بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Prevalence of *Helicobacter pylori* among People with Gastritis in Elsayal Elkapeer Village - River Nile State

معدل انتشار البكتريا الحلزونية البوابية لدى الأشخاص المصابين بإلتهاب المعدة من سكان قرية السيال الكبير بولاية نهر النيل

A thesis Submitted in Partial Fulfillment of the Requirements of M.Sc. Degree in Medical Laboratory Science (Microbiology)

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December 2019

بسم الله الرحمن الرحيم

الآية

قال تعالى:

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّن الْعِلْمِ إِلاَّ قَلِيلاً)

صدق الله العظيم

سورة الإسراء الآية (85)

Dedication

To my parents and my Husband for their patience, encouragement and infinite support.

To my Brothers, sisters

And

To Every one from whom

learned

Acknowledgment

First all thank to Almig ty ALLAH to reconcile me to complete my study
I am indebted to**Dr Youssef Fadlallah Hamedelneel** for his great help in supervising and guiding my work**.**
I would like to thank all person helping me and understand my research questions.
My thanks also are extended to all staff of lab at Shendi Thank to my dear husband **Hassan Ali Abu zed** to encouragement me. thank to my family, to all those who helped me and not mentioned.
Thank to Shendi University of Medical Laboratory Sciences for providing me good environment for practical work..

Abstract

This is descriptive and cross sectional study aimed to determine the frequency of Anti *H .pylori* Ig G antibody using ELISA technique among people suffering from gastritis(symptom such as vomiting ,stomach pain, bloating, belching, o black stool).This study was carried out in Elsayal Elkabeer Village in River Nile State, from April to June 2016.

A total of one hundred and fifty tested by ICT. Take ninety serum sample positive by ICT retested by ELISA technique found 76 (84.4%) positive and 14(15.6%) negative classified to male32(35.5%) female44(48.9%).According to this study a majority of positive are female among poor participant and then used *H .pylori* and stool Ag from positive ELISA46 is positive by stool Ag and 30(33.3%) negative from negative ELISA 3(3.3%)is positive.

According to this study there was no significant correlation between *H. pylori* Sero positive I gG Economic status (p 0.6), Age group (p0.3) family history (p 0.1).But found correlation between *H. pylori* infection and gender (p 0.04).age group 10-30 is more infected than other age group 46(51.1%).

Finally the ICT ,ELISA ,and stool Ag is more sensitive ,specific and high accuracy predictive value technique to diagnose *H pylori* infection.

ملخـــص الدراسة

أجريت هذه الدراسة الوصفية المقطعية الآنية للكشف عن مدي انتشار البوابية الحلزونية في قرية السيال الكبير بولاية نهر النيل، وسط الأشخاص الذين يعانون من اعراض التهاب المعدة (الم في المعدة ،نفاخ، طمام واستفراغ ، تجشؤ ،او براز اسود) مستخدماً تقنية الانزيم المناعي المرتبط لنوع الاجسام المضادة (الإليزا IgG) والرحلان المناعي (ICT)ا وفحص الفسحة للجرثومة في الفترة من أبريل إلى يونيو لعام 2016م.

اختيرت 90من العينات الايجابية من اجمالي مائة وخمسين التي تم فحصها مسبقا بواسطة ICT الرحلان المناعي للفحص بواسطة الانزيم المناعي المرتبط اظهرت نتائج ايجابية76(84.4%)وسلبية 14(15.6%)عدد الرجال الايجابي 32(35.5%)والنساء 44(48.9%).واستخدم فحص الفسحة للكشف عن الانتيجين وجد ان (51.1%)46عينة ايجابية من ايجابي المفحوص بواسطة الانزيم المناعي المرتبط بينما (30)33.3% سلبي و3 من سلبي الانزيم المناعي ايجابي بواسطة فحص الفسحة للجرثومة وان الفئة العمرية10_30هم اكثر اصابة من غيرهم من الفئات العمرية الأخرى 46(51.1%).بناء علي هده الدراسة وجد ان النساء الفقيرات هن اكثر اصابة من غيرهن.

اثبتت الدراسة انه لاتوجد علاقة سببية بين الاصابة بجرثومة المعدة والحالة الاقتصاديةالقيمة (6.)العمر(3.)والتاريخ الاسري(1.).لكن توجد علاقة سببية بين الجنسين حيث ان النساء هن اكثر عرضة للاصابة بجرثومة المعدة.(p0.04.)

اثبتت هده الدراسة ان التشخيص للإصابة بواسطة الإنزيم المناعي المرتبط والرحلان المناعي وفحص الفسحة للانتيجين كلها حساسية ونوعية ومضبوطة.لكن فحص الفسحة للجرثومة أكثرها فعالية p(,007)

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List of abbreviation

Abbreviation	Complete Word
Ab	Antibody
Ag	Antibody
Cag A	Cytotoxic – Associated Gene A
ELISA	Enzyme Linked Immune Sorbent Assay
H.pylori	*Helicobacter pylori*
ICT	Immune Chromatography Test
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LPS	Lipo polysaccharide
PCR	Polymerase Chain Reaction
TMB	Test Methyl Benzaldehyed

Chapter one

Introduction

Chapter I

1. Introduction

1.1 Background:

Helicobacter *.pylori* previously named *Campylobacter .pylori*, is Gram negative, micro aero philic spiral rod. Shaped bacteria that lives just beneath the enteral gastric mucous layer, on the surface of epithelial cell.

It was identified in 1982 by Australian scientists Barry Marshall and Robin Warren with further research led by British scientist Stewart Good win, who found that it was present in patients with chronic gastritis and gastric ulcer. The Condition not previously believed to have a microbial cause (Gi ustic 2004).

Helicobacter *is* main causative agent of gastro intestinal disease including chronic gastritis, peptic ulcer, duodenal ulcer, duodenal carcinoma, stomach cancer that lead to morbidity and mortality rate (Black 2004, Baik *et al*., 2012).

H.pylori also associated with (MALT) mucosa associated lymphoid tissue over 80% of individuals infected with the bacterium asymptomatic (Covacci *et al* 1999).

Approximately, 50% of the world population is believed to be infected with *H.pylori* most infection are probably acquired in childhood (Czesnik jewicz – Guzik *et al*., 2004). It may be play a major roll in the natural stomach ecology (Blaser 2006, Graham *et al*., 1991).

More than 50% of the world population harbor *H.pylori* in their upper gastro intestinal tract infection in high prevalence in developing countries (Bail *et*

al., 2012). Accoding to Yamoka(. 2008)80 of infecctioni in adult Is male 22D.

However, the encidance in support of *H.pylori* infection as a cause of the non-gastro intestinal tract disease is not widely understood (Betting and Alexander., 1999).

H.pylori can also affect organ system outside of the gastro intestinal tract it is now apparent that can infect skin, liver and heart and these infections may produce different disease states (Utas, *et al*., 1999).

On the other hand in developing county up to 10yers is more infected with *H.* the infection involve up to 92% of all children elder than 10 years. (Graham *et al*, 1991).

Person to person transmission of *H.pylori* is likely because interfamilial clustering occur (Brown 2000).

Infection of *H.pylori* was diagnosed by invasive technique used biopsy based test (Uerase test, direct stain, culture) also western immune blotting, polymerase chain reaction and serological test are non-invasive technique (Rocha *et al*., 2003).

Specific immunoglobulin (1g M) antibody can be detected shortly after infection occurred, but I g A, I g G antibody titer used to determine prevalence of acute and chronic infection (Crabtree *et al*., 2003).

Measurement of specific antibody is serum or antigen in or antigen in stool by using immuno chromatography have been used as non – invasive method (Crabtree *et al*., 2003).

The Enzyme Linked Immuno Sorbent Assay (ELISA) test is rapid non expensive and high sensitive and specific and widely use in epidemiological studies (Rocha *et al*., 2003).

1.2 Rationale

H .pylori is human pathogens that cause several illness and it is the commonest bacterial infection world wide.

The annul incidence rate of it is 4 – 15% in developing countries (including Sudan) companied with 0.5% in industrial countries (according to Center for Disease Control and prevention in 1998).

H.pylori antibodies occur in about 70% of patient with chronic active gastritis and are associated with ulcer condition in 60% - 90% of all cases.

H.pylori one of important cause of gastric cancer. So proper and rapid laboratory assay are needed to diagnose infection in order to treatment and management (Rocha *et al*., 2003)

The large majority of infection is asymptomatic and the late consequences are MALT lymphomas and adenocarcinoma.

A complete and permanent eradication of the bacteria in diagnosed *H pylori* infection in children ,adolescent ,and adult lead to reduction of relapsing rate of 80% in case of peptic ulcers and20% of duodenal ulcer. *H pylori* positive Sudanese patient with dyspepsia comparable group matched of age ,sex and endoscopy with United kingdom found 17%of British patient have intestinal metaplasia compared to only 2.4of Sudanese patient(Nuha and Fedial2000,).

Several technique including culturing and molecular method are present to identify organism the pathogen is difficult to grow serological tests are less expensive and less stressful for the patient especially for children. So sero diagnostic method have been attempted to detect infection (Wood *et al*. expen2003).

1.3 Objectives

1.3.1 General objective:

To detect Sero prevalence of *H .pylori* I g G antibody among people with gastritis in Elssaya l ELkapeer village in River Nile State

1.3.2 Specific objectives:

1. To detect prevalence of Ig G antibodies of *H .pylori* in people with gastritis by using Enzyme Linkage Immuno Sorbent A ssay (ELISA) ICT and stool Ag
2. To determine the correlation between sero positive IgG and serum immune chromatography .and tool Ag.
3. To determine relationship between positive I g G *Pylori* and gender, family history- , age group and socioeconomic status in the development of disease,

Chapter two

Literature Review

CHAPTER II

2.1 Back ground:

Helicobacter pylori was first discovered in stomachs of patient with gastric and stomach ulcer in 1982 by Barry Marshall and Robin Warren in Perth western s.

That condition not believed to home microbial cause (Giustic, 2004).

Helicon bacter Pylori previously named campyloloced pylori is gram negation spiral rod in shape, micro Europhilic bacterium found in the stomach on the surface of epithelial cell (Gustic 2004).

Consensus development conference cone lured that *H.pylori* infection is major cause of peptic ulcer and gastro intestinal disease and it also developed of duodenal ulcer lead to duodenal carcinoma and stomach can ser lead to morbidity and mortality in human (Black 2004; Baik *et al*., 2012; Yamada *et al*., 2006).

More than 50% of world population her boor *H.pylori*.

H -pylori normally infect your stomach during child hood. While infections with this strain of bacteria typically don't cause symptoms, these bacteria can change the environment around them and reduce its acidity so they can survive. The shaped it help them to penetrate your stomach lining where they are protected by mucous and your body's immune cell notable to reach them (Helen college and Ja cquelyn cafasso2005).

2.2 Scientific classification:

Domain = Bacteria.

Phylum = Protco bacteria.

Class = Epsilon proleo bacteria.

Order = campylo bacterales.

Family= Helico bacteria care.

Genus = Helio bacter.

Species = H-Pylori.

(Goodwin *et al*., 1989).

Table I: - characteristic of selected helico bacter SPP, (Kusters *et al,* 2006):-

Species	Primary mammalian host	Pathology	Animal mode
H.Pylori	Human, primate	Gastritis, peptic ulcer, gastric disease, adenocarcinoma not MALT lymphoma.	Mouse guinea pig, biotic piglet.
H.felis	Cot dog-mouse	Gastritis in natural host maycause peptic ulcer or gastric adeno carcinoma in mouse	Mouse
H.mustelae	Ferret	Gastritis, peptic ulcer disease, gastric adeno carcinoma MALT lymphoma	Mouse
H.acinonychis	Cheetah, tiger, other pig cats	Castritis – peptic ulcer disease	Mouse
H.heulmanril	Human, doge, cat monkey. Cheath rat	Gastritis, dyspeptic symptoms, MALT lymphoma	Mouse
Enterohepatic helicopater SPP			
H.hepaticey	More, other rodent	Pholifrative typhplocoletis, hepatitis hepato cellular carcinoma	

2-3 Bacteriology:

Pylori is oral x- shaped (classified as curved rod not spirochetes). Gram negative bacterium about 3 µm long with diameter of about 0.5, micrometer. It is micro aerophilic that require oxygen, but at low concentration than is found in the atmosphere.

It contains hydrogenase which CAMB used to obtain energy by oxidizing molecular (H_2) producing by intestinal bacteria. (olson and maier 2002).

It is capable to form biofilms and can convert from spiral to a possibly viable but not culturable coccoid form, both likely to favor its survival and be factor sin epidemiology of the bacterium (HYonezawa *et al*., 2007).

H.pylori possesses five major outer membrane protein families. The largest families include known and putative adhesions.

The other four families are porins, iron transport, flagellum associated protein and proteins of unknown function. Like other typical gram negative bacteria, the outer membrane of *H.pylori* consist of phospholipid and lipopoly sacchayide (LPS). The oantigen of LPS may be fucosylated (kuster *et al*) and mimic lewis blood group antigen found on the gastric epithelium (Chang and parsonnet 2010). The outer membrane also contain cholesterol glycosides. Which are found in few other bacteria. *H pylori* has 4 – 6 lophotrichous flagella; all gastric and entero hepatic *helico bacter* species are highly motile (Josen home *et al*., 2000). The characteristic sheathed flagellar filaments of *Helico bacteria* are composed of two copolymerized flagellin. Fla A and fla B (Surendra G *et al*., 2016).

2.4 Microscopy:

H.pylori can be demonstrated in tissue by gram stain, Giemsa stain, haematoxylin- eosin stain, warthin – starry silver stain, acridine orange stain, and phase contrast microscopy (Brown 2002).

2.5 H. Pylori genome:

Was organallys classified in the genus campylobacter but is now known to be different from campylobacter in 16 SRNA sequence the genetic diversity of H. pylori can be exploited by using molecular typing based on DNA analysis. PCR technique which are able to isolate and replicate Helicobacter DNA in biological material and then detect the genetic variation between different strains of Helicobacter (Arora, 2006).

Study of *H. pylori* genome is contended on attempts to understand pathogenisity, ability of it to cause disease. Approximately 29% of loci in the pathogenic category of the genome data loose. Both sequence strain have an approximately 40 kb – long cog A pathogmisity rsland (medisand marshall, 2008).

2.6 Host range:

Man is principle host of *H. pylori* occasionally strain identified as *H.pylori* have been isolated from domestic cats and other animals including pig and monkey (Owen *et al*., 2001).

2.7 Signs and symptoms:

Up to 85% of people infected with *H.pylori* never experience symptoms or complications (Brown, 2002). Acute infection may appear as an acute gastritis with abdominal pain or nausea (Bytzer *et al*., 2001). Where this develops into chronic gastritis, the symptoms, if present those of non-ulcer dyspepsia.

Stomach pain, nausea, bloating, belching, and some time vomiting or black stool (Butcher and Graham, 2003).

Individual infected with *H.pylori* have 10 – 20% lifetime risk of developing peptic ulcer and 1 to 2% risk of acquiring stomach cancer (Chang and parsonnet, 2010).

Inflammation of the corpus (body of the stomach) is more likely to lead to gastric ulcer and gastric carcinoma (kusters *et al*., 2006).

However *H.pylori* possibly play a role only in the first stage that lead to common chronic inflammation, but not in further stage leading to carcinoma genesis (Yamoka, 2008).

H.pylori have also been associated with colorectal polyps and colorectal cancer (Suerbaum and michetti, 2002)

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2.8 Rout of transmission:

H.pylori is commonly transmuted person to person by saliva or fecal oral route is most likely. Consistent with these transmission routes the bacteria have been isolated from feces, saliva and dental plaque of some infected people.

Findings suggest *H.pylori* is more easily transmitted by gastric mucous and saliva (Yamoka, 2008). Transmission occurs manly within family in developing orations, yet can also be acquired from the community in developing countries.

Hygienic environment could increase risk of *H.pylori* infection (Yamoka, 2008)

Latrogenic spread through contaminated endoscopes has been documented as route of transmission (*Dunn et al*., 2001).

2.9 Pathophysiology:

To avoid the acidic environment of the interior of the stomach (lumen), *H.pylori* uses its flagella to burrow in to the mucus lining of the stomach to reach the epithelial cell underneath, where the Ph is more neutral (Amiera and ELomer, 2008).

H.pylori is found in the mucous on inner surface of the epithelium, and occasionally inside the epithelial cells themselves. It adheres to the epithelial cell to producing adhesions, which bind to lipid and carbohydrate in the epithelial cell membrane (Madndan *et al*., 2002)

2:10 Pathogenicity:

2:10:1 Peptic ulcer:

The relationship between *H.pylori* and peptic ulcer disease has been more difficult to establish.

Ulceration develop in only few people harboring the organism. The reason of these unknown but possibilities include differences in host defenses, strain of *H.pylori* and environmental factors. (Torres *et al*., 2002). .

Several factors may operate such as production of ammonia lead to toxic of mucosa, or other substances as lipo polysuccaride that activate inflammatory cell, stimulation of auto immune response by the production of antigen that cross react with internal gastric Ag and degeneration mucous by protease (Arora, 2006, Wood *et al*., 2003).

2.10.2 Gastritis:

H.pylori has factors that help it to survival in side epithelial cell of stomach like urease and protease production. Urease leads to toxic by break down urea to carbondioxide and ammonia protease vacillating cytotoxic A (vacA). Also certain phospho lipases damage the epithelial cell (Ottemann and Lowenthal, 2002).

Gastritis have two types acute gastritis and chronic.Accute phase of colonization associated with non-specific dyspeptic symptoms such as fullness, nausea and vomiting. This phase associated with hypochlrohydria which can last for month (Prez- prez *et al*., 2003.

The chronic phaseClose correlation exists between the level of acid secretion and distribution of gastritis. Result from the concentration affect of acid on bacterial growth (Kuipers *et al*., 1995).

Subject when acid impaired due to whatever mechanism have more even distribution of bacteria in antrum and corpus, bacteria in corpus are in closer contact with mucous lead to carpus predominant gastritis disease (Kuipers *et al*., 1995).

2.10.3 Skin Diseases:

Pathogenic strains of H.pylori have been shown to activate the epidermal growth factor receptor (EGFR), a membrane protein with tyrosine kinase domain. Activation of (EGFR) by *H.pylori* is associated with altered signal transduction and gena expression in host epithelial cells that may contribute to pathogenesis.

It has also been suggested that ac. Terminal region of the cag A Protein (aminoacid 873 – 1002) can regulate host cell gene transcription independent of protein tyrosine posphorylation (Vaira *et al*., 1999).

2.10.4 Cancer:

Two related mechanisms by which *H. pylori* could promote cancer are under investigation one mechanism involves the enhanced production of free radical near *H. pylori* and increased rate of host cell mutation. The other proposed mechanism called " perigenetic and involves enhancement of the transformed host cell phenol type by means of alteration in cell proteins such as locally high levels of TNF α and / or interleukin 6 (IL – 6). (Guo *et al*., 2011).

2.10.5: Vascular disease:

Several epidemiological studies have been carried out on the association between ischemic heart disease (IHD) and *H. pylori* infection (Arthur *et al*., 1999).

2.10.6 Immunological Disease:

Several clinical observation suggest a role fir *H. pylori* infection in various immunological disorder, some report have shown healing of some autoimmune disease such as (Henoch – schonlein purpura, siogren syndrome, and autoimmune thrombocytopenia) after eradication of *H. pylori*. Furthermore the observation of complete disappearances of some cases of extra gastric mucose associated with lymphoid tissue (MALT) lymphoma (Owens and smith 2011).

2.10.7: Liver and Biliary tract:

A higher prevalence of *H. pylori* infection has been described in patient with liver cirrhosis than in age and sex matched controls (Anderson and Esperson, 1992).

2.10.8 Other extra-gastro duodenal disease:

H. pylori infection is reported to be more highly frequent in patients with sideropenic anemia compared with health controls (Hgri- Ashtiani and monajemzadeh, 2008).

2.11 Immunity:

Patient infected with *H. pylori* develops IgM antibody response to the infection. Subsequently IgG, IgA are produced and persist. Both systemically and at the mucosa in high titer infected persons (Wood *et al*., 2003).

The most common result from the immunity is chronic superficial gastritis also the host could be harmed by the immune response due to direct damage of epithelial cells, which affect their function (Wood *et al*., 2003).

2.12 Other body site *H. pylori* may found:

Stomach was supposed to be the only reservoir of infection in human. Nevertheless *H. pylori* infection was detected in other site recently it was found in dental plaque and oropharyngeal lymphatic tissue. The question of direct contribution of *H. pylori* to oral and oropharyngeal disease was not resolve (Braden *et al*., 2012).

2.13 Epidemiology.

At least half the world's population is infected by the bacterium, making it the most widespread infection in the world. Actual infection rates vary from nation to nation; the developing world has much higher infection rates than the West (Western Europe, North America, Australasia), where rates are estimated to be around 25%. The age at which this bacterium is acquired seems to influence the possible pathologic outcome of the infection: people infected with it at an early age are likely to develop more intense inflammation that may be followed by atrophic gastritis with a higher subsequent risk of gastric ulcer, gastric cancer, or both. Acquisition at an older age brings different gastric changes more likely to lead to duodenal ulcer (Yamoka, 2008). Infections are usually acquired in early childhood in all countries (Chang and Paresonnet, 2010). However, the infection rate of children in developing nations is higher than in industrialized nations, probably due to poor sanitary conditions, perhaps combined with lower antibiotics usage for unrelated pathologies. In developed nations, it is currently uncommon to find infected children, but the percentage of infected people increases with age, with about 50% infected for those over the age of 60 compared with around 10% between 18 and 30 years (Smoot, 1997). The higher prevalence among the elderly reflects higher infection rates in the past

when the individuals were children rather than more recent infection at a later age of the individual (Chang and Parsonnet, 2010).

In the United States, prevalence appears to be higher in African – American and Hispanic populations, most likely due to socioeconomic factors. The lower rate of infection in the West is largely attributed to higher hygiene standards and widespread use of antibiotics. Despite high rates of infection in certain areas of the world, the overall frequency of *H. pylori* infection is declining. However, antibiotic resistance is appearing in *H. pylori*; many metronidazole- and clarithromycin- resistant strains are found in most parts of the world (Smoot, 1997).

2.14 Previous studies of *H. pylori* in Sudan.

The study compared *H. pylori positive* Sudanese patients with dyspepsia comparable group matched of age, sex and endoscopy finding from United Kingdom. It was found that 17% of British patients have intestinal metaplasia compared to only 2.4% of Sudanese patients (Nuha and Fedail, 2000).

Clutter and Harvested in their study done in 192 patients evaluate the accuracy of ICT kit compared with ELISA. They found that sensitivity of ICT, specifity and accuracy was 99%, 86.6%, and 93.3% respectively (Culter and Havestead, 2000).

Another study conduct by study on the office of triple therapy in eradication of *H. pylori* in Sudanese patients with peptic ulcer. In these study 33 patients with endosmotically confirmed peptic ulcer and how had positive rapid urease test were include, amoxicillin, metronidazole and ranitidine were used in treatment. 75% of patient's bad complete healing ulcer, 15% patients had reported healing ulcer and 5% had no healing ulcer (Ahlam and Fedail, 2001).

Mirghani et al studied the prevalence of *H. pylori* in 100 Sudanese subjects with gastroduodinal inflammation. In this study two methods were used to detect *H.pylori* (rapid urease test and culture).

H. pylori were found in 80% of patients with gastritis and 60% of duodenitis, 56% with duodenal ulcer and 16% in normal individual (Mirghani et al., 2002).

Francis and his worker in their study culture and susceptibility testing of *H.pylori* strain was performed in multicenter randomized clinical trial. Culture was carried out in gastric biopsy samples obtained from 516 patients. When urea breath test was used as reference (Mergrand *et al*., 2003).

2.15: Diagnosis:

Multiple invasive and non-invasive methods are available for detection of *H.pylori* Diagnosis of infection is usually made by checking for dyspeptic symptoms and there doing test which can suggest *H. pylori* infection. With is usually grossed in are of these ways (Kusters *et al* 2006 " table 1 – 2 ").

2.15.1: Invasive tests through endoscopy:

2.15.1.1 Biopsy and histo pathology:

The definitive diagnosis of *H. pylori* and the evidence of the consequence of infection can be made reliably only is endoscopy with multiple biopsy specimen obtained from one or more region of the stomach (Dohil *et al*., 1999).

Stomach biopsy taken from the lining of the stomach and duodenum several different test may be done on biopsy, it may be treated with chemical to seen of enzyme that break down urea is present. The presence of enzyme indicates *H.pylori* infection. The biopsy sample may be stained by special stain e.g. warthin- starry silver stain and examined under microscope to show *H. pylori* Bacteria.

In this case the sample will be placed in container that promotes the growth of *H. pylori* bacteria and then do sensitivity test. Stomach biopsy is the most difficult and expensive method to test *H. pylori* infection (Vaira *et al*., 1999).

Biopsy specimen may be cultured on Colombia blood agar contain 7% difibrnated blood with 5% O2 and 10% CO. The organism can be identified as *H. pylori* based on colonial morphology gram stain and production of urease, oxidase. Catalase.

Culture of organism is least sensitive diagnostic test approximate 70 – 80% positive 100% specific (Perez – Prez *et al*., 2001).

2.15.1.2: Rapid urease test:

Measuring urease production can be accomplished with several expensive kits that use a PH sensitive colorimetric indicator e.g. phenol phthaline, mixed in to a gargle containing urea (Elomar *et al*., 2011). With *H. pylori* infection is tested bacterial urease hydrolyze urea, resulting in production of bicarbonate and increasing the PH. Result indicate by the color of medium within 20 minute and always within 24 minute (Elomar *et al*., 2011).

2.15.2: Noninvasive test: Both European consensus and American Gastroenterological Association position statement recommended the use of non-invasive tests for diagnosis of *H. pylori* (Walsh and Peterson, 2005).

2.15.2.1 Immune Chromatography Test (ICT):

The KT fir anti *H. pylori* antibody detection is based on the principle of reverse, flow immunochromatography. The kits included high molecular masscult associated with protein (Hm. CAP) on Antigen highly specific to *H. pylori* as the target antigen to detect *H. pylori* specific I g G antibodies in the serum

(Manfred *et al*., 2001).

2.15.2.2: Immune blotting technique:

Serological test that enable the detection of antibodies against specific *H. pylori* antigen (Abusi yanik *et al*., 2004).

In this method electrophoreticaly separated component are transferred from cell to solid support and probed with reagent that is specific for particular sequences of amino acid.

Western blotting is there for extremely use of will for identification and quantification of specific protein in complex mixture of protein that not ratio labeled. Because electrophoretic separation of protein is almost carried under denaturizing condition, any problem in solublization, aggregation and precipitation of the target protein are eliminated (Abu si yanik. *et al*., 2004).

2.15.2.3: Saliva and urine test:

Similar to serological test saliva based test also detect the presence of *H. pylori* specific I g G antibody. The test is easy to perform, painless, but expensive.

Saliva test is sensitive to serum or whole blood (Braden *et al*., 2012).

2.15.2.4: Stool Antigen test:

Stool antigen test is an accurate and precise method this accuracy is influenced by several limiting factors: upper gastro intestinal bleeding, antibiotic consumption bowel movement and also proton pump inhibitor uptake, non-invasive (T .Shimoyama *et al.*, 2013)

Also use for monitoring of the success of eradication therapy. Stool refrigerated to test in more difficult (Oderda *et al*., 2000).

2.15.2.5 Polymerase Chain Reaction (PCR):

The polymerase chain reaction (PCR) is able to isolate and replicate *H. pylori* DNA in biological material. PCR technique has been used to detect genetic variation between different strains of *H. pylori* (Wood *et al*., 2003).

2.15.2.6 Enzyme Linked Immune Assay (ELISA).

It is most extensively used in serological detection of *H. pylori* and had been show to produce constant and reliable results (Abusiyanik *et al*., 2004).

It is based on purified *H. pylori* antigen coated on the service of micro wells. Diluted patients serum is added to the well and *H. pylori* immunoglobulin specific antibody, if present, bind to the antibody – antigen complex. Excess enzyme conjugate is washed off and Tetra Methyl Benzaldehyde (TMB) substrate is added. The enzyme conjugate catalytic reaction is stopped at specific time. The intensity of the color generating is proportional to the amount of antigen specific antibody in the sample.

The result is read by micro well reader compared in parallel manner with calibrator and control (Graham, 2004).

2.15.2.7 Breath test.

The breath test utilizes the ability of *H. pylori* to produce large quantities of urease as diagnostic characteristic the patient is required to drink solution of urea labeled with C^{13} or C^{14} isotopes (Tsuji *et al*., 2003).

If an *H. pylori* infection is present the urea will be metabolized by the bacteria producing ammonia and labeled carbon dioxide, which can be detected in the patients, exhaled breath by radioactive counting or mass spectrometry.

Breath test based on C^{13} labeled urea and using infrared spectrophotometer to measure the ratio of C^{13} or C^{12} in breath sample.

Breath test has been found to have high sensitivity and specificity 94 – 98% and can be applied at moderate cost. It is suitable for monitoring the effectiveness of treatment. As it is specific to active infections and can be used to confirm eradication (Tsuji *et al*., 2003).

Table (2-2): Diagnosis of H pylori infection

Diagnostic method	Sensitivity and specifity	Typical application	Remark
Invasive method			
Histology	>95%	"Gold stander" in routine hospital diagnosis	Requires expert pathologist; alsopoides histological data on inflammation and atrophy
Biopsy culture	>95%	Alternative gold standard	Allow for testing of microbial sensitivity requires specific microbiological expertise.
Rapid urease test	>90%	Cost effective and rapid test	Requires an additional test for confirmation of *Hpylori* infection
Noninvasive methods			
Urea breath test	>95%	Alternative gold standard	Very useful, reliable test to evaluate success of eradication treatment of *Hpylori* limited availability due to requirement of expensive equipment.
Fecal antigen	>90%	Not widely used	Simple test but may not be

test		yet	reliable for evaluation of success of eradication treatment of *Hpylori*
Serology	80-90%	Mainly used for epidemiological studies	Insufficient reliability for routine screening; cannot prove ongoing infection due to immunological memory.

2.16 Treatment:

Once *H. pylori* is detected in a person with a peptic ulcer, the normal procedure is to eradicate it and allow the ulcer to heal. The standard first- line therapy is a one- week "triple therapy" consisting of proton pump inhibitors such as Omeprazole and the antibiotics Clarithromycin and amoxicillin. Variations of the triple therapy have been developed over the years, such as using a different proton pump inhibitor, as with Pantoprazole or Rebeprazole, or replaxing amoxicillin with Metronidazole for people who are allergic to penicillin. Such a therapy has revolutionized the treatment of peptic ulcers and has made a cure to the disease possible. Previously, the only option was symptom control using antacids, H2-antagonists or proton pump inhibitors alone (Malfertheiver *et al*., 2007).

An increasing number of an increasing number of infected individuals are found to harbor antibiotic- resistant bacteria. This results in initial treatment failure and requires additional rounds of antibiotic therapy or alternative strategies, such as a quadruple therapy, which adds a bismuth colloid, such as bismuth subsalicylate (Tsuji *et al*., 2003). For the treatment of

Clarithromycin-resistant strains of *H. pylori,* the use of Levofloxacin as part of the therapy has been suggested (Malferteiver *et al*., 2007).

Ingesting lactic acid bacteria exerts a suppressive effect on *H. pylori* infection in both animals and humans, and supplementing with *Lactobacillus*- and *Bifidobacterium*- containing yogurt improved the rates of eradication of *H.pylori* in humans. Symbiotic butyrate-producing bacteria which are normally present in the intestine are sometimes used as probiotics to help suppress *H. pylori* infections as an adjunct to antibiotic therapy. Butyrate itself is and antimicrobial which destroys the cell envelope of *H. pylori* by inducing regulatory T cell expression and synthesis of an antimicrobial peptide, against the *H. pylori* (Tsuji *et al*., 2003).

1.17 Prevention:

H. pylori are a major cause of certain diseases of the upper gastrointestinal tract. Rising antibiotic resistance increases the need to search for new therapeutic strategies; this might include prevention in form of vaccination. Much work has been done on developing viable vaccines aimed at providing an alternative strategy to control *H. pylori* infection and related diseases, including stomach cancer (Selgrad and Malfertheiner, 2008). Researchers are studying different adjuvant, antigens, and routes of immunization to ascertain the most appropriate system of immune protection; however, most of the research only recently moved from animal to human trials. An economic evaluation of the use of a potential *H. pylori* vaccine in babies found its introduction could, at least in the Netherlands, prove cost-effective for the prevention of peptic ulcer and stomach cancer. A similar approach has also been studied for the United States (Blaser, 2006).

The presence of bacteria in the stomach may be beneficial, reducing the prevalence of gastro esophageal reflux disease and esophageal cancer by influencing systemic immune responses (Blaser, 2006).

Chapter three

Materials and Methods

Chapter III

3. Materials and Methods

3.1 Study design*:

This was a descriptive prospective cross sectional study. Laboratory based.

3.2 Study area:

The study was conducted in Elsyal Village in River Nile State.

3.3 Study population:

Sample had been collected from people with gastroenteritis in Elsayal village.

3.4 Study duration:

Study was carried out during 3 months from April to June 2016.

3.5Ethical consideration:

Permission to conduct this study was obtained from college of Graduate studies, Sudan University of Science and Technology. All volunteer were informed about the importance and benefits of the study.

3.6 Inclusion criteria:

People suffer from symptom of gastritis and were *H. pylori* ICT positive.

3.7 Exclusion criteria:

Peoples suffer from symptom of gastritis and ICT of *H. pylori* Negative.

3.8 Data collection:

Data were collected using questionnaire (Appendix I) with informed consent.

3.9 Sample size:

A total of 150 persons were enrolled in this study.

3.10 Sample collection:

Under a septic condition after the wearing gloves, alcohol antiseptic (70%) was used to clean the skin. Venous blood (2.5ml) was obtained from patients. Serum was collected into the collection tubes and left to settle for 30 minutes in rack to clot and then centrifuged at 3000 rpm for 5minute. Sample was kept at -20^{o}c until used. Stool sample take about 5mg in dry clean container.

3.11 Laboratory Methods.

3.11.1 Immune Chromatography Test.

3.11.1.1 Principle of test.

Rapid *H. pylori* antibody test employs chromatographic lateral flow test device in a cassette format.

Colloidal gold conjugated *H. pylori* antigens (Au-Ag) are dry-immobilized at the end of nitrocellulose membrane strip. *H. pylori* antigens are bound at the Test Zone (T). When the sample was added; it was migrates by capillary diffusion rehydrating the gold conjugate. If anti- *H. pylori* antibodies present in sample, antibodies will bind with the gold conjugated antigens forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by *H. pylori* antigens generating a visible red line. If there is no anti- *H. pylori* antibodies in sample, no red line are formed in the Test Zone (T). A built-in control line will always appear in the Control Zone (C) when the test has performed properly, regardless of the presence or absence of anti- *H. pylori* antibodies in specimen.

3.11.1.2 Storage and stability:

The sealed pouches in the kit may be stored between 3 – 4°C for the duration of the shelf life as indicated on the pouch.

3.11.1.3 Procedure of test:

The kit components were brought to reach room temperature if necessary.

The pouch and card were opened. Once opened, the test card must be used immediately.

The test card was labeled with patient's identity.

2- Drops (80 µl) of serum were applied to the sample well marked as (S).

At the end of 10 minutes the results were read. Strong positive sample may show earlier.

11.1.4. Interpretation of the results:

Negative

Only control line appears.

Positive

Both control line and the test line appear. It indicates the antibodies to *H. pylori* have been detected.

Invalid result:

If after 10 minutes on line is visible within the control zone, the result is invalid. The test should be repeated with a new test card.

- Positive ICT samples were retested by (ELISA).

3.11.2 Enzyme Linked Immuonosorbant Assay (ELISA):

3.11.2.1 Principle of ELISA

IgG

- The ELISA test kit (EUROMMUN) provide quantitative in vitro assay for human antibodies of the IgG class against *H. pylori* in serum. The test kit contains micro-titer strips each with 8 break-off reagent wells coated with *H. pylori* antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the cause of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound

antibodies, second incubation is carried out using an Enzyme- labeled anti-human Ig G (Enzyme conjugate).

Catalyzing the color reaction.

3.11.2.2 Storage and stability:

The test kit was stored at temperature between +2°C to +8°C. , all test kit component are stable until he indicated expiry data.

3.11.2.3 Procedure of the test

Purified *H. pylori* antigen was coated on surface of micro wells. Diluted patient samples1:101 in sample buffer (appendix 3), was added to the well *H. pylori* IgG specific antibody, if present, will bind to the antigen all unbound materials were washed away. Enzyme conjugate was added, which binds to the antibody – antigen complex. Excess enzyme conjugate was washed off and the chromogen (substrate reagent) was then added. The enzyme catalytic reaction was stopped at specific time by sulphuric acid.

According to manufactures protocol, 100µl from positive and negative control and diluted patient samples were transferred into the micro plate wells then incubated for 30 minutes at room temperature. The wells were then emptied and subsequently manual washed 3 times using 300µl of wash buffer (appendix4). Then 100µl of enzyme conjugate was added into each of micro plate wells and incubated for 30 minutes at room temperature. The wells were then emptied and subsequently washed 3 times using 300µl of wash buffer (appendix 4). Then 100µl of TMB chromogenic substrate solution was added into each of micro plate wells and incubated for 30 minutes at room temperature. The reaction was stopped by adding 100µl of stop solution (sulphuric acid) into each micro plate wells in the same order and the same order and the same speed the substrate solution was introduced. Photometric

measurement of the color intensity was done at wavelength of 450 nm and reference wavelength between 620nm within 30 minutes of adding stop solution. Prior to measuring the micro plate was slightly shake to ensure homogenous distribution of the solution.

3.11.2.4 Reading of the results

Results can be evaluated by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator.

EUROIMMUN recommends interpreting results as following:

Ratio < 0.8: negative.

Ratio ≥ 0.8 to < 1.1: borderline.

Ratio ≥ 1.1: positive.

3.12 Data analysis:

All data were analyzed using Statistical Package for Social Science (SPSS)Correlation ,One way A nova sample T test and graph .The obtained data analysis was analyzed in parameter of gender, family history, economic status, age group, figure were performed by using Microsoft office and Exile soft were.

Chapter four

Results

Chapter IV

Results

Total of one hundred and fifty serum sample were collected from people with gastritis from peoples in Elsayal Elkapeer village in River Nile State were tested by ICT .After that take a ninety sample positive by ICT and retested for IgG using ELISA procedure and stool Ag to detect recent infection.

H pylori was more prevalent in females44/53(48.9)(table4-1)

Table (4-1): The Relationship between *H. pylori* and gender.

Gender	Positive ELISA	%	Negative	%
Male	32/37	35.5%	5/37	5.6%
Female	44/53	48.9%	9/53	10%

p. value = 0.04

The majority of infected patient with *H pylori is* female in poor economic status (48/76) (53.3%). Table (4- 2)

Table (4-2): The Relationship of *H. pylori* and economic status.

Economic status	High	%	Medium	%	Poor	%
Positive	6	6.67%	22	24.4%	48	53.3%
Female	0	0	3	3.3%	11	12.2%

P. value = 0.1 not significant different at level 0.05.

Table (4-3): The Relationship between *H. pylori* and family history.

Family history	Positive ELISA	%	Negative	%
Yes	43 / 76	56.6%	5 / 14	35.7%
No	35 / 76	43.4%	9 / 14	64.3%

P. value = 0.1, not found significant correlation between family hi story and *H. pylor*i infection.

The *H pylori* was more common in patients with infection is present in family (43/76) (56.6%)(table4- 3).

The age group10-30 year were more infected by *H pylori* (46/76) (51.1%) table(4-4)

Table (4-4): The Relationship between *H. pylori* and age group.

Age by year	Positive ELISA	%	Negative	%
10-30	46	51.1%	9	10%
:31-50	24	26.6%	5	6.7%
:More than 50	6	6.8%	0	0%

P (0.3)

Table (4.5): The Relationship between ELISA and stool antigen.

Stool Ag	+ve ELISA	%	-ve ELISA	%
Positive	46	51.1%	3	3.3%
Negative	30	33.3%	11	12.3%
Total	76	84.4%	14	15.6%

P 0.007

From76 positive ELISAfound46 is positive by stool Ag and 30 negative .Whilefrom14 negative ELISA found 3positive stool Ag and 11negative too.

Chapter five

Discussion

Conclusion

Recommendations

Chapter V

5.1 Discussion

The prevalence of *H. pylori* infection show large geographical distribution especially in developing countries.

80% of people were infected in the world wide. The serological test give high sensitivity is detection *H. pylori*.

This study investigated ICT and ELISA IgG, stool Ag and risk factor associated with *H. pylori* among people in El sayal GL kappear village.

The sample were tested by ELISA I g G to show prevalence of *H. pylori* in the village.

In this study have showed positive correlation between gender and *H. pylori* infection ($p<.05$) this disagree with (Khan and Ghazi, 2007; Marie, 2008; Jafar *et al*., 2013). Found that the *H. pylori* is lower is male than female.

The study revealed that female was more affected than male.

This agree with study in Egypt by Manal *et al* (2007) and other study by Huang *et al* (2004) in Malysia, kikuchia and Dore (2005) in Iran and (2005) in Newther Land.

The result was dis agree to Nirhhani *et al* (2007) and his worker in Sudan they found no significant difference between gender and *H. pylori* antibody I g G.

Also versalovic and fox (2003), reported that *H. pylori* was more frequent in males than female and was disagreed with present result. The difference in result m

y be due to the immunity status to female environmental exposures in and hormonal differences between two genders as recent studies identified an important role of oxytoxic in the gastric evaluation rate (G E R).

In this study show no significant correlation between *H. pylori* antibody and socioeconomic status. Found poor person (low class) who more affected than high social class people. With No significant correlation that agreed with study done by H.M. Malaty, Dy Graham.

In this study the age is one of risk factor age group (10 – 30) is more affected than other age group this agree with study done by Malco (Metal. (15)

With no significant correlation and the *H. pylori* infection decreased with age a lower prevalence rate of *H. pylori* in the elderly has also been reported by other and two hypotheses have been proposed to explain these finding: *H. pylori* could have been present in the past in small number or low activation with might not have been detected and *H. pylori* could have been present in the past, but was eliminated on account of the development of an favorable gastric environment with age at the mean there is a progressive gastric migration a proximal direction in resent study founded relation between *H. pylori* and family history with no significant correlation between it this study is agree with.

Recently in some study which aimed to determine the *H. pylori* infection in patient using HPSA, and *H. pylori* (IgG, IgM and IgA), the result showed that the sensitivity, specefity and accuracy of stool antigen test gave highest value between these test this due to the nature of the patient who were infected with *H. pylori* (Bedir 2012). But in other studies the western blot and immune florescence IgG is very high sensitive and accuracy (leal et al; 2008), and it is most reliable test in diagnosis of *H. pylori* infection. Study ELISA, IgG is

more effective test used to diagnosis *H. pylori* infection compared with ICT and stool Ag this agree with man's studies. (Best *et al*. 2014).

In this study stool Ag is more reliable test that agree with (Braden *et al.* 2012).

5.2. Conclusion

In this study concluded the females in poor people were more affected than males.

The result show significant correlation between gender and no significant correlation in other risk factors, socioeconomic status, family history, age group and presence of *H. pylori* antibody.

All tests that used in this study (ELISA,, Stool Ag, ICT) showed high sensitivities. Specify accuracy and predictive values.

5.3 Recommendations

Based on this study results the following recommendations are to be considered:

1- High number of samples is needed to verity these result.
2- ELISA I g G is most reliable test in diagnosis of *H. pylori* infection but is expensive and need technical demand, stool Ag might be more suitable for us is developing countries.
3- More studies about risk factors and mode of transmission of *H. pylori* are needed to determine the extent of this disease.
4- Enhance prevention strategy to reduce *H. pylori* infection.

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Appendices

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بسم الله الرحمن الرحيم

Appendix1

Sudan University of Science and Technology

College Of Graduate Study

Prevalence of *H pylori* among People with Gastritis in ELsayal ELkapeer Village in River Nile State

معدل انتشار البكتريا الحلزونية البوابية لدى الأشخاص المصابين بإلتهاب المعدة من سكان قرية السيال الكبير بولاية نهر النيل

الاسم/ ..... Name ……

العمر.....Age

الحالة الاقتصادية :..... Economic status ……

Are you infected with *H pylori*

هل يوجد اعراض للاصابة بجرثومة المعدة؟ .....

Do you have any symptom.??

في حالة الاصابة متي ظهرت؟.....

If there …how long.?

هل يوجد اعراض للاصابة بمشاكل الجهاز الهضمي مثل حرقانفي فم المعدة او لون البراز اسود .....

Do you have any GIT symptom like **stomach acidity pain or dark stool ?**

هل يوجد احد المخالطين لديه مشاكل معدة ويتعاطي علاجات.....

Any one at home has thesesymptom. And received treat ment of stomach problem

نتائج فحص الجرثومة بواسطة الانزيم المناعي المرتبط والرحلان المناعي وفحص الفسحة

Result of *Hpylori* dignosed by ELISA, ICT and stool Ag

…….

Appendix II

بسم الله الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Prevalence of *H .pylori* among People with Gastritis in ELsayal ELkapeer Village in River Nile State

معدل انتشار البكتريا الحلزونية البوابية لدى الأشخاص المصابين بإلتهاب المعدة من سكان قرية السيال الكبير بولاية نهر النيل

اقرار موافقة:

بهذا أؤكد أني فهمت الهدف من الدراسة وماهو مطلوب مني لقد اتيحت لي فرصة كافية لمراجعة المعلومات المتوفرة، واعلم ان مشاركتي هي أمر اختياري وان بوسعي الانسحاب في اي وقت .

واخيرا اوافق علي الاسهام في البحث المزكور اعلاه

I confirm that I understand the purpose of the study and what is require from me .I understand that my participation is voluntary and that I am free to withdrow at any time.

I agree to take part in above research study

Name of participant: ……اسم المشارك:

Name of uardian :…….....اسم ولي الأمر

Signature:……..……التوقيع:

اسم الباحث : تيسير بابكر محمد

Appendix III

ELISA sample dilution

Diluted1:101in sample buffer

Serum…….10ul

Sample buffer……..1.0ml

Mix well by vortexing

ELISA Buffer

Carbonate bicarbonate buffer (coating buffer)PH:6.9

NaCO3anhydrous ……1.59mg

NaCO3……2.93mg

Dissolve in leterDW.adjust PH to py NaOH

Phosphate buffer saline (PBS) Teen-20(washing buffer)PH7.4

NaCl ……..8gm

Na2HPO4……2.9mg

KH2PO4…….0.2mg

KCL……0.2mg

Dissolve in liter disteled water then add 0.01v/v polyoxy ethylene sorbitol monourate (Teen 20)adjust PH by NaOH OR HCL

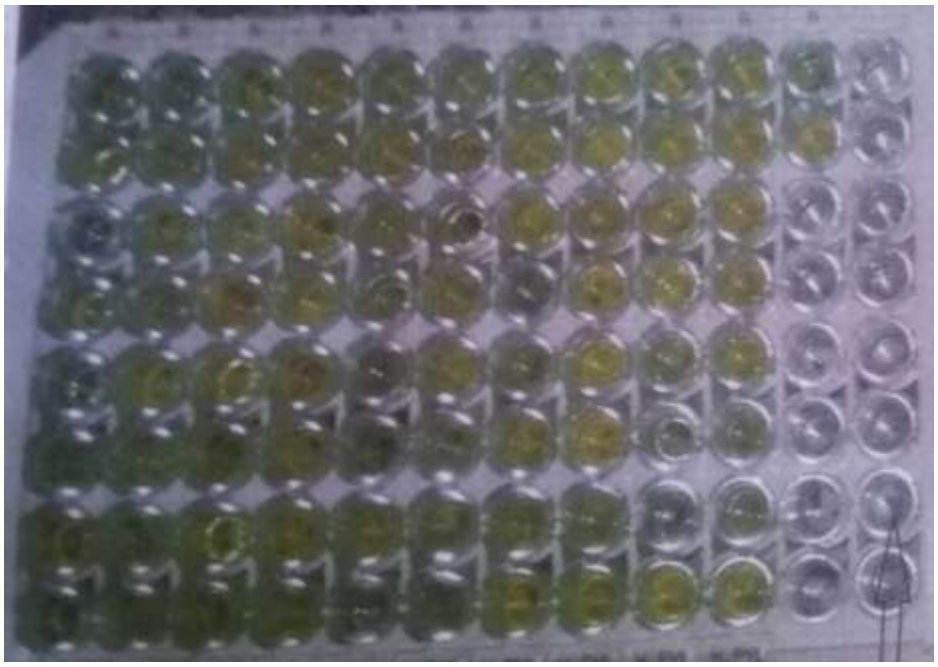
H2SO4 (stopping buffer)

20%H2SO4

20ml……H2SO4

80ML……D.W

Appendix IV:



Picture of ELISA IgG result

Yellow colour :=+ve

White colour:=-ve

Appendix V

ELISA washer

Appendix VI

ELISA Reader