POTENTIAL USE OF *HELICOBACTER PYLORI* WESTERN BLOT FOR SERODIAGNOSIS

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ABSTRACT: *Helicobacter pylori* infection has been known to associate with gastric and peptic ulcer, mucosa-associated lymphoid tissue, lymphoma and gastric carcinoma. The hyperimmune serum prepared from six New Zealand White rabbits, by immunizing four times, biweekly with *Helicobacter pylori* bacterial extracts were used in this assay. The antisera were collected every week during week 0-11. The Western blots demonstrated at least 13 reactive bands with distinct molecular masses of approximate 80, 78, 73, 70, 63, 60, 28, 27, 24, 23, 18, 15 and 11 kDa. Increased antibody reactivity of hyperimmune sera against *H. pylori* developed intensive bands between 15- 28 kDa which were depended on time and dose exposures. This preliminary study suggested that the Western blot could be used as a potential serodiagnosis of *H. pylori* infection in further veterinary public health study.

Keywords: *Helicobacter pylori*, rabbit, Western blot

INTRODUCTION: *Helicobacter pylori* (*H. pylori*) infection is associated with gastric, peptic ulcer, mucosa-associated lymphoid tissue lymphoma and gastric carcinoma[1-4]. Recently, the noninvasive diagnostic tests for *H. pylori* infection have significantly gained[5]. Immunodiagnosis of *H. pylori* infection is attractive for the investigation of upper gastrointestinal symptoms in comparison with other non-invasive diagnostic methods[6]. It has been suggested that animals are the reservoir of *H. pylori* which may be of importance in human infection and the role of *Helicobacter spp.* in gastrointestinal diseases in dogs and cats is uncertained[7]. It has been known for years that gastric Helicobacter-like organisms (HLO) are commonly present in stomach of dogs but the relationship between these organisms and gastric diseases has never been resolved[8-13]. The invasive *Helicobacter spp*. infection diagnosed by histopathology and PCR technique have been investigated in necropsied dogs[14]. The detection of antibody against *H. pylori* in rabbit serum samples during immunization using indirect immunofluorescent antibody assay was developed for the use of non-invasive method[15]. The Western blot methods have been successfully performed for the serodiagnosis of many bacterial diseases such as human brucellosis, *Helicobacter pylori*, and leptospirosis[16]. This study demonstrates that hyperimmune sera against *H. pylori* could be used as an in-house antiserum reference in future investigation of antibody response in infected animals.

MATERIALS AND METHODS: *H. pylori* was kindly obtained from the Department of Microbiology, Chulalongkorn Memorial Hospital, Bangkok, Thailand. The bacterial cells were harvested, washed three times in phosphate-buffered saline (PBS; pH 7.2), and disrupted by sonicator. After centrifugation at 600x g for 10 min at 4°C, the sonicated protein content in supernatant was determined, calculated by the standard use of bovine serum albumin. The solubilized bacterial material was aliquoted and stored at -20°C until used as previous described[15]. Six, four-month-old female New Zealand Whiterabbits were immunized subcutaneously and intramuscularly at four different injection sites: both scapula regions of fore limbs and thigh muscle of hind limbs. Five hundred microlitres of *H. pylori* cell lysate was diluted (by

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volume) with Freund's complete adjuvant and 0.5 ml was used for each injection site. The immunization took place 4 times, biweekly (week 0, 2, 4 and 6). During week 0-11, the 72 sera were collected and each serum samples was consequently used for Western blot analysis. The experiment protocol was approved by the Animal Care Committee Guidelines of Chulalongkorn University.

One dimensional SDS-PAGE and Western blot was performed using a discontinuous buffer system with resolving and stacking gel containing 10% and 4% polyacrylamide gel respectively. Bacterial antigens and standard molecular weight markers (Amersham Biosciences) were loaded onto the stacking gel and electrophoresed using a constant voltage of 200 V for 1 h according to recent work[17]. After electrophoresis, the separated antigens were transferred onto poly-vinylidene fluoride membrane (PVDF) (Millipore®) and primarily immunostained with in-house produced rabbit antisera and secondarily probed with goat anti-rabbit immunoglobulins conjugated with horse-radish per-oxidase enzyme (DAKO®). The membranes were placed in DAB, 3, 3'- Diaminobenzidine Tetrahydro-chloride, (Sigma®). visualization solution until the bands produced adequate intensity. The interpretation of the results was performed by a standard linear curve as described[18].

RESULTS AND DISCUSSION: The reactivities of rabbit anti - *H. pylori* antisera on Western blots of *H. pylori* derived antigens showed 13 prominent reactive bands of approximate molecular weights of 80, 78, 73,70, 63, 60, 28, 27, 24, 23, 18, 15 and 11 kDa (Figure 1). The specificity of the test was evaluated in comparison to blots with sera from *H. pylori* in non - immunized rabbits (day 0). This provided a satisfactory result and in a good corresponding with previous report of 28 and 31 kDa of *H. pylor*[19]. Although some intense bands reactive between 60-80 kDa were observed in both *H. pylori*-immunized and non-immunized rabbits, these antigenic activities among 43-66 kDa proteins were also found reactive in certain bacteria, such as the flagellin of *Treponema pallidum, Borrelia burgdorferi* or heat shock proteins (HSPs) of *Pseudomonas aeruginosa* and *Campylobacter jejuni*[20]. The three specific bands of 25, 30 and 37 kDa have been potentially used to differentiate between *H. pylori* infected dogs and non-infected ones[20]. Furthermore, the bands of 26, 33 kDa have been specifically demonstrated in *H. pylori* infected mice[21]. In human, the polypeptides with molecular masses of 120, 50 and between 19, 36 kDa were suggested to be the most specific antigens for diagnosis of *H. pylori* infections[22]. The immunoreactive bands, approximately between 15-28 kDa were significantly detected after immunization at week 4 of the experiment, which were depended on time and dose exposures. The obtained results could be suggested the potential of the Western blot for serodiagnosis of *H. pylori* infection in future veterinary public health study.

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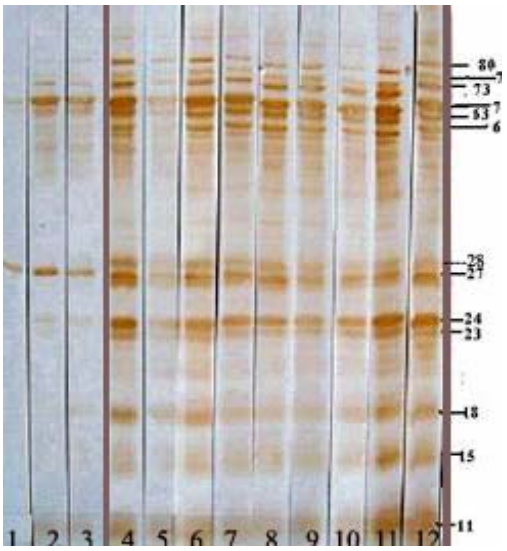

Figure 1 Western blot of *H. pylori* cell extracts with rabbits antisera obtained from week 0 -11 (lane 1 -12, respectively) show 13 prominent bandings, approximately at the molecular weights of 80,78,73, 70, 63, 60, 28,27, 24, 23, 18, 15 and 11 kDa.

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ศักยภาพในการใช้ Western Blot ของเชื้อเฮลิโคแบคเตอร์ ไพโรไล เพื่อการวินิจฉัยทางซีรัมวิทยา

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บทคัดย่อ: การติดเชื้อเฮลิโคแบคเตอร์ ไพโรไล ทำให้เกิดพยาธิสภาพบริเวณกระเพาะอาหารและลำไส้เล็กส่วนต้น เช่น แผลหลุม มะเร็งเม็ดน้ำเหลืองของเยื่อบุทางเดินอาหาร มะเร็งของกระเพาะอาหาร แอนติบอดีที่ได้เตรียมจากซีรัมที่สร้างขึ้นจากการฉีดเชื้อเฮลิโคแบคเตอร์ ไพโลไร ในกระต่ายพันธุ์ New Zealand White จำนวน 6 ตัว โดยฉีดเชื้อ 4 ครั้งทุก 2 สัปดาห์ และเก็บซีรัมกระต่ายทุกสัปดาห์ ระหว่างสัปดาห์ที่ 0 - 11 ผลการตรวจสอบแอนติบอดีในซีรั่มกระต่ายด้วยวิธี Western blot พบแถบปฏิกิริยาจำเพาะจำนวน 13 แถบ ซึ่งมีมวลน้ำหนักโมเลกุล ดังนี้ 80, 78, 73, 70, 63, 60, 28, 27, 24, 23, 18, 15 และ 11 กิโลดาลตัน โดยแถบปฏิกิริยาที่ตรวจพบชัดเจนระหว่าง 15-28 กิโลดาลตัน ขึ้นอยู่กับระยะเวลาและปริมาณของเชื้อที่ได้รับ การศึกษานี้เป็นรายงานเบื้องต้นซึ่งแสดงถึงศักยภาพของการใช้ Western blot ในการวินิจฉัยทางซีรัมวิทยาเพื่อศึกษาการติดเชื้อ เฮลิโคแบคเตอร์ ไพโรไล ทางสัตวแพทยสาธารณสุขต่อไป

คำสำคัญ: เฮลิโคแบคเตอร์ ไพโลไร กระต่าย เวสเทิร์นบลอท

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