

RESEARCH ARTICLE

The Differential Binding Capacities of Protein A, Protein G, Antibovine IgG and Anticaprine IgG for Serum Samples from Eld's Deer and Hog Deer in Thailand

Jaruwee Kayman¹, Nikorn Thongtip², Orawan Boodde³, Manakorn Sukmak¹, Waradee Buddhakosai^{4,5}, Yuttamol Muangkram^{6,7}, Worawidh Wajjwalku^{1,6,7,*}

Abstract

Objective—To detect immunoglobulin G in serum samples of wild animals in the order Artiodactyla (Eld's deer and hog deer) in Thailand with protein A, protein G, antibovine IgG and anticaprine IgG.

Materials and Methods—Six serum samples were selected at random from each animal species and coated on a microplate. The binding capacities were tested using an direct ELISA method.

Results—The results revealed that protein G had a high binding capacity to immunoglobulin G, followed by anticaprine IgG, in serum samples of both Eld's deer and hog deer. Protein G and anticaprine IgG showed no statistical difference ($p>0.05$) in the optical density (OD) value at 650 nm between these two secondary antibodies in serum samples of Eld's deer.

Conclusion—Protein G and anticaprine IgG can be used for the effective serological detection and surveillance of infectious disease in captive and free-ranging Eld's deer, hog deer, and other non-domestic ruminants.

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Keywords: Eld's deer; Enzyme-linked immunosorbent assay; Hog deer; Protein A; Protein G

¹Center of Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140 and Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok, Thailand.

²Department of Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kamphaeng Saen Campus, Kasetsart University, Nakhon Pathom, 73140. ³Department of Farm Resources and Production

Medicine, Faculty of Veterinary Medicine, Kamphaeng Saen Campus, Kasetsart University, Nakhon Pathom, 73140. ⁴Interdisciplinary Graduate Program in Genetic Engineering, Graduate School, Kasetsart University,

Bangkhen campus, Bangkok, 10900. ⁵Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand, 10530. ⁶The Graduate School, Bangkaen Campus, Kasetsart University, Bangkok, 10900.

⁷Department of Pathology, Faculty of Veterinary Medicine, Kamphaeng Saen Campus, Kasetsart University, Nakhon Pathom, 73140.

*Corresponding author E-mail: fvetwww@ku.ac.th

การประเมินแอนติบอดีทุติยภูมิ โปรตีนเอ และโปรตีนจี ต่อการจับกับอิมมูโนโกลบูลินของละมั่งและเนื้อทรายในประเทศไทย

จารุวี ค่ายมัน¹, นิกร ทองทิพย์², อรวรรณ บุตรดี³, มานะกร สุขมาก¹, วราตี พุทธโกสัย^{4,5},
ยุทธมล ม่วงคราม^{6,7}, วรวิทย์ วัชชวัลคุ^{1,6,7*}

บทคัดย่อ

วัตถุประสงค์ เพื่อประเมินความสามารถต่อการจับของอิมมูโนโกลบูลินจีในซีรัมของละมั่งและเนื้อทรายในประเทศไทย ด้วยโปรตีนเอ โปรตีนจี แอนติบอดีจำเพาะต่ออิมมูโนโกลบูลินของโคและแพะ **วัสดุอุปกรณ์ และวิธีการ** เก็บตัวอย่างซีรัมของละมั่งและเนื้อทรายแบบสุ่มชนิดละ 6 ตัวอย่าง ทำการศึกษาโดยวิธี direct ELISA ประเมินผลการศึกษาของโปรตีนเอ โปรตีนจี แอนติบอดีจำเพาะต่ออิมมูโนโกลบูลินของโคและของแพะ ต่อการจับกับอิมมูโนโกลบูลินในละมั่งและเนื้อทราย ด้วยการวัดค่าการดูดกลืนของแสงที่ความยาวคลื่น 650 นาโนเมตร

ผลการศึกษา พบว่าโปรตีนจีมีความสามารถต่อการจับกับอิมมูโนโกลบูลินสูงที่สุดทั้งในละมั่งและเนื้อทราย รองลงมาคือ แอนติบอดีจำเพาะต่ออิมมูโนโกลบูลินของแพะ นอกจากนี้ยังพบว่า โปรตีนจีและแอนติบอดีจำเพาะต่ออิมมูโนโกลบูลินของแพะมีความสามารถต่อการจับกับอิมมูโนโกลบูลินในละมั่งแตกต่างกันอย่างไม่มีนัยสำคัญทางสถิติ ($p>0.05$)

ข้อสรุป โปรตีนจี และแอนติบอดีจำเพาะต่ออิมมูโนโกลบูลินของแพะสามารถนำมาประยุกต์ใช้ในการตรวจทางซีรัมวิทยาในการตรวจโรคติดเชื้อในละมั่ง และเนื้อทราย ซึ่งอาจประโยชน์ต่อสัตว์กับเด็วอื่นๆ ทั้งในส่วนเพาะเลี้ยง และในธรรมชาติ

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คำสำคัญ: เนื้อทราย โปรตีนจี โปรตีนเอ ละมั่ง อิมมูโนโกลบูลิน แอนติบอดีทุติยภูมิ

¹ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140 และศูนย์ความเป็นเลิศด้านเทคโนโลยีชีวภาพเกษตร สำนักพัฒนาบัณฑิตศึกษาและวิจัยด้านวิทยาศาสตร์และเทคโนโลยี สำนักงานคณะกรรมการอุดมศึกษา กระทรวงศึกษาธิการ, ²ภาควิชาเวชศาสตร์คลินิกสัตว์ใหญ่และสัตว์ป่า คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140, ³ภาควิชาเวชศาสตร์และทรัพยากรการผลิตสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140, ⁴โครงการสหวิทยาการระดับบัณฑิตศึกษาสาขาพันธุวิศวกรรม บัณฑิตวิทยาลัยมหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตบางเขน กรุงเทพมหานคร 10900, ⁵คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีมหานคร กรุงเทพมหานคร 10530, ⁶บัณฑิตวิทยาลัย มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตบางเขน กรุงเทพมหานคร 10900, ⁷ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140

*ผู้เขียนที่ให้การติดต่อ E-mail: fvetwww@ku.ac.th

Introduction

The hog deer (*Axis porcinus*) is a member of the Cervidae family [1]. It is a small deer whose coat is quite thick and generally dark-brown in color except for the under parts of the body and legs which are lighter. These deer are predominantly found in Northern India, Pakistan and Southeast Asia [2-5]. The hog deer is listed as endangered by the International Union for the Conservation of Nature and Natural (IUCN) Resources Species Survival Commission [6]. Eld's deer (*Cervus eldii*) are distributed throughout Southeast Asia [2-5,7]. The species is listed in Appendix I of the Convention on International Trade in Endangered Species (CITES) and is listed as endangered by the IUCN [8]. Populations of Eld's deer and hog deer have been reduced by illegal hunting, an increase in the rate of habitat loss and they may be affected by the transmission of infectious diseases from domestic livestock [9-15].

Enzyme-linked immunosorbent assay (ELISA) is a serological assay for the detection and screening of human and animal populations for infectious diseases [16,17-21]. ELISA tests have been reported to provide an economical, high sensitivity, short duration serological test that can be easily automated [22,23]. They are therefore widely used for the detection, screening and serological surveillance of infectious disease in captive and free-ranging animal populations [24]. However, the application of ELISA assays may be limited as they require a labeled secondary antibody or protein (enzyme conjugate) that is specific for each species tested [25]. Moreover, little is known about the binding capacities of secondary antibody or protein to the immunoglobulins of wild animals such as non-domestic ruminants in the order Artiodactyla commonly found in Thailand. This is particularly important as infectious diseases such as bovine tuberculosis (*Mycobacterium bovis*) can be maintained in non-domestic hoofstock and transmitted to domestic livestock. Eradication of the disease would then depend on the serologic screening and surveillance of wild hoofstock such as hog deer. Further information on binding capacities could be used for this purpose. Although the ELISA assay may be limited by a requirement for specific antisera to use as a conjugate for each species, it is difficult and expensive to produce [25]. For this reason, it is preferable to use labeled secondary antibody or enzyme conjugates such as protein A, protein G, antiovine IgG and anticaprine IgG for the serologic detection of infectious disease in domestic ruminants.

Protein A is found on the cell wall and is a virulence factor for *Staphylococcus aureus* that can induce hypersensitivity and histamine release from basophils [26,27]. It has the capacity to bind to immunoglobulin G (IgG) via the Fc portion of the immunoglobulin [28-30]. Previous studies have revealed the ability of protein A to bind to IgG in 65 mammal species, e.g., Japanese monkey, nutria [31]. Protein A is also able to bind to IgE, IgA and IgM in domestic dogs [32]. Protein G is found in bacterial cell walls and also has the capacity to bind to IgG [33-35]. It can be derived from group C and G streptococci. Similar to protein A it also binds to the Fc portion of IgG and IgG subclasses [36,37]. Protein G has been shown to have a high binding affinity for IgG in many species including e.g., antelope, oryx, sheep, muntjac, and impala. It also binds to IgG subclasses in many domestic species, e.g., rat, mouse [25]. Previous studies have reported the application of protein G to the detection of antibodies in several non-domestic artiodactyl species. For example, horseradish peroxidase-conjugated protein

G was used in serological tests of tuberculosis (TB) in reindeer [38]. Antibovine IgG is used for the serological surveillance of selected viral agents in captive and free-ranging populations of Arabian oryx (*Oryx leucoryx*) [37]. There are no previous reports of the use of antibovine IgG or anticaprine IgG for serological surveillance in wildlife animals within Thailand. Similarly, there are no studies of the binding capacity of protein A or protein G to IgG for non-domestic ruminants in Thailand.

In this study we used protein A, protein G, antibovine IgG and anticaprine IgG to detect IgG in serum samples of Eld's deer and hog deer in Thailand.

Materials and Methods

Serum samples

Twelve serum samples were collected from the jugular vein of clinically normal adult Eld's deer (*Cervus eldii*) (n=6) and hog deer (*Axis porcinus*) (n=6) held in a semi-captive environment in Thailand. The two species are from the same taxonomic family (Cervidae) within the order Artiodactyla. The 3 domestic animals species including cattles (n=3), goats (n=6) and dogs (n=2) were obtained as a control.

Indirect ELISA

Serum samples were diluted in coating buffer (pH 9.5) to a 1:1000 dilution. Each well of a polystyrene microtiter plate (Nunc, New York, USA) was coated with 120 μ l of a diluted serum sample. The coated plates were incubated for 1 hour at 37°C. After incubation the plate wells were washed 3-5 times with phosphate-buffered saline (PBS-T) containing 0.15 M sodium phosphate, 0.2 M sodium chloride, 0.05% Tween 20, pH 7.2 and then blocked with 0.5% casein (Oxiod, Cambridge, UK) in PBS. The wells were filled completely with 100 μ l of blocking solution per well and incubated at 37°C for 1 hour. In the next step, each well was washed 3-5 times with PBS and 100 μ l of a 1:5000 dilution of commercial peroxidase-conjugated protein A, protein G, antibovine IgG and anticaprine IgG (Pierce, Rockford, USA) added to each well. After incubation at 37°C for 30 mins the wells were washed 3-5 times with PBS-T to remove unbound conjugate and 100 μ l of HRP substrate tetramethylbenzidine (Pierce, Rockford, USA) added to each well. The plates were then incubated at room temperature for 15 mins. In the final step the enzyme reaction was stopped by adding 50 μ l of 5% sodium dodecyl sulfate. Mean optical density (OD) values were measured at 650 nm with an ELISA reader (Tecan, Männedorf, Switzerland). Duplicate measurements were taken for each individual sample.

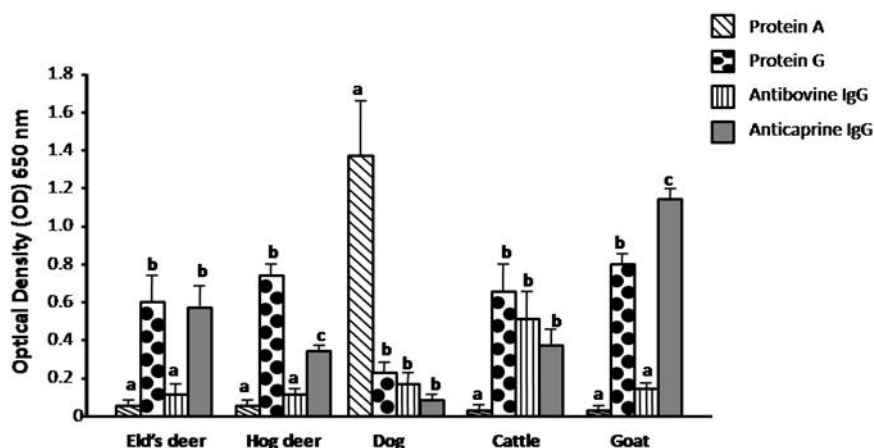
Statistical analysis

Statistical analysis was performed with NCSS® for Windows™ (Kaysville, Utah, USA). All data throughout are expressed as mean values \pm SD. The difference in IgG binding capacity between the four types of enzyme conjugate for each species was evaluated by Multiple-Comparison Tests, and statistical significance was considered at $\alpha = 0.05$.

Results

The mean optical density (OD) values at 650 nm are shown in Figure 1. Protein G and anticaprine IgG had the highest mean OD values, and thus the highest binding capacity, in Eld's deer and hog deer. There was no significant difference between the binding capacity of protein G and anticaprine IgG in Eld's deer. However, the serum samples from hog deer showed a significantly higher binding ability to protein G than protein A and the other secondary antibodies.

Figure 1. Bar Graph Showing the Mean Binding Capacities of 4 Different Types of Secondary Antibody



Letters a-c refer to the statistical difference among each type of secondary antibody or protein (enzyme conjugate) within the same species ($\alpha = 0.05$).

Discussion

Both of Protein G and anticaprine IgG showed the high binding capacity to IgG in the serum samples from Eld's deer, while only protein G alone showed high binding capacity to IgG in hog deer. Although Eld's deer and hog deer are non-domestic ruminants from the same taxonomic family (Cervidae) but they are not categorized in the same genus. Eld's deer is belonged to genus *Cervus*, whereas hog deer is belonged to genus *Axis*. Moreover, Eld's deer is more genetically related to family Caprinae than hog deer [39]. This may be effect on the binding capacity to IgG between these two different genus. Therefore, protein G could be used for disease serosurveillance in species within the genus *Cervus* and *Axis* [21,25,31], whereas anticaprine IgG could only be used for species from the genus *Cervus*. As well

as cattle and goat, protein G is also showed the high binding capacity to IgG [36], while anticaprine IgG was showed the significantly high binding capacity in goat which specific to their genus. Protein G may be used as the universal secondary antibody among Artiodactyls [21,25,31].

Antibovine IgG showed high binding capacity but not different with protein G and anticaprine IgG in cattle serum samples. The results of antibovine IgG showed different in the other three species that are member of order Artiodactyla; Hog deer, Eld's deer, and goat, showed low binding capacity to antibovine IgG.

Protein A showed low binding capacity to IgG in serum samples of Eld's deer and hog deer, especially when compared to binding capacities in the domestic dog [21,32]. Therefore, protein A cannot be recommended as a useful serological assay for infectious disease serosurveillance in non-domestic artiodactyls animals but appropriate for carnivores [21,31,32].

However, previous studies report that binding capacity is affected by other factors such as buffer pH, which affects binding of IgG to the polystyrene plate. This may mean that the labeled secondary antibody or protein (enzyme conjugate) used in this study may need different conditions for optimal binding [32]. Other factors that may be important are serum IgG concentrations and the quality of the enzyme conjugate.

The results of this study suggest that protein G and anticaprine IgG can be used for the effective serological detection and surveillance of infectious disease in captive and free-ranging Eld's deer, hog deer, and other non-domestic ruminants.

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