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Structure and antigenicity analysis of the IgG gene for *Nyctereutes procyonoides*

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Abstract

Objective: *Nyctereutes procyonoides immunoglobulin G (IgG) gene is partially cloned.*

Material and methods: *In order to obtain a certain length (966bp) of Nyctereutes procyonoides immunoglobulin G (IgG), two pairs of primers are designed according to the conserved nucleotide sequence of canine (GenBank:AF354265, AF354265, AF354266, AF354267) and mink (GenBank: L07789). Using Bioinformatics technology and Western-blot to analyze antigenicity of Nyctereutes procyonoides IgG-B gene.*

Results: *The homology for nucleotide sequence of IgG between Nyctereutes procyonoides and canine (IgG A, IgG B, IgG C, IgG D), mink, Homo sapiens, Oryctolagus cuniculus, Mus musculus, Anas platyrhynchos and gallus were respectively (88.1%, 93.6%, 85.4%, 87.2%), 83.7%, 74.8%, 71.8%, 69.2%, 51.6%, 48.4%. It can be seen that there was high homology of aminoacid sequence between IgG of Nyctereutes procyonoides and IgG (A, B, C, D) of canine. And the serum antibody of Nyctereutes procyonoides had obviously cross-reaction with HRP conjugated rabbit anti-dog IgG,compared with those of canine, oryctolagus cuniculus, mus musculus, mink, gallus.*

Conclusions: *We successfully got Nyctereutes procyonoides immuneglobulin G (IgG) gene (GenBank: KM010191). There is the closest ties of consanguinity of IgG exist between Nyctereutes procyonoides and canine among the mammal through the genetic evolution.The detection and treament of canine distemper can be used on Nyctereutes procyonoides.*

Key words: *gene cloning, western blot, genetic evolution analysis, nyctereutes procyonoides, IgG.*

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Introduction

In recent years,the breeding industry of fur-bearing animal such as ussuri raccoon, fox, and so on has become a bright spot in the characteristic breeding in china due to the short breeding cycle and high economic benefit. But once the fulminating infectious diseases such as canine distemper, and so on break out, which will result in dying like flies of fox and ussuri raccoon [1, 2]. The special virus detection kit and colloidal gold test strip for the *Nyctereutes procyonoides* are not popularized yet, and therefore the fast detection on the diseases of the *Nyctereutes procyonoides* cannot be carried out conveniently. Diagnostic reagent market of caine diseases is prone to perfecting, and therefore it is important that whether the detection reagent for the canine distemper can be used for detecting the disease of the *Nyctereutes procyonoides* or not.

Antibodies (immunoglobulins, Ig) are used by the immune system to identify and neutralize foreign objects and are responsible for antigen-binding and effector functions. they are a special class of glycoproteins presented on the surface of B-cells as membrane-bound receptors and in blood serum and tissue fluid as soluble molecules, and are the most important factors of the specific humoral immunity [3]. They induce a particular immune response, e.g. trigger the classical scheme of complement activation. The route by which an antigen enters body and its chemical composition steers the (secondary) immune reaction into preferential patterns of class switching. Besides direct B-cell triggering by the antigen itself, a number of secondary signals will influence differentiation of the B-cell, including recognition by pattern-recognition receptors like Toll-like receptors and cytokines produced by other lymphocytes and antigen-presenting cells [4, 5].

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Material and methods

Ethics statement

The experimental procedures were carried out in accordance with standard guidelines for the care of animals. All efforts were made to minimize the number of animals used as well as their suffering.

Sample collection and tissue preparation

The spleen of *Nyctereutes procyonoides* was obtained from a farm, Zhucheng, China and stored in –20°C.

Total RNA isolation and synthesis of cDNA

Total RNA samples were extracted from spleens using Trizol (TransGen) and the cDNA pool was obtained using the PrimScript RT reagent Kit (TaKaRa).

The amplification of cDNA sequence

Two pairs of homologous primers (Table 1) were designed with DNASTAR 5.0 software in the conserved region of canine (GenBank: AF354265, AF354265, AF354266, AF354267) and mink (GenBank: L07789). All primers used in this study are listed in Table 1. With the primers, a cDNA fragment was amplified by RT-PCR using the first strand cDNAs as templates. The PCR reaction was performed under the following conditions in a thermal cycle: initial dematuration at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s; annealing at 54°C for 30 s and extension at 72°C for 1 min: and extension at 72°C for 10 min. PCR products were analyzed by electrophoresis in 1% agarose, and purified by Agarose Gel DNA Extraction Kit (Shanghai Sangon Biotech Co. Ltd). The products were cloned by pMD18-T (TaKaRa) and sent to Shanghai Sangon Biotech CO., Ltd. for sequencing.

Table 1. Conserved sequence amplification PCR products using forward and reverse primer sequences

Primer name	Primer sequences
F1	5'-GAAGGGCCGATTCACCAT-3'
R1	5'-TGAACACTGGCTTGTCTACTTT-3'
F2	5'-GGCCAGCAACACTAAAG-3'
R2	5'-CTCCACATCAATGTCAGGTG-3'

Multiple alignment and phylogenetic sequence analysis

The nucleotide sequence of *Nyctereutes procyonoides* IgG gene, along with that of avian and several mammalian species from GenBank, were aligned by DNASTAR 5.0 software. Sequence analysis of the predicted *Nyctereutes procyonoides* IgG protein translated from the nucleotide sequence of *Nyctereutes procyonoides* IgG fragment was performed using the NCBI and ExPaSy software.

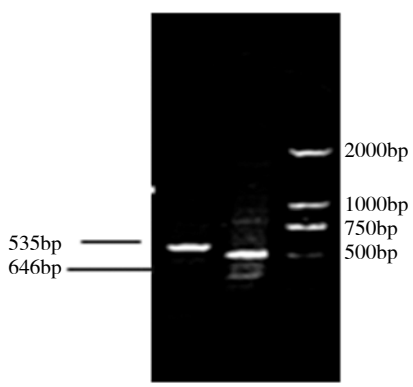


Fig. 1. RT-PCR amplification result of the conserved sequence IgG

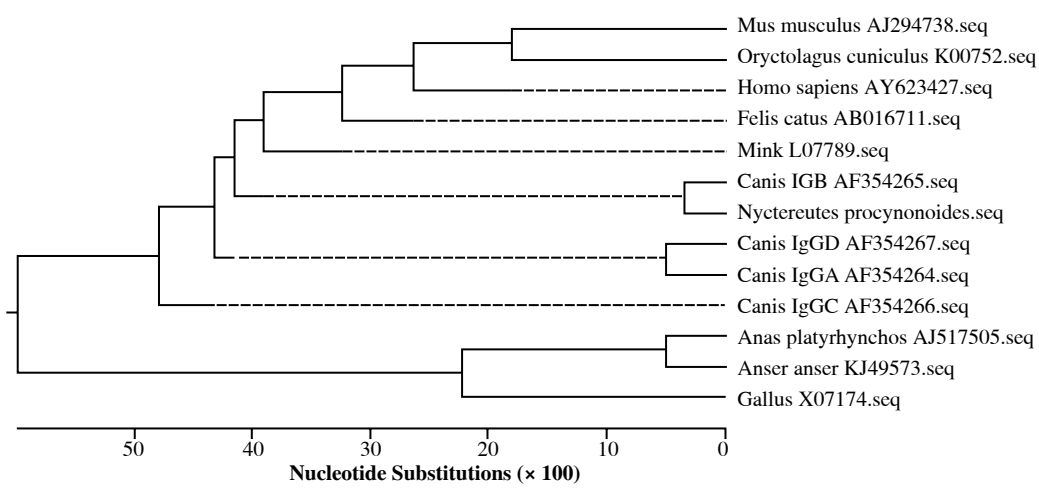


Fig. 2. The evolutionary tree of *Nyctereutes procyonoides* compared with other animals from Genbank

Western-blotting analysis

The cross immunogenicity of the IgG of *Nyctereutes procyonoides*, canine, mink, *Homo sapiens*, *Sus scrofa*, *Oryctolagus cuniculus*, *Mus musculus*, *Anas platyrhynchos*, gallus is detected through the Western blot. Experimental basis is provided for the clinical detection of *Nyctereutes procyonoides* diseases and serology treatment.

Results

Molecular cloning and analysis of *Nyctereutes procyonoides* IgG

The nyctereutes procyonoides IgG of 464 bp and 535 bp (Fig. 1) were obtained from RT-PCR that were identified from the spleen cDNA library of nyctereutes procyonoidess which are homologous to canine and mink. After splicing, a certain length (966 bp) of gene was sucessfully obtained. It covers the complete CH1,Hinge,CH2,CH3 region [6] and the partial VH region. It encodes a protein 322 amino acids in length.

Sequence analysis

The homology between the nucleotide sequence of the *Nyctereutes procyonoides* and the nucleotide sequence of canine (IgG A, IgG B. IgG C, IgG D), mink, *Homo sapiens*, *Oryctolagus cuniculus*, *Mus musculus*, *Anas platyrhynchos* and gallus are respectively (88.1%, 93.6%, 85.4%, 87.2%), 83.7%, 74.8%, 71.8% ,69.2%, 51.6%, 48.4%. (Figs. 2 and 3). The analysis of genetic tree showed that the IgG relationship of *Nyctereutes procyonoides* and canine had high homology.

Aminoacid sequence analysis

The deduced aminoacid sequence of *Nyctereutes procyonoides* IgG has an estimated isoelectric point and Mr of 6.16 and 35.2 KD, respectively. The number of negatively charged residues (Asp + Glu) in the sequence is 9. The total number of positively charged residues (Arg + Lys) is 10, indicating that the protein has an overall positive charge.

The sequence was compared with those of different subtypes of canine IgG using DNASTAR5.0 (Fig. 4). The fully automatic procedure on the SWISS-MODEL server was used to construct a 3D structural model of *Nyctereutes procyonoides* IgG and canine IgG (Fig. 5).

Western-blot analysis

The result of Western blot can be seen that the Rabbit anti-dog IgG-HRP could be reacted with the heavy and light chain of canine IgG. It has significant cross immune response to *Nyctereutes procyonoides* IgG heavy chain, has some degree of cross immune reaction with mink, *Oryctolagus cuniculus*, *Mus musculus* and has weak cross-immunity with gallus (Fig. 6).

Discussion

The gene of *Nyctereutes procyonoides* IgG has been partially determined. There was no report about *Nyctereutes procyonoides* IgG so far. The homology of nucleotide and amino acid sequencesfor IgG between *Nyctereutes procyonoides* and canine was higher than other animals. By means of Western blot, obvious cross-reaction between the serum antibody of the *Nyctereutes procyonoides* and HRP conjugated rabbit anti-dog IgG were observed. It was proved that IgG antibodies of *Nyctereutes procyonoides* and canine maybe interact with their respective receptors

	1	2	3	4	5	6	7	8	9	10	11	12	13	
1		85.9	85.1	86.4	52.9	54.3	49.0	79.2	70.3	68.6	68.3	80.1	85.4	1
2	15.7		90.8	87.4	51.4	52.4	48.9	82.3	70.3	70.2	71.0	81.4	87.2	2
3	16.7	9.8		88.5	51.0	52.3	48.7	81.6	68.0	71.0	70.8	80.1	88.1	3
4	15.1	13.8	12.6		49.8	51.1	48.0	84.7	68.5	75.7	71.7	83.2	93.6	4
5	74.5	78.4	79.7	82.9		90.8	66.5	50.2	42.1	50.9	47.2	45.8	50.7	5
6	70.8	75.5	76.1	79.4	9.8		66.8	50.7	43.3	51.7	47.2	48.0	51.6	6
7	85.7	86.1	86.8	88.9	44.5	43.9		47.3	39.9	48.8	43.4	44.7	48.4	7
8	24.5	20.3	21.3	17.2	82.0	80.3	91.1		69.0	74.6	69.6	82.8	83.7	8
9	38.2	38.2	42.1	41.1	110.8	105.8	121.2	40.1		71.8	71.5	69.8	69.2	9
10	40.9	38.1	37.0	29.4	79.7	77.6	86.2	31.2	35.7		73.1	78.4	74.8	10
11	41.3	36.7	37.1	35.6	91.5	91.3	105.6	39.1	36.0	33.4		77.1	71.8	11
12	23.2	21.5	23.2	19.0	96.3	88.8	100.4	19.6	38.7	25.5	27.3		83.6	12
13	16.3	14.1	13.0	6.8	80.4	77.8	87.6	18.5	39.9	30.7	35.4	18.6		13
	1	2	3	4	5	6	7	8	9	10	11	12	13	

Canis IgGC AF354266.seq
Canis IgGD AF354267.seq
Canis IgGA AF354264.seq
Canis IgGB AF354265.seq
Anser anser KJ49573.seq
Anas platyrhynchos AJ517505.seq
Gallus X07174.seq
Mink L07789.seq
Mus musculus AJ294738.seq
Homo sapiens AY623427.seq
Oryctolagus cuniculus K00752.seq
Felis catus AB016711.seq
Nyctereutes procyonoides .seq

Fig. 3. Homology comparisons of nucleotide sequence among nyctereutes procyonoides, canine, mink and other animals IgG from GenBank

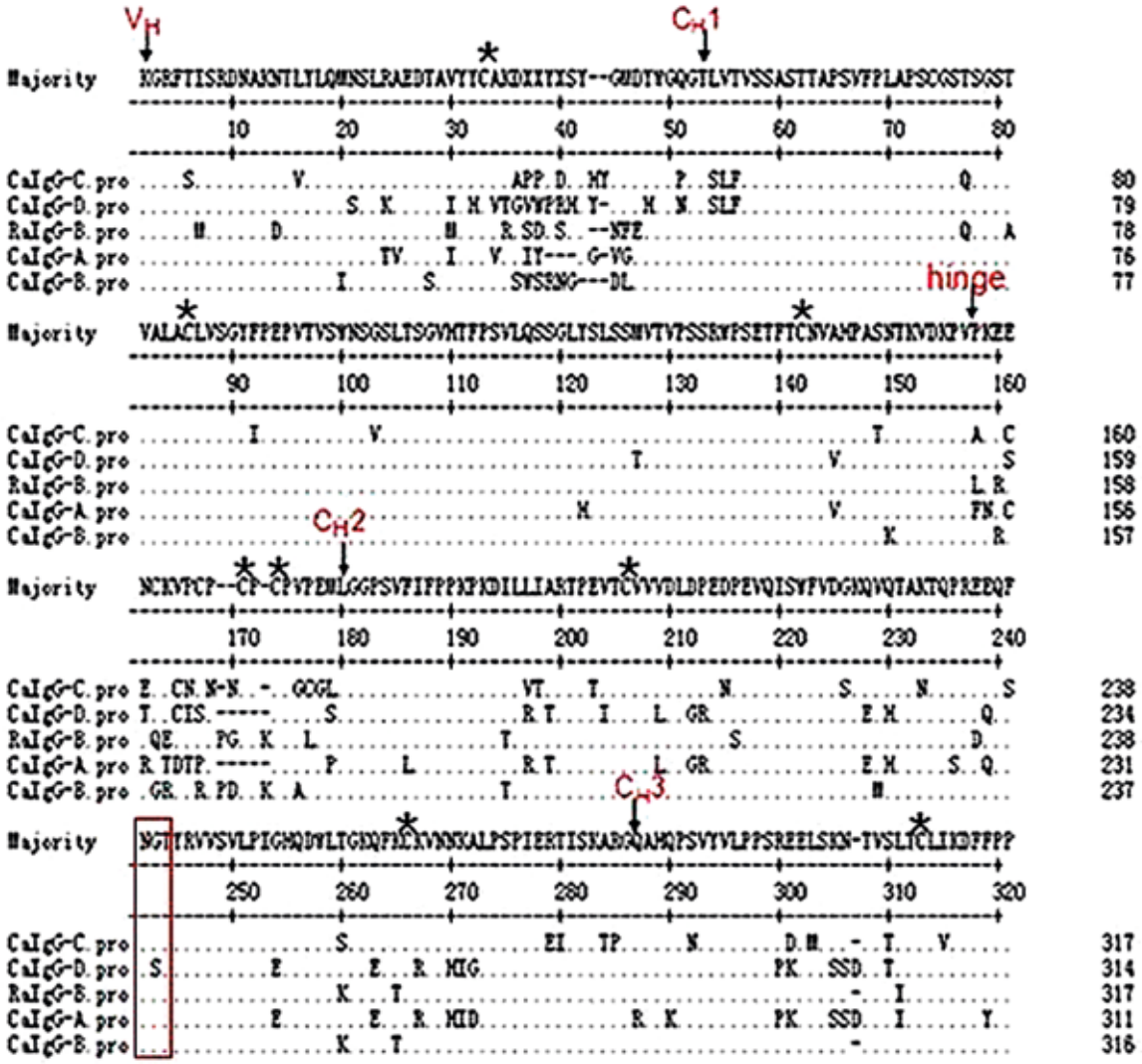
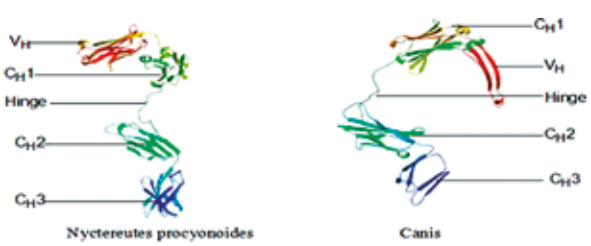
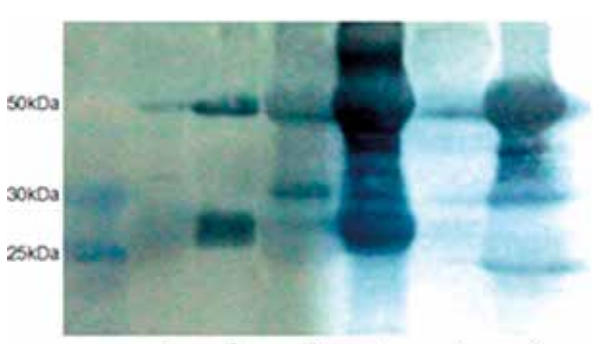


Fig. 4. Comparison of the primary structure of *Nyctereutes procyonoides* IgG and canine IgG (A,B,C,D). There is a N-glycosylation site inlocated in 241-243 amino acid residues site(maked with red box). Eight conservative cysteines are marked with * High homology between *Nyctereutes procyonoides* IgG and canis IgG (A–D) can be seen in the figure



CaIgG-A – Canis IgG A; CaIgG-B – Canis IgG B; CaIgG-C – Canis IgG C; CaIgG-D – Canis IgG D; RaIgG-B – Nyctereutes procyonoides IgG

Fig. 5. Comparison of the protein tertiary structure to IgG of *Nyctereutes procyonoides* and canine



1: Gallus (10 µl); 2: Mus musculus (10 µl); 3: Mink (10 µl); 4: Canis (10 µl); 5: Oryctolagus cuniculus (10 µl); 6: Nyctereutes procyonoides (10 µl)

Fig. 6. The cross-reaction of different animal serum with anti-dog IgG-HRP

in a broadly similar manner: the positive residue in the amino acid sequence of canine IgG being accommodated by a complementary site in the canine receptor, while other pairings remain identical [7].

Similar or identical antigenic determinants are sometimes found in association with widely different molecules or cells.This cross-reactivity is important in protection against organisms with cross-reactive antigens and in autoimmune diseases induced by infectious organism bearing antigens cross-reactive with normal self antigens.

As an essential consistent of canine distemper virus particles ,nucleocapsid protein (N protein), which has wrapped and protection of its internal genes associated with viral infection, is the major cross-antigen of hemp-epidemic virus [8, 9]. Hamburger *et al.* found that CDV virulence were closely linked with its N protein and it would act on the central nervous system and cause persistent infections. N protein can stimulate the body to produce strong immune responses [10]. F protein, as one of the canine distemper virus surface glycoprotein, is a major cross-shaped immune antigen with a high degree of homology epitopes.It mediates mutual integration between its envelope with the cell membrane, so that the virus has the host body diffusion capacity [11-13]. Therefore,the serum antibody stimulated *in vivo* by N protein and F protein of CDV on canine can be used on *Nyctereutes procyonoides*.

Furmore, SLAM (signal lymphocyte activation molecule) is the specific receptor to CDV [14]. The result for comparison of the nucleotidesequences of SLAM gene among canine, foxes, nyctereutes procyonoides and mink shown that the homology SLAM nucleotide sequence between canine, fox and nyctereutes procyonoides is higher than 98.6%, while the homology between mink and the above three kinds of animal is less than 86% [15]. Therefore, it constitute a genetic branch in the evolutionary tree as CDV infected host.

So that, the antigenic cross-reactivity of CDV from different species, the high homology of IgG and SLAM between canine and *Nyctereutes procyonoides* as well as strong cross-reactivity between serum antibody of nyctereutes procyonoides with HRP conjugated rabbit anti-dog IgG, all these have been proved that it was available that the kit for canine distemper can be used for detecting the disease of the *Nyctereutes procyonoides* and the colloidal gold test paper for diagnosing canine distemper can be used for diagnosing related diseases of the *Nyctereutes procyonoides* in clinical practice.

The authors declare no conflict of interest.

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