further novel immunological investigations on tigers and related felid species.

Introduction
Tigers (Panthera tigris) are important members of their ecosystem, and their state indicates the health of the ecosystem (Sanderson et al. 2010). The International Union for Conservation of Nature (IUCN) Red List of Threatened Species divides the tiger into six extant subspecies based on the distinctive molecular genetic markers identified by Luo et al. (2004). It has been estimated that there are only approximately 3200 tigers remaining in the wild globally (Goodrich et al. 2015). Despite significant regional and international support for conservation of these magnificent beasts, the tiger population in the wild has been decreasing due to habitat loss and prey depletion caused by intense poaching (Dinerstein et al. 2007). Consequently, the tiger has been listed not only as an endangered species by the IUCN but also as being at a crisis point (Sanderson et al. 2010; Goodrich et al. 2015).

Presently, tigers in zoological parks are susceptible to several infectious diseases, including canine distemper virus (CDV) (Appel et al. 1994; Nagao et al. 2012), feline panleukopenia virus (FPV) (Duarte et al. 2009; Dissanayake et al. 2017), feline calicivirus (FCV) (Tian et al. 2016), Cytauxzoon felis (Jakob and Wese-meier 1996; Lewis et al. 2012), Toxoplasma gondii (Yang et al. 2017) and even avian influenza (Keawcharoen et al. 2004; Hu et al. 2016). One explanation for the recent emergence of infectious diseases is that global changes coupled with human activities have potentially altered the interactions between the disease components (Daszak et al. 2000; de La Rocque et al. 2008; Harvell et al. 2009; Gallana et al. 2013; Lindahl and Grace 2015). Hence, endangered species will have a tendency to acquire virulent pathogens, which would cause a decline in their number or an increase in the number of species becoming extinct (McCallum and Dobson 1995). Vaccination could protect such animals from infectious diseases. Moreover, it could prevent disease transmission to humans or animals, protect animal health, and prevent economic losses (Cross et al. 2007; Plumb et al. 2007).

In the past several decades, vaccination of tigers has been practiced in zoological parks in accordance with the vaccinations recommended for exotic cats by Bittle (1993). A previous study, however, reported the deaths of captive tigers despite vaccination (Wang et al. 2017). However, evidence with regard to the efficacy of the vaccine was not demonstrated in that case. Antibody titres of the vaccine might help to resolve the issue. Likewise, antibody titres of pathogens might also help in the detection of disease in future surveillance and epidemiological studies. Unfortunately, current serological assays to

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evaluate immune responses in tigers are restricted to seroneutralization (Nagao et al. 2012; Risi et al. 2012; Sadler et al. 2016) and hemagglutination assays (Hu et al. 2016; Yang et al. 2017) due to a lack of suitable detecting antisera (Anti-tiger immunoglobulin: Ig). It is interesting to note that an anti-Asian elephant IgG antibody was recently produced for endangered Asian elephants (Elephas maximus) for the development of serological diagnostic tests (Kania et al. 1997; Vongchan 2013; Humphreys et al. 2015). To address the limitations of serological diagnostic tools for tigers, alternative serological assays urgently need to be developed. To the best of our knowledge, specific serology assays for the detection of tiger IgG antibody have not yet been reported. Therefore, the present study aimed to produce a rabbit polyclonal anti-tiger IgG antibody and to determine its specificity against the IgG of Bengal tigers and other species.

Materials and methods

Animal sera

Sera of wild felid species, including the orange-haired and white-haired Bengal tiger (Panthera tigris tigris), snow-white tiger (Panthera tigris), golden tabby tiger (Panthera tigris), Siberian tiger (Panthera tigris altaica), panther (Panthera pardus), leopard (P. pardus), jaguar (Panthera onca), lion (Panthera leo), white lion (P. leo), clouded leopard (Neofelis Nebulosa), Asian golden cat (Catopuma temminckii), leopard cat (Prionailurus Bengalensis), and jungle cat (Felis Chaus), were obtained from the sero bank of Tiger Kingdom, Chiang Mai Night Safari, and Chiang Mai Zoo, Thailand. Furthermore, sera of the domestic species provided by the sera bank of the Veterinary Diagnostic Laboratory Center, Faculty of Veterinary Medicine, Chiang Mai University were also used; these were sera of the domestic cat (Felis catus), pig (Sus Scrofa domesticus), cow (Bos taurus), chicken (Gallus gallus domesticus), sheep (Ovis aries), and goat (Capra hircus).

Animal immunoglobulin G (IgG) preparation

Pooled sera of each animal species were subjected to IgG isolation and purification using the Melon™ Gel IgG Spin Purification Kit (Pierce Biotechnology, Rockford, USA) according to the manufacturer’s instructions. The concentration of IgG was measured by UV spectrophotometry at 280 nm and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purified IgG was adjusted to a concentration of 2 mg/ml and then kept as an aliquot at −20°C until further analysis.

Rabbit anti-Bengal tiger IgG polyclonal antibody production

The purified orange-haired Bengal tiger IgG from the previous step was mixed with an equal volume of Montanide (Seppic, Paris, France; 1:1 v/v). Three New Zealand White rabbits (mixed gender and age) were subcutaneously immunized with purified tiger IgG in Montanide (100 µg/dose 1 ml) at one-week intervals. Before each immunization, 2 ml of blood was collected from the saphenous veins, and the antibody titre was determined weekly using an indirect enzyme-linked immunosorbent assay (ELISA). The immunization was terminated when the optical density (OD) values reached 2.5000. The indirect ELISA assay was carried out as previously described by Vongchan (2013). Briefly, 96-well immunoplates (Nunc-Immuno Plate MaxiSorp, Intermed, Roskildes, Denmark) were coated with purified orange-haired Bengal tiger IgG (10 µg/ml in 0.05 M carbonate/bicarbonate buffer pH 9.6) and then incubated overnight at 4°C. The plates were washed three times with phosphate buffer saline (PBS) containing 0.05% Tween-20 (PBST) and blocked with a blocking buffer (5% skim milk in PBS). Serial 10-fold dilutions of pre-immunized and post-immunized rabbit sera were added to the blocking buffer and incubated at 37°C for 1 hr. The plates were developed with secondary goat anti-rabbit IgG horseradish peroxidase (HRP) conjugated antibody (Dako Cytomation, Glostrup, Denmark; 1:2000 dilution) and incubated again at 37°C for 1 hr. The reaction was revealed by the addition of 3,3′,5,5′-tetramethybenzidine (TMB) substrate (SeraCare Life Sciences, Gaithersburg, MD, USA), and the plates were incubated at room temperature for 15 min away from the light. Finally, the reaction was stopped with 2 N H₂SO₄ and the optical density (OD) was measured spectrophotometrically by using the Accu Reader Microplate reader M965 (Metertech, Taipei, Taiwan R.O.C.) at 450 nm.

One week after the last immunization, the final blood sample was collected by exsanguination from the jugular vein. Then, the rabbit antisera were isolated and purified using the Melon™ Gel IgG Purification Kit according to the manufacturer’s instructions. The rabbit anti-Bengal tiger IgG polyclonal antibody concentration was measured by the BCA method and analyzed by SDS-PAGE. The purified rabbit anti-Bengal tiger IgG polyclonal antibody was adjusted to 2 mg/ml, and then the aliquot was stored at −20°C for further analysis.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis for immunoglobulin G detection

Either purified animals’ IgG antibody or rabbit anti-Bengal tiger IgG antibody were subsequently analyzed by SDS-PAGE according to Laemmli (1970). Briefly, the samples were solubilized in SDS-PAGE sample buffer (50 mM Tris, 20% glycerol, 0.005% bромphenol blue, 4% SDS) with or without 5% β-mercaptoethanol as a reducing agent, boiled at 95°C for 5 min, and then separated electrophoretically on a 10% SDS-PAGE gel using the Mini-PROTEAN® electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA). The separated proteins were stained with Coomassie Brilliant Blue R-250 (Sigma-Aldrich) for protein band detection.

Evaluation of binding capacity of rabbit anti-Bengal tiger IgG using western blot analysis

The separated proteins of purified animal species’ IgG were electrically transferred onto a nitrocellulose membrane (Merck Millipore™, Merck KGaA©, Darmstadt, DEU) using a western blot apparatus as described by Towbin et al. (1979). The blotting
time was 60 min at a constant voltage of 10 V. The membranes were blocked with a blocking buffer (1% bovine serum albumin (BSA) in PBS) for 1 hr at room temperature. After washing three times with PBST, the membrane was probed with rabbit anti-Bengal tiger IgG polyclonal antibody and incubated with gentle rocking at room temperature for 1 hr. The membrane was washed three times again and subsequently incubated with HRP-conjugated goat anti-rabbit IgG (1:2000 dilution) for 1 hr at room temperature. Finally, the reactions were visualized using a solution containing 3,3′-diaminobenzidine tetrahydrochloride (DAB; Invitrogen, Carlsbad, CA, USA) and hydrogen peroxide (H₂O₂; Merck, Germany).

**Optimization of concentration of reagents for indirect ELISA testing**

To determine the optimum concentration of the coating antigen, sera and conjugate, a checkerboard titration was performed according to Crowther (2001). Ninety-six well immunoplates (Nuncimmuno™ plate, Denmark) were coated with different concentrations of purified orange-haired Bengal tiger IgG (2.0, 1.0 and 0.5 µg/ml) in 0.05 M carbonate/bicarbonate buffer (pH 96). The plates were incubated overnight at 4°C, washed with 150 µl/well of PBST (pH 72) three times, and blocked with 200 µl/well of the blocking buffer followed by incubation at 37°C for 1 hr and subsequent washing three times with PBST.

Thereafter, purified rabbit anti-Bengal tiger IgG was serially diluted ten-fold in the blocking buffer 1:10² A volume of 100 µl of each diluted serum was added to each well as a primary antibody and then incubated at 37°C for 1 hr. After washing three times with PBST, the plate was incubated with 50 µl/well of HRP-conjugated goat anti-rabbit IgG as a secondary antibody. The rabbit anti-Bengal tiger polyclonal antisera was added by using an automatic ELISA plate reader.

**Determination of rabbit anti-Bengal tiger IgG polyclonal antibody species specificity and cross-reactivity to IgG of wild felid species and other domestic animals**

To determine the species specificity and cross-reactivity of the rabbit anti-Bengal tiger IgG antiserum, an indirect ELISA assay and western blot were performed as described above. For indirect ELISA, the optimal reagent concentrations were used. Briefly, a flat-bottom 96-well plate was coated in duplicate overnight at 4°C with purified orange-haired Bengal tiger IgG and IgG from each of the animals (05 µg/ml). The negative control wells consisted of no protein coating. The rabbit anti-Bengal tiger IgG polyclonal antibody at a dilution of 1:100 (20 µg/ml) was added and incubated at 37°C for 1 hr. After washing three times with PBST, the rabbit anti-Bengal tiger IgG was detected using HRP-conjugated goat anti-rabbit IgG at a 1:4000 dilution at 37°C for 1 hr. The reaction was developed by adding TMB peroxidase substrate for 30 min at room temperature under light protection conditions and subsequently stopped with 2 N H₂SO₄. The absorbance was read at 450 nm by using an automatic ELISA plate reader.

**Results**

**Generation of rabbit polyclonal antibody against purified orange-haired Bengal tiger IgG**

To produce rabbit polyclonal antibody against purified Bengal tiger IgG, the orange-haired Bengal tiger IgG needed to first be isolated and purified. The analysis of purified orange-haired Bengal tiger IgG using SDS-PAGE is shown in Figure 1 (A). The results revealed a protein band of approximately 170 kDa under non-reducing conditions as the expected size of the IgG. Additionally, protein bands of approximately 50 and 25 kDa were observed under reducing conditions corresponding to the γ heavy and κ light chains of immunoglobulin, respectively. The purified IgG antibody with Montanide adjuvant was used to immunize the rabbits. The purified rabbit antisera were analyzed under non-reducing conditions, revealing a protein band of approximately 170 kDa on the Coomassie Blue-stained SDS-PAGE gel (Figure 1(B)). Subsequently, the binding capacity of rabbit anti-Bengal tiger IgG antibody was evaluated using western blotting. The reactivity was observed at approximately 170 kDa (Figure 2) on the membrane, demonstrating the capturing ability and specificity of the antibody. The rabbit anti-Bengal tiger polyclonal antisera were subjected to further analysis.

**Optimization of indirect ELISA for development of alternative diagnostic tool**

To the authors’ knowledge, there are no available specific serology assays for detection of the tiger IgG antibody. Therefore, an indirect ELISA was conducted with serial dilutions of the rabbit anti-Bengal tiger IgG serum against orange-haired Bengal tiger IgG-coated immunoplate. The optimal dilution volumes of the ELISA reagents were determined and were chosen using the

![Image](334x118 to 538x235)

**Figure 1.** Analysis of orange-haired Bengal tiger IgG. (A) The purified orange-haired Bengal tiger IgG was analyzed under reducing and non-reducing conditions using SDS-PAGE and stained with Coomassie Brilliant Blue. Lanes M: molecular mass standards; 1: Non-reducing conditions; 2: Reducing conditions. (B) The non-purified and purified rabbit anti-Bengal tiger IgG polyclonal antibody were analyzed using SDS-PAGE and stained with Coomassie Brilliant Blue. Lanes M: molecular mass standards; 1–2: Non-purified rabbit anti-tiger IgG; 3–4: Purified rabbit anti-tiger IgG.
OD values as summarized in supplemental Table S1. Considering the dilution of the plate-coating antigens, the OD values did not have significant differences with regard to OD profiles between dilutions (Figure 3(A-C)). Consequently, the lowest dilution of 0.5 µg/ml was chosen for the coating antigen. The rabbit anti-Bengal tiger IgG showed saturated binding to Bengal tiger serum IgG at dilutions of 1:10, 1:100, and 1:1000. As a result, dilutions of 1:10 and 1:1000 showed a reduction in the OD values detected by the 1:6000 dilution of HRP-conjugated goat anti-rabbit IgG. Accordingly, this study selected the dilution of 1:100 for rabbit anti-Bengal tiger IgG and the dilution of 1:4000 for HRP-conjugated goat anti-rabbit IgG (Figure 3(A)).

**Species specificity and cross-reactivity of purified rabbit anti-Bengal tiger IgG**

For the wild felid species, the specificity of rabbit anti-Bengal tiger IgG was examined using an indirect ELISA. The optimal dilution quantities of the ELISA reagents were applied in this analysis. The OD values are shown in Table 1. The rabbit anti-Bengal tiger IgG showed strong reactivity with the *P. tigris* subspecies IgG. Furthermore, the rabbit anti-Bengal tiger IgG also cross-reacted strongly with other wild felid species.

In addition, cross-reactivity of rabbit anti-Bengal tiger IgG was observed among domestic species, as shown in Table 2. Relative to the homologous reactivity of rabbit anti-Bengal tiger IgG with Bengal tiger IgG, the highest percentage cross-reactivity was with the domestic cat (63.58%), followed by the pig (38.45%). In contrast, low cross-reactivity of rabbit anti-Bengal tiger IgG was observed with cow, goat, sheep, and chicken. To confirm the cross-reactivity, western blotting was performed (Figure 4). As expected, the results showed that...
aimed to develop new reagents to detect and characterize tiger immunoglobulin G. In this study, we evaluated the cross-reactivity of rabbit anti-Bengal tiger IgG polyclonal antibody reactivity with domestic animals. The cross-reactivity was strong and has the longest serum half-life of all immunoglobulin isotypes (Leusen and Nimmerjahn 2013). There may be a relationship between IgG level and severity of diseases or immune responses to vaccines (Kumagai et al. 2001; Isa et al. 2002; Oliveira et al. 2009; de la Torre et al. 2016; Mbengue et al. 2016). Therefore, IgG plays a critical role in the protection against diseases, and it can be of value in revealing immunological responses (Kinyanjui et al. 2004; Stanisic et al. 2009). Presently, little is known concerning the Bengal tiger’s immune responses. Generally, an intact IgG shows variation in the molecular mass, depending upon the species, with an average of approximately 150 kDa (Janeway et al. 2001). Consistent with previous reports, the molecular weight of Bengal tiger IgG in the present study was approximately 170 kDa (Mandal et al. 2013). These results indicated that purification of orange-haired Bengal tiger IgG using the Melon™ Gel IgG Purification Kit was successful in the present study.

Cross-reactivity of anti-immunoglobulin with immunoglobulins of different species has been reported previously (Neoh et al. 1973; Jefferis et al. 1982; Lastras et al. 2000; Nollens et al. 2008). In the current study, the cross-reactivity to IgG of wild felid species was determined by indirect ELISA. The observed cross-reactivity could be explained by the fact that the rabbit anti-Bengal tiger IgG produced in this study is a polyclonal antibody that displays diverse antibody specificity against many antigenic epitopes. Thus, the cross-reactivity often occurs more readily than in the case of monoclonal antibodies (Frank 2002). However, there are other factors involved. Evolutionary history could be one explanation for the cross-reactivity. Consequently, it is not surprising that the anti-Bengal tiger IgG produced from the orange-haired Bengal tiger IgG in the present study showed an extremely strong reaction to the homologue IgG derived from other pantherine subspecies white, golden tabby, and snow-white tigers). Furthermore, the cross-reactivity with the Panthera lineage (lion, jaguar, leopard, panther, and clouded leopard) IgG may be due to the evolutionary relationship within the monophyly of the Panthera genus (Johnson et al. 1996, 2006; Yu and Zhang 2005; Christiansen 2008; Davis et al. 2010). Interestingly, the anti-Bengal tiger polyclonal antibody was able to strongly cross-react with purified sera IgG from other wild felid species beyond the Panthera lineage. This implies that the polyclonal antibody has the ability to detect wild felid species’ IgG. Remarkably, these results reflect the phylogenetic relationship within the family Felidae. Fossil records reveal that the common ancestor of the modern felid species came into existence approximately 11 million years ago in the late Miocene. With the Panthera lineage began the first divergence, followed

**Table 1.** Species specificity of rabbit anti-Bengal tiger immunoglobulin G and cross-reactivity among wild felid species (family Felidae).

| Animal | OD  | %   |
|--------|-----|-----|
| Bengal tiger: orange hair | 1.194 | 100.00 |
| Bengal tiger: white hair | 0.903 | 75.63 |
| Golden tabby tiger* | 0.893 | 74.79 |
| Snow-white tiger* | 0.849 | 71.11 |
| Siberian tiger* | 0.868 | 72.70 |
| Lion | 0.641 | 53.69 |
| Lion: white hair | 0.692 | 57.96 |
| Panther* | 0.813 | 68.10 |
| Leopard* | 0.794 | 66.50 |
| Jagua | 0.892 | 74.71 |
| Clouded leopard* | 0.844 | 70.69 |
| Asian golden cat | 0.776 | 64.99 |
| Leopard cat | 0.815 | 68.26 |
| Jungle cat | 0.851 | 71.27 |

*Panthera tigris* subspecies.

Table 2. Cross-reactivity of purified rabbit anti-Bengal tiger immunoglobulin G with domestic animals’ immunoglobulin G.

| Animal | OD  | %   |
|--------|-----|-----|
| Bengal tiger | 0.593 | 100.00 |
| Domestic cat* | 0.377 | 63.58 |
| Pig* | 0.228 | 38.45 |
| Cow | 0.062 | 10.46 |
| Sheep | 0.043 | 7.25 |
| Goat | 0.051 | 8.60 |
| Chicken | 0.041 | 6.91 |

* indicates that the cross-reactivity was observed in this study by the rabbit anti-Bengal tiger IgG polyclonal antibody reactivity with domestic animals’ IgG.

**Figure 4.** The immunoblotting analysis of rabbit anti-Bengal tiger IgG polyclonal antibody cross-reactivity with domestic animals. The cross-reactivity was strong with the cat IgG and faint with the pig IgG.

rabit anti-Bengal tiger IgG cross-reacted strongly with domestic cat. In addition, slight cross-reactivity was observed with the pig, while no cross-reactivity was detected with cow, goat, sheep or chicken.

**Discussion**

Due to the combination of novel diseases emerging in the tiger coupled with the lack of serological assays to detect either seroconversion from pathogen exposure or vaccination, this study aimed to develop new reagents to detect and characterize tiger immune responses. To the best of the researchers’ knowledge, this study presents the first attempt at development of rabbit anti-tiger IgG polyclonal antibody for further immunological research or laboratory investigation. Not only the anti-tiger IgG can be applied to evaluate the antibody titre against vaccines in tigers and related wild felid species including FPV, FCV, feline herpes virus (FHV), and feline leukemia virus (FeLV) but also to detect certain disease such as feline infectious peritonitis (FIP), feline immunodeficiency virus (FIV) infection (Risi et al. 2012; Lammerski 2015).

IgG is the predominant immunoglobulin in humoral immunity and has the longest serum half-life of all immunoglobulin isotypes (Leusen and Nimmerjahn 2013). These results indicated that purification of orange-haired Bengal tiger IgG using the Melon™ Gel IgG Purification Kit was successful in the present study.
by further diversification into seven lineages (Johnson et al. 2006). In support of the evolutionary relationships, the family Felidae shows high levels of genomic similarity, with very low levels of genetic diversity (Kim et al. 2016). Therefore, it is probable that immunoglobulins are related to other species because they originated from a common ancestral precursor gene (Hill et al. 1966), allowing related felid species’ IgG to be definitively assigned as a homologue of Bengal tiger IgG.

Western blotting was used to confirm the observed reactive bands with domestic cat and pig serum IgG. In comparative phylogenetic relationships, the domestic cat is obviously more closely related to the tiger than other domestic species (Gaphodatsky et al. 2011). In addition, a previous comparison of genomic sequences revealed 95.6% similarity between the tiger and the domestic cat (Cho et al. 2013). Therefore, it is not surprising that cross-reactivity was observed with domestic cat IgG. A close phylogenetic relationship is one possible explanation for the occurrence of the cross-reactivity. The results from this study indicate that the rabbit anti-Bengal tiger IgG polyclonal antibody generated in this study was highly specific to the Felidae species.

Although the domestic pig is an outgroup to the Felidae, low-level cross-reactivity was observed on western blotting, indicating that the rabbit anti-Bengal tiger polyclonal IgG antibody was also somewhat specific to the Suidae species. Previously, the cytochrome b gene was used for identifying and investigating the evolutionary relationships among endangered species Irwin et al. 1991; Janczewski et al. 1995; Masuda et al. 1996). The construction of a phylogenetic tree based on cytochrome b revealed that the pig has a relatively closer relationship to felid species than to other Cetartiodactyla species such as cows, sheep, and goats, based on lower genetic distance (Hsieh et al. 2001). Therefore, it is possible that this evolutionary relationship may explain the immunological cross-reactivity. Unfortunately, the whole-genome reference sequence is limited for the tiger in regard to investigating the evolutionary relationships or carrying out genetic analyses (Cho et al. 2013). Nevertheless, it is an interesting observation that the IgG from the cat and the pig exhibited binding to the HCMV FcR, in contrast to the IgG from sheep, goats, cows, and chickens (Antonsson and Johansson 2001). It is possible that the antigenic determinants of both the cat and the pig IgG Fc regions are identical by way of sharing homologies in the amino acid sequence of the antigen (Hill et al. 1966; Esteves and Binaghi 1972). These results might support the fact that both the pig and the cat IgG could cross-react with the rabbit anti-Bengal tiger IgG in this study. However, because whole orange-haired Bengal tiger IgG was used to immunize, it is difficult to know whether the cross-reactivity occurred with the antigenic epitopes on the heavy chains or those on the light chains. To gain a more comprehensive understanding of these phenomena, further studies on the antigenic determinants of the immunoglobulin responsible for cross-reactivity will be needed.

Conclusions
In conclusion, the rabbit anti-Bengal tiger IgG polyclonal antibody was successfully produced, and the antibody was highly specific to the P. tigris tigris IgG. Interestingly, the antibody could strongly cross-react with other animals’ IgG, particularly those of the family Felidae. The importance of the characteristics of cross-reactivity for this polyclonal antibody might lie in the fact that it has the potential to be a useful research tool for further application in qualitative and quantitative analyses of IgG in felid species.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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