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PRODUCTION OF ANTI-*HELICOBACTER PYLORI* IGY IN EGG YOLK OF LAYING HENS IMMUNIZED WITH THE BACTERIAL WHOLE CELL LYSATE

By

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ABSTRACT

Production and evaluation of specific antibodies from egg yolk (IGY Abs) of immunized hens are attracting scientific trends, as indicated from the extensive IGY literature. Considering animal care, high productivity and special suitability for certain diagnostic purposes, hen's eggs represent an alternative that offers some advantages over mammals. Even though, IGY antibodies still represent an underused resource which can be due to the lack of information concerning the possibility of production, application and IGY isolation from the egg yolk. The main purpose of the present work was to produce IGY specific to *Helicobacter pylori* (*H.* pylori), the bacteria of peptic ulcer, chronic gastritis and gastric cancers. The produced antibodies are to be further employed for diagnostic or therapeutic purposes.

Polyclonal anti-*H. pylori* IGY antibodies were successfully produced, purified and titrated in this study. This was accomplished through immunization of laying hens with the bacterial cell lysate and isolation of the anti-*H. pylori* IGY by ammonium sulphate precipitation and affinity chromatography. The purified IGY was proved to be reactive with the bacterial cells and with high titre as indicated by ELISA and microplate agglutination.This article comes in accordance with the European Centre for the Validation of Alternative Methods (ECVAM) concerning alternative methods of value to the bioscience and medical fields that call for refining or replacing the use of laboratory animals.

Keywords:

Egg Yolk, IGY, *Helicobacter pylori*, Ammonium Sulphate, ELISA.

INTRODUCTION

The major antibody in chickens is called immunoglobulin Y (IGY). The acquisition of passive immunity in birds was first described in 1893 by demonstrating the transfer of immunity against tetanus toxin from the hen to the chick through IGY passage from the mother to the offspring **(Klemperer, 1893).**

The chicken immune system has been intensively studied and added a lot of knowledge to the fundamental concepts of immunology. However, the chicken-originated antibodies used in laboratories are not extensively considered as they should be. It became well known that a laying hen transfers its IGG into the egg yolk (IGY) in a level more than a rabbit can produce over a similar time period. Moreover, the animal care costs are lower for the chicken if compared to the rabbit. Fortunately, the IGY antibodies can be purified in large amounts from egg yolk, making laying hens highly efficient producers of polyclonal antibodies **(Carlander *et al.*, 1999).**

The use of egg yolk antibodies (IGY) instead of IGG from mammalian species offer several advantages for the diagnostic immunoassays. The animal suffering does not exist, as antibodies are obtained directly from the egg **(Davalos-Pantoja *et al.*, 2000**).

In addition, a hen lays approximately 20 eggs per month the IGY and 2 gram of egg yolk IGY corresponds approximately to the IGY content of 300 ml of serum or 600 ml concentration in chicken serum. Moreover, when compared to rabbits, the chicken antibodies are ten times less expensive **(Carlander, 2002)**.

IGY technology has many applications in human and veterinary medical fields. It can be used for therapeutic purposes as it has been useful in the prevention of various types of diseases. In addition, by employing different immunological techniques, IGY can detect several classes of antigens, such as microorganisms, tumor markers and certain substances of medical significance. Due to both phylogenetic and structural divergence, IGY is more specific for diagnostic use than its mammalian IGG counterpart **(Pereira *et al.*, 2019).**

IGY has been used in veterinary practice for passive immunization against bacterial or viral infectious diseases. Enteropathogenic *Escherichia coli* (EPEC) IGY was successfully obtained from the egg yolk of immunized hens. The results confirmed the efficacy of the immunization and the adequacy of the method **(Amaral *et al.*, 2002).**

The utilization of IGY in different immunological assays without loss of specificity and sensitivity is of the most important advantages of chicken antibodies over their mammalian

counterparts. Therefore, the European Centre for the Validation of Alternative Methods (ECVAM) specified that yolk antibodies could be used instead of mammalian antibodies for animal welfare seek **(Dias Da Silva and Tambourgi, 2010)**.

Uses of IGY antibodies in the detection and treatment of viral, bacterial, fungal and parasitic diseases in humans and animals are summarized in an article issued by **Lee *et al.* (2021).**

Helicobacter pylori (*H. pylori*), Gram-negative bacterial species, that colonizes on the human gastric mucosa is confirmed as the main pathogenic factor of chronic active gastritis, peptic ulcer, gastric mucosa-associated lymphatic tissue lymphoma and gastric cancer. In 1994, a joint report issued by world health Organization/International Agency for Research on Cancer has listed *H. pylori* as Class I carcinogen **(Wang *et al.*, 2014)**.

Anti - *H. Pylori* IGY antibodies have been utilized for therapeutic purposes in animals **(Nomura *et al.* 2005)** and humans **(Shimamoto *et al.* 2002)**. Antibodies with specificity against immunodominant proteins of *H. pylori* were effective as prophylactic biological **(Shin *et al.*, 2003).** Generally, no complete *H. pylori* eradication could be achieved in view of the increasing bacterial resistance and therefore, the use of specific IGYs minimizes the need for antibiotics. Drinking yogurt fortified with anti *H. pylori* urease IGY, showed a significant decrease in urea breath values of the treated volunteers **(Horie *et al.*, 2004)**.

Diagnostic methods for detecting *H. pylori* are classified into invasive (Endoscopy) and non-invasive methods such as urea breath test, serology, stool antigen detection and molecular techniques **(Hirschl and Makristathis, 2007).**

In 2015, the stool antigen detection (SAT) method of *H. pylori* infection using specific antibodies was reviewed by the Canadian Agency for Drugs and Technologies in Health. The pooled sensitivity and specificity of the test in children were 92.1 and 94.1%, respectively. Monoclonal *H. pylori* SAT tests were better than the polyclonal ones. Stool antigen screening and repeat testing after eradication treatment were found to be the most cost-effective approach for reducing the future burden of peptic ulcer disease and gastric cancer in human **(Mentis *et al.*, 2015)**.

This study aimed at production and evaluation of IGY antibodies against *H. pylori* for their future utilization to develop immunodiagnostic methods for the direct detection of the bacterial targets in clinical specimens of human or animal origins.

MATERIAL AND METHODS

Laying hens:

Thirty hens of a pure white Leghorn strain at the maximum lying age (45 weeks old), purchased from a local farm at Giza Governorate, and were used in this study. The hens were divided into two groups (15 each) and left two weeks for adaptation before immunization. Optimum rearing conditions concerning feed, water and lightning were considered.

H. pylori:

H. pylori used in this study was a local isolate recovered from an endoscopic gastric biopsy of a dyspeptic male patient. The isolate was recovered and identified at the Microbiology Department, Faculty of Veterinary Medicine, Cairo University during a cross sectional study on *H. pylori* infection in human patients **(Rabiea *et al.*, 2021)**.

Immunogen preparation and immunization:

The *H. pylori* isolate was propagated on Columbia blood agar base supplemented with 7 % sheep blood and campylobacter enrichment supplement. The inoculated plates were incubated at 37ºC for 10 days in microaerophilic conditions (5-15% O2 and 10 % CO2) using Campy pack and anaerobic jar **(Mégraud and Lehours, 2007)**.

Suspension was made from the bacterial colonies in sterile normal saline and carefully sonicated using short jets while kept on ice. The bacterial cell lysate was adjusted to a concentration of 200 µg protein/ ml saline employing Bradford method. For the first shot, equal volumes of the bacterial lysate and complete Freund's adjuvant were mixed until emulsification while incomplete Freund's adjuvant was used for the booster shots.

Hens of one group were immunized subcutaneously with 0.5 ml immunogen / bird while the other group was left as a non-immunized control.The booster injections were given at 15, 30 and 45 days after the first immunization **(Carlander *et al.*, 1999).** Blood samples were collected through the wing vein puncture to separate sera and eggs were collected, labeled and refrigerated until needed for IGY extraction.

Serum monitoring:

Serum samples of immunized and control hens were screened for anti-*H. Pylori* immunoglobulins employing microplate agglutination test using bacterial suspension as an agglutinogen **(Mony *et al.*, 2019)**.

Ammonium sulfate extraction of IGY from egg yolks **(Ren *et al.*, 2016).**The collected egg yolks were diluted 1:5 with distilled water stirred for 2 hours and kept at -20 ºC for at least

three days. The frozen egg yolk was thawed and centrifuged at 2800 xg for 20 minutes and the supernatant was aspirated. For each millilitre of the supernatant 0.27 g of ammonium sulfate was added followed by stirring for two hours and centrifugation at 10,000 xg for 20 minutes. Ammonium sulfate (2M) was added to the sediment (24 ml/1 egg yolk) and the mixture was stirred for 40 minutes followed by centrifugation at 10,000 xg for 15 minutes. The supernatant was discarded and the precipitate was dissolved in PBS (2.5 ml per egg yolk) and dialyzed against PBS at 4ºC for 48 hours. The solution is then dialyzed thoroughly (Three buffer changes, at least 150 times volume) against PBS in a dialyzing tube with molecular cut off weight (MCOW) of 14 KDa After dialysis the protein concentration was measured using the Biuret method and the extracted IgY was kept at -20ºC until needed.

Chromatographic purification of anti-*H. Pylori* polyclonal IGY:

The anti-*H. Pylori* IGY was secured from the whole IGY extract using the polystyrene affinity chromatography. Polystyrene PS 158K granulated raw material (10 g) was mixed with 50 ml of carbonate buffer (pH 9.6) containing 175 mg of the bacterial cell lysate. The suspension was shaken for 1 hour at room temperature and left overnight at 4ºC. The polystyrene beads were washed using distIlled water. After blocking with bovine serum albumin, the beads were packed into a 10 ml syringe barrel and the IGY extract was allowed to slowly pass through. The unbound IGY molecules were washed away using PBS with tween 20. The anti-*H. Pylori* IGY polyclonal molecules were eluted and received on neutralizing buffer, quantitated and frozen for further investigation **(Staak, 1996).**

Evaluation of the anti-*H. Pylori* IGY polyclonal immunoglobulins:

Indirect ELISA (Kazimierczuk *et al.*, 2005).

An indirect ELISA was adapted and used to test the purified anti-*H. Pylori* polyclonal antibodies for titre. The bacterial cell suspension was the coating antigen where each well received 25 ng/well. Microtitre plates were coated with 100 µl antigen, diluted to the desired concentration in NaHCO3 (0.1 M and pH 9.5).The plate was incubated for an overnight in 4°C followed by washing three times with PBS containing 0.01% Tween 20, pH 7.2 (PBS-T). The plate wells were blocked with 150 µl of 1 % bovine serum albumin in carbonate buffer (pH 9.5) for 30 minutes at 37°C on an orbital shaker. After 3 PBS-T washes, 100 µL of different 2 fold dilutions of the IGY preparation in PBS was delivered into the assigned wells. The plate was incubated for one hour at room temperature on an orbital shaker and washed

three times with PBS-T. Rabbit anti-chicken IGG HRP-conjugate (Chongqing Biospes Co. Ltd, China) was diluted to 10^{-4} in PBS as directed by the manufacturer and 100 µl was delivered into each well of the test plate. After 30 minutes incubation at 37 ºC, 100 µl of diluted (DAB) chromogenic substrate were delivered to each well and the colour was let to develop for 15 minutes in a dark place. The reaction was stopped by adding 50 µl of distilled water and the OD values were recorded at a 450 nm wavelength employing an ELISA reader.

Microplate agglutination:

H. pylori cells were inactivated by formalin 0.1% followed by 2 days incubation at room temperature. The cells were pelleted down at 2700 xg for 20 minutes and the pellet was re-suspended in PBS, treated with 0.1% formalin and refrigerated to be used as an antigen.

To evaluate the anti-*H. Pylori* IGY agglutinability, the inactivated bacterial cells were diluted in the optimal dilution in PBS. A 100 µl volume of the suspension was delivered into each well of a 96-well U-shaped microtitre plate and incubated at room temperature for 2 hours. Then, 100 µl aliquots of IGY preparation of different 10 fold dilution were added to each followed by incubation for 2 hours at room temperature. The aggregation was observed which was represented by sheath formation with no pellet in the well bottom **(Mony *et al.*, 2019).**

Ethical considerations:

This study was approved by the Research Ethics Committee (Process number Vet CU12/10/2021/374).

RESULTS

Purified anti-*H. Pylori* immunoglobulins from egg yolk:

Ammonium sulphate precipitation and polystyrene affinity chromatography resulted in purified immunoglobulins from the immunized chicken egg yolks in a protein concentration of 1.9 mg/ml from eggs collected during the second week after the second booster.

The same period showed the highest agglutinability as indicated by monitoring of the sera collected from immunized hens Fig. (1).

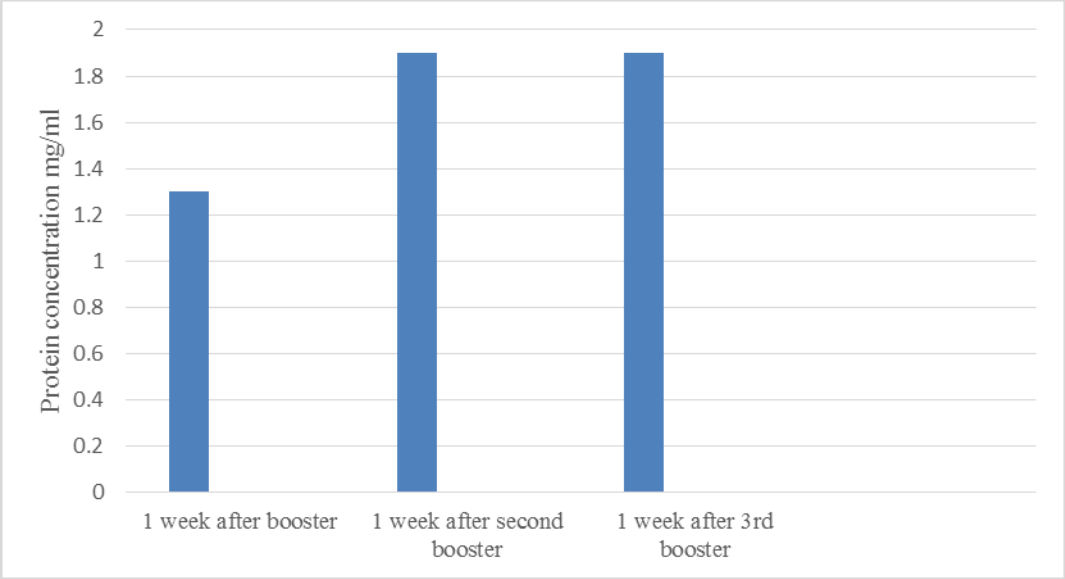

Fig. (1): Protein concentration of purified anti-*H. Pylori* IGY from hen eggs collected at different post immunization intervals

Indirect ELISA:

The cut off value of the ODs recorded at 450 nm was calculated by subtracting 2SD from the mean of ODs of the control negative. OD values more than the cut of value indicated positive results. Anti-*H. Pylori* IGY showed extensive reactivity as it resulted in positive ELISA results till the dilution 1/1280 of the IGY preparation.

Microplate agglutination test:

The results obtained with 96-u shape microtitre plate agglutination revealed that the purified anti-*H. Pylori* IGY was able to agglutinate the bacterial suspension diluted down to 1/10240 in PBS.

DISCUSSION

Chicken egg yolk IGY is a good alternative for the mammalian IGG for immunological diagnostic, prophylactic and therapeutic purposes. IGY is also more stable than mammalian IGG at different pH and temperature and more tolerant to proteolytic enzymes. Despite of being both isotypes similar in their biological functions, IGY has advantages of acid and alkali resistance, high temperature resistance, high output, easy collection, good stability and no induction of the human body's autoimmune disease, characteristics not existing in IGG **(Wang *et al.*, 2014).**

In addition, more advantages of chicken antibodies over mammalian antibodies include

reduction in animal use because since chickens produce larger amounts, the elimination of painful blood collection in animals, the utility of IGY in various assays without loss of specificity and sensitivity, and the lower cost of rearing chickens **(Dias Da Silva and Tambourgi, 2010)**. This means that, the IGY-technology as an alternative method can particularly integrate the three R requirements of animal protection (Reduce, Replace, Refine), science (Characteristics of avian immune system and resulting properties of IGY) and economy (Amount of IGY produced from one chicken) **(Schade and Hlinak, 1996).**

IGY technology has been applied extensively to produce anti-mammals polyclonal antibodies such as anti-human IGY antibodies from egg yolk to obtain monospecific Coombs reagent **(Gutierrez Calzado *et al.*, 2003)**. Anti-mouse IGY polyclonal antibodies was also produced and conjugated with fluorescein isothiocyanate (FITC).In egg yolk, specific antibodies appear 2 weeks after the first immunization, reached a plateau after week 11 and remained high until week 20 **(Kritratanasak *et al.*, 2004)**.

Diagnostically, IGY antibody has been widely used in the detection of viruses, cancers, parasites and chemical contaminants. IGY has therapeutic applications as well. IGY is usually used to treat rotavirus and alimentary tract diseases. The use of egg yolk antibodies (IGY) instead of IGG from mammalian species may present several advantages in the development of routine diagnostic immunoassays. On the one hand, the animal suffering is reduced, as antibodies are obtained directly from the egg. Results were compared to those obtained by IGG. Interesting differences were observed (Which mainly arise from the differences in molecular structure between IGY and IGG), suggesting that IGY is a more hydrophobic molecule than IGG. In addition, colloidal dispersions of IGY-covered latex particles are more stable (At pH 8) than those sensitized by IGG **(Davalos-Pantoja *et al.*, 2000)**.

In a review article, it was reported that IGY had been used for routine diagnostic work covering the following subjects: 1.Identification of the host species from abdominal blood of haematophageous insects; 2.IGY-anti-horse-Ig-PO conjugate for ELISA on dourine; 3.FITC-conjugated IGY-antirabies for diagnostic work on rabies; 4.FITC-conjugated IGY against avian virus diseases (Newcastle, Infectious Bronchitis, and Gumboro). In all cases satisfactory results were achieved **(Staak, 1996).**

In this study, laying hens responded properly and produced antibodies against *H. pylori* whole cell lysate as indicated from the results of serum monitoring using adapted microplate agglutination **(Mony *et al.*, 2019).**

The bacterial whole cell sonicate prepared from a local isolate was used as the immunogen. A previous extensive study compared crude sonicates with ultracentrifuged whole cell sonicates and acid-glycine extracts and antigen fractions separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis.In such study,the greatest discrimination between *H. pylori* positive and negative sera was found with high molecular mass fraction antigens, but whole cell sonicates were better than acid-glycine extracted antigens **(Newell *et al.*, 1988; Xiang *et al.*, 1993)**.

Hens immunized in the current study produced good titres of anti-*H. Pylori* IGY from the first post immunization and up to the end of the experiment. Local post immunization reaction was mild to moderate and the general conditions of the immunized hens were good. However, the number of eggs laid by immunized hens decreased to about 80 %, which can be attributed to the stress of immunization on the hens especially with being the bacterial antigen more foreigner. In addition, Fruend's complete adjuvant may exert an additional stress on the hens.

Eggs were collected and kept refrigerated starting one week after immunization in week-based batches. The collection was synchronized with the results of serum monitoring as it is well known serum immunoglobulins are readily transferred from hen serum to the yolk while the egg is still in the ovary and IGY is found at levels equal to those in hen serum **(Tizard, 2000)**. Ammonium sulfate precipitation was found efficient in extraction of egg yolk IGY **(Schwarzkopf and Thiele, 1996).** Using an affinity chromatographic method adapted by **Staak (1996)**, the specific anti-*H. Pylori* IGY immunoglobulins were separated through column containing bacterial lysate-coated polystyrene particles.Egg yolk IGY antibodies were easily eluted and the protein concentration was considerably high. This might be attributed to the unique nature of IGY which facilitates its reaction and elution. The good yield of IGY antibodies from egg yolk confirm the advantages of chickens over rabbits **(Davalos-Pantoja *et al.*, 2000; Gutierrez Calzado *et al.*, 2003)**.

In 1997, detection of *H. pylori* antigens in stools was carried out using polyclonal anti-*H. pylori* antibodies and showed sensitivity and specificity of 88.8 % and 94.5%, respectively **(M´egraud and Lehours, 2007).**

ELISAs have been used to study the IGY activity in most studies targeting the production of specific IGYs. In this study, an indirect ELISA was adapted to titrate the anti-*H. Pylori* IGY level in the egg yolk extracts. The titre was found very high as the IGY preparation showed

positive results until the dilution 1/1280. Moreover, microplate agglutination was also employed for the same purpose. Although a secondary immunological test, the microplate agglutination showed higher titre of the anti-*H. Pylori* IGY preparation which could agglutinate the bacterial cell suspension with a very high dilution (1/10240). In a similar study, an indirect enzyme immunoassay for rapid detection of *Campylobacter jejuni* subsp. *jejuni* was developed. Polyclonal chicken IGY and rabbit anti-IGY antibody-horseradish peroxidase conjugate were employed and polyclonal antibody was specific for homogeneous antigen **(Hochel *et al.*, 2005)**.

Also,**Mony *et al.*(2019)** evaluated anti-*H. Pylori* urease IGY using bot ELISA and microplate agglutination and both assays recorded high titre of the IGYantibodies. In another study, an in-house ELISA was developed, standardized, validated and evaluated to detect monoclonal and polyclonal IGG antibodies using *H. pylori* whole-cell antigen from strains isolated in Brazil. The sensitivity and specificity of polyclonal and monoclonal antibodies were (78.9% and 90.6%) against (84.3% and 88.6%), respectively **(OgataI *et al.*, 2018)**.

IGY serves as suitable and reliable alternatives to IGG in immunoassays. In an in-house ELISA to titrate anti-Staphylococcus aureus protein A IGY, antibody titers reached up to 1:64,000 dilutions after administration of three antigen doses by intramuscular route of laying hens **(Kota *et al.*, 2020).**

Conclusively, anti-*H. pylori* IGY was successfully produced in this study in pretty good titre and efficiency. The IGY produced in this study will be utilized to diagnose, treat or protect from *H. pylori* infection in human and animals.

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REFERENCES

Amaral, J. A., Tino De Franco, M., Carneiro-Sampaio, M. M. and Carbonare, S. B. (2002): Anti-enteropathogenic Escherichia coli immunoglobulin Y isolated from eggs laid by immunised Leghorn chickens, Res. Vet. Sci., 72 (3):229 - 34.

Carlander,D.(2002): AvianIGYAntibody In vitro and in vivo.ActaUniversitatis Upsaliensis,Uppsala, ISSN 0282-7476, ISBN 91-554-5227-2.Printed in Sweden by Uppsala University, Tryck and Medier, Uppsala.

Carlander,D.,Stalberg,J.and Larsson,A.(1999): Chicken antibodies: a clinical chemistry perspective. Ups. J. Med. Sci., 104 (3):179 - 89.

Davalos-Pantoja, L, Ortega-Vinuesa, J. L., Bastos-Gonzalez, D., Hidalgo-Alvarez, R. (2000): A comparative study between the adsorption of I GY and IGG on latex particles, J. Biomater. Sci. Polym. Ed. 11(6):657-73.

Dias Da Silva, W. and Tambourgi, D. V. (2010): IGY: A promising antibody for use in immunodiagnostic and in immunotherapy. Veterinary Immunology and Immunopathology, 173-180.

Gutierrez Calzado E., Cruz Mario, E., Samon Chavez, T., Vazquez Luna, E., Corona Ochoa, Z. and Schade, R. (2003): Extraction of a monospecific Coombs-reagent from chicken eggs, Altex, 20 (1):21-5.

Hirschl, A. M. and Makristathis, A. (2007): Methods to detect *Helicobacter pylori*: from culture to molecular biology. Helicobacter, 12, 6-11.

Hochel, I., Viochna, D., Skvor, J. and Musil, M. (2005): Development of an indirect competitive ELISA for detection of Campylobacter jejuni subsp.jejuni O: 23 in foods, Folia Microbiol. (Praha), 49(5):579-86.

Horie K., Horie, N., Abdou, A. M., Yang, J. O., Yun, S. S., Chun, H. N., Park, C. K., Kim M. and Hatta, H. (2004): Suppressive effect of functional drinking yogurt containing specific egg yolk immunoglobulin on *Helicobacter pylori* in humans. J Dairy Sci 87:4073 - 4079.

Kazimierczuk, K., Cova, L., Ndeboko, B., Szczyrk, U., Targosz, A., Brzozowski, T, and Sirko, A. (2005): Genetic immunization of ducks for production of antibodies specific to Helicobacter pylori UreB in egg yolks. Acta Biochimica Polonica, Vol. 52 No. 1: 261-266.

KlempererF.(1893): Über naturliche immunität und ihre verwertung für die immuniserungstherapie. Archiv für die experimentelle pathologie und pharmakologie. 1893; 31, 356-382.2.

Kota, R. K., Reddy, P. N. and Krupanidhi Sreerama, K. (2020): Application of IgY antibodies against staphylococcal protein A (SpA) of *Staphylococcus aureus* for detection and prophylactic functions. Applied Microbiology and Biotechnology, 104:9387-9398.

Kritratanasak,S.,Chiampanichayakul,S. and Kasinrerk,W.(2004): Production of IgY anti-mouse IGG antibodies from chicken eggs, Asian Pac. J.Allergy Immunol.,22 (1):61-8.

Lee, L., Samardzic, K.Wallach, M., Frumkin,L.R. and Mochly-Rosen,D. (2021): Immunoglobulin Y for potential diagnostic and therapeutic applications in infectious diseases. Front. Immunol. 09 June, 2021 doi: 10.3389.

M´egraud, F. and P.Lehours, P.(2007): Helicobacter pylori detection and antimicrobial susceptibility testing. Clinical Microbiology Reviews, .20 (2): 280-322.

Mégraud, F. and Lehours, P. (2007): Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev; 20: 280-322.

Mentis, M.,Lehours,P.and Megraud, F.(2015): H. pylori Epidemiology and Diagnosis. Helicobacter 20 (Suppl. 1): 1-7.

Mony, T. J., Kwon, H., Won, M., Kang, Y., Lee, S., Kim, S., Baek, D. and Elahi, F. (2019): Anti-urease immunoglobulin (I GY) from egg yolk prevents Helicobacter pylori infection in a mouse model. Food and Agricultural Immunology 2019, Vol. 30, No. 1, 662-676.

Newell, D. G., Johnston, B. J., Ali, M. H. *et al.* (1988): An enzyme-linked immunosorbent assay for the serodiagnosis of *Campylobacter pylori*-associated gastritis. Sc and J Gastroenterol (Suppl); 142:53-7.

Nomura, S., Suzuki, H., Masaoka, T., Kurabayashi, K., Ishii, H., Kitajima, M., Nomoto, K. and Hibi, T.(2005): Effect of dietary anti-urease immunoglobulin Y on Helicobacter pylori infection in Mongolian gerbils. Helicobacter 10:43 -52.

OgataI, S. K., Camorlinga-Ponce, M., Granato, C. F. H., Rohr, M. R. Da Silveira, Neto, R. A. and Kawakami,E.(2018): Development and validation of a whole-cell ELISA for serologically diagnosing *Helicobacter pylori* infection in Brazilian children and adults: a diagnostic accuracy study. Sao Paulo Med J.; 136 (5):442-8.

Pereira, E. P. V., van Tilburg, E. P. V., Florean, E. O. P. T. and Guedes, M. I. F. (2019): Egg yolk antibodies (IGY) and their applications in human and veterinary health: A review. International Immunopharmacology 73: 293-303.

Rabiea, E. A., Abdelmalek, S. and Hashad, M. E. (2021): Bacteriological and molecular detection of *H. pylori* in endoscopic biopsies from dyspeptic patients recruited to a governmental hospital at Cairo, Egypt. Journal of the Egyptian Veterinary Association, 81 (1).

Ren, H. Yang, W., Thirumalai, D., Zhang, X. and Schade, R. (2016): A Comparative Evaluation of Six Principal IGY Antibody Extraction Methods. ATLA 44, 11-20.

Schade, R. and Hlinak, A. (1996): Egg Yolk Antibodies, State of the Art and Future Prospects, ALTEX, 13 (5):5-9.

Shimamoto, C.,Tokioka, S., Hirata, I., Tani, H., Ohishi, H. and Katsu, K. (2002): Inhibition of Helicobacter pylori infection by orally administered yolk derived anti-*Helicobacter pylori* antibody. Hepatogastroenterology 49:709 -714.

Shin, J. H., Nam, S. W., Kim, J. T., Yoon, J. B., Bang, W. G. and Roe, I. H. (2003): Identification of immunodominant *Helicobacter pylori* proteins with reactivity to H. pylori-specific egg-yolk immunoglobulin. J Med Microbiol 52:217-222.

Staak, C. (1996): Egg Yolk Antibodies (IGY) in Routine Diagnostic Work, ALTEX, 13 (5):73-75.

Tizard, I. R.(2000): Veterinary Immunology: An Introduction, 6th Edition, W. B. Saunders Company, and Philadelphia, USA.

Wang, B., Yang, J., Cao, S., Wang, H., Pan, X., Zhu, J., Zhou, Y., GAO, L., Li, W. and Li, M. (2014): Preparation of specific anti-*Helicobacter pylori* yolk antibodies and their antibacterial effects. Int J Clin Exp Pathol; 7 (10): 6430-6437.

Xiang, Z., Bugnoli, M., Ponzetto, A. *et al*. (1993): Detection in an enzyme immunoassay of an immune response to a recombinant fragment of the 128 kilodalton protein (CagA) of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis 1993; 12: 739-45.