"SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR"

By

Dr. AYSHA YASMEEN, ***MBBS***

DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

TAMAKA, KOLAR, KARNATAKA

In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE (MD)

IN

MICROBIOLOGY

Under the Guidance of

Dr. S.R.PRASAD, *MD*

Professor

DEPARTMENT OF MICROBIOLOGY,

SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

KARNATAKA

APRIL 2017

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH, TAMAKA, KOLAR, KARNATAKA.

DECLARATION BY THE CANDIDATE

I HEREBY DECLARE THAT THIS DISSERTATION ENTITLED **'SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR'**

AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

IS A BONAFIDE AND GENUINE RESEARCH WORK CARRIED OUT BY ME UNDER THE DIRECT GUIDANCE OF

Dr. S.R.PRASAD, *MD*

PROFESSOR,

DEPARTMENT OF MICROBIOLOGY,

SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

Date:

Place: Kolar **Dr. AYSHA YASMEEN**

CERTIFICATE BY THE GUIDE

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED

'SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR'

AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

IS A BONAFIDE RESEARCH WORK DONE

BY

Dr. AYSHA YASMEEN

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

M.D IN MICROBIOLOGY

DATE:

PLACE: KOLAR

Signature of the Guide

Dr. S.R. Prasad, *MD*

Professor

Department of Microbiology

ENDORSEMENT BY THE CO-GUIDE

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED

'SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR'

AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

IS A BONAFIDE RESEARCH WORK DONE

BY

DR AYSHA YASMEEN

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

M.D IN MICROBIOLOGY

Date:

Place: Kolar

Signature of the Co-Guide

Dr. S.R. Sheela, *MS*

Professor,

Department of Obstetrics & Gynaecology

ENDORSEMENT BY THE CO-GUIDE

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED

'SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR'

AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

IS A BONAFIDE RESEARCH WORK DONE

BY

DR AYSHA YASMEEN

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

M.D IN MICROBIOLOGY

Date:

Place: Kolar

Signature of the Co-Guide

Dr. KRISHNAPPA.J, ***MD***

Professor,

Department of Pediatrics

ENDORSEMENT BY THE HOD, PRINCIPAL / HEAD OF THE INSTITUTION

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED

"SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR"

AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

IS A BONAFIDE RESEARCH WORK DONE BY

DR AYSHA YASMEEN *MBBS*

UNDER THE GUIDANCE OF

DR S.R.PRASAD, *MD*

PROFESSOR

DEPARTMENT OF MICROBIOLOGY

SEAL & SIGNATURE OF THE HOD	SEAL & SIGNATURE OF THE PRINCIPAL
Dr BEENA P.M	**Dr M.L. HARENDRA KUMAR**
Date:	Date:
Place: KOLAR	PLACE: KOLAR

SRI DEVARAJ URS MEDICAL COLLEGE, TAMAKA, KOLAR.

ETHICAL COMMITTEE

CERTIFICATE

THIS IS TO CERTIFY THAT, THE ETHICAL COMMITTEE OF

SRI DEVARAJ URS MEDICAL COLLEGE, TAMAKA, KOLAR

HAS UNANIMOUSLY APPROVED

DR AYSHA YASMEEN,

POST GRADUATE STUDENT IN THE DEPARTMENT OF MICROBIOLOGY OF

SRI DEVARAJ URS MEDICAL COLLEGE

TO TAKE UP THE DISSERTATION WORK ENTITLED

'SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR'

AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

TO BE SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF

HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR.

MEMBER SECRETARY

COPYRIGHT

DECLARATION BY THE CANDIDATE

I HEREBY DECLARE THAT

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR, KARNATAKA

SHALL HAVE THE RIGHTS TO PRESERVE, USE AND DISSEMINATE

THIS DISSERTATION,

IN PRINT OR ELECTRONIC FORMAT, FOR ACADEMIC / RESEARCH PURPOSE.

DATE: SIGNATURE OF THE CANDIDATE

PLACE: KOLAR **Dr AYSHA YAMEEN**

ACKNOWLEDGEMENT

It is a pleasure to thank the many people who made this dissertation possible. I thank each and every one for the knowledge they imparted to me and for the time they so graciously gave me.

I wish to express my humble, sincere indebtedness to ***Dr.S.R.PRASAD****, Professor, Department of Microbiology, SDUMC, Kolar, who encouraged me to take the subject of this study and who has been my teacher and supervisor in this scientific work. I thank him for his excellent and tireless guidance, encouragement and patience throughout the years of the study.*

I wish to express my deepest gratitude to ***Dr. BEENA.P.M****, Professor and HOD, Department of Microbiology, for able guidance, constant support and encouragement during the period of this study.*

I am grateful to ***Dr. ARVIND.N, Dr. PARIMALA, Dr.MAMATHA KALE, Dr.SAVITHA.N,*** *and* ***Dr. ANITHA. D,*** *Faculty members, Department of Microbiology, for their invaluable guidance, kind support, co-operation and timely advice.*

My sincere thanks to my colleague ***Dr. SAGAR MALI,*** *and my seniors* ***Dr.SUDHA,*** and ***Dr. BIPIN,*** *for giving constant support and encouragement.*

I would like to acknowledge the help rendered by all the ***technicians*** *in the Department of Microbiology during my study. I also thank all* ***non teaching staff*** *for their kind co-operation.*

I thank the Almighty God for all his blessings and my family for the constant support and encouragement throughout this study.

Date: Signature of the candidate

Place: KOLAR **Dr. AYSHA YASMEEN**

LIST OF ABBREVIATIONS

'r'	Pearson's correlation co-efficient
µm	Micrometer
^{0}C	Degree Celsius
AIDS	Acquired immunodeficiency syndrome.
APC	Antigen presenting cell
BID	two times a day
CD	Cluster of differentiation
CDC	Centers for Disease Control and prevention
Cm	Centimeter (10^{-2} meter)
CSF	Cerebro Spinal Fluid
CT	Computed Tomography
Dc	Dentritic cell
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
HAART	highly active anti retroviral therapy
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
HRP	horse radish peroxidase
i.e.	that is
IFAT	Indirect Fluorescent Antibodies Test
IFN γ	Interferon γ
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	interleukin
ISAGA	Immunoglobulin M immunosorbent agglutination assay
IU/ml	International units per milli litre
KDa	Kilo Dalton
mg	milligram

MHC	Major Histocompatibility Complex
ml	milli litre (10^{-3} Litres)
MRI	Magnetic resonance imaging
NK	Natural Killer
NO	Nitric oxide
^{O} F	degree Fahrenheit
OD units	Optical Density units
PAS	Per-iodic acid Schiff
PCR	Polymerase Chain Reaction
QID	four times a day
RNA	Ribonucleic Acid
T.gondii	*Toxoplasma gondii*
TLR	Toll like receptor
TMB	Tetramethylbenzidine
USG	Ultrasonography
Vs	versus
∴	Therefore

ABSTRACT

INTRODUCTION:

Toxoplasmosis is a zoonotic disease, caused by the protozoan parasite *Toxoplasma gondii (T.gondii)*, affecting one third of the world's population. It has been included by Centers for Disease Control and prevention (CDC), Atlanta, USA, under neglected parasitic infections. Primary infection in pregnant women by *T. gondii* can result in vertical transmission causing foetal loss or congenital malformations in the newborn. In India, the prevalence of toxoplasmosis in pregnant women is around 22 %. The prevalence of anti-toxoplasma antibodies in pregnant women of Kolar region and the incidence of vertical transmission to their babies is not known. We estimated the prevalence of anti-toxoplasma IgG and its titres in pregnant women admitted for delivery, and tested the cord blood samples of babies for IgM antibodies to *T.gondii* to detect vertical transmission.

MATERIALS AND METHODS:

In this observational cross sectional study, 251 venous blood samples from pregnant women admitted for labour at R. L. Jalappa Hospital, Kolar, from December 2014 to October 2016, and cord blood samples from their respective newborn babies were tested for IgG and IgM anti- toxoplasma antibodies respectively by using IgG ELISA NOVATEC IMMUNDIAGNOSTICA kit and IgM μ capture ELISA NOVATEC IMMUNDIAGNOSTICA kit.

The mothers of babies who gave a positive IgM test in the cord blood were also tested for IgM anti- toxoplasma antibodies. Anti-toxoplasma IgG titres were also estimated in the cord blood samples of babies born to seropositive mothers

RESULTS:

The prevalence of anti-toxoplasma IgG antibodies among 251 pregnant women admitted for delivery was 21.1%. The mean antibody titre was 167±86 IU/ml. None of the mothers had a titre above the mean plus 3 Standard deviations, suggestive of recent infection. The seropositivity was significantly higher in women belonging to lower socioeconomic strata.

We could not find any association with multigravida, previous history of abortion, gestational age, mode of delivery (normal vs. caesarean), level of education, occupation, owning cats, drinking untreated water, and consumption of salad or raw meat with the seropositivity. There was no significant difference among women who consumed salad and drank untreated water. None of the pregnant women gave history of consumption of raw meat.

Out of 251 cord blood samples, 5(2 %) samples gave a positive IgM reaction. They were considered to be false positives; as their respective mothers lacked IgG antibodies to *T.gondii* or on follow up, the infant was found to be negative for both IgG and IgM antibodies to the parasite.

CONCLUSION:

In the study presented here a seroprevalence of 21.1% detected by us is similar to the average prevalence of 22 % reported among pregnant women from other parts of the country. Pregnant women from lower socioeconomic status had a significantly higher prevalence. We found a false positive IgM positive reaction in 5 (2%) of the cord blood samples tested.

Our study could not detect any vertical transmission of toxoplasma infection; it emphasizes the problem of false positivity encountered in μ capture ELISA for

detection of IgM antibodies against *T.gondii*. Routine screening of a larger number of pregnant women, follow up of those who have IgM antibodies and testing a larger number of cord blood samples not only for IgM, but also for IgA and IgE antibodies to *T.gondii* may provide a clearer picture of vertical transmission of toxoplasma infection in Kolar region.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Parasitic zoonosis is a global health problem. The disability due to them is high, especially, in low income countries.[1] Toxoplasmosis is a zoonotic disease, caused by the coccidian protozoa *Toxoplasma gondii (T.gondii).* Toxoplasmosis is often asymptomatic in the immunocompetent people. It is one of the most prevalent chronic infections affecting one third of world's population. It has been included by Centers for Disease Control and prevention (CDC), Atlanta, USA, under neglected parasitic infections.[2, 3]

T.gondii is an obligate intracellular parasite, which can infect all warm blooded animals including humans, mammals, and birds. It is an important component of TORCH group of infections which can cause congenital malformations, and ranks among *Treponema pallidum,* Rubella, Cytomegalovirus, Herpes simplex virus and Hepatitis–B virus. The factors favouring the transmission of disease are the close proximity between humans and animals.[1, 2, 4] Cats and other felines are the only definitive hosts and thus the only source of infective oocysts, but other mammals and birds can develop tissue cysts, and can also act as an infective source.[3]

Human infection generally occurs through consumption of food or water contaminated with oocysts, and tissue cysts from undercooked meat. Congenital transmission and organ transplantation are also other routes of infection.[2] Toxoplasmosis can result in untoward consequences in immunocompromised patients and seronegative pregnant women.[3] Primary infection in pregnant women by *T.gondii*, can cause vertical transmission of the parasite to the foetus which may result in miscarriages, stillbirths, premature births and congenital malformations in the newborn.[1, 2]

There are a few reports on seroprevalence of toxoplasma antibodies in women of child bearing age and case reports describing congenital toxoplasmosis from India. Seroprevalence of toxoplasmosis seems to vary with the geographical region. To the best of our knowledge, we did not find any study involving testing of newborns for vertical transmission of toxoplasmosis from India.

There are no studies so far on the prevalence of toxoplasma infection among pregnant mothers or incidence of toxoplasmosis in newborns from Kolar region. Our study fills the gap in this area by screening the pregnant women for IgG class of antibodies to *T.gondii* by quantitative enzyme linked immunosorbent assay (ELISA) and testing the cord blood samples of babies born to them for IgM class of antibodies to *T.gondii* by μ capture ELISA.

OBJECTIVES

1. To screen the pregnant women from Kolar region for IgG class of antibodies to *T.gondii*
2. To screen the cord blood of their newborn babies for IgM class of antibodies to *T.gondii*

REVIEW OF LITERATURE

HISTORY

Discovery of *T.gondii*, the causative agent of toxoplasmosis was an offshoot of the research work which was being carried out for leishmaniasis at the Pasteur institute in Tunis (Africa). Charles Nicolle and Louis Manceaux in 1908 were the pioneers who detected the parasite in the tissues of an African hamster-like desert rodent named gundi (*Ctenodactylus gundi*). The rodent *Ctenodactylus gundi* was used for leishmaniasis research.[5, 6] This protozoan parasite was named by them as *Toxoplasma gondii* based on the crescent shape of the parasite they saw (toxo = arc/bow, plasma = form/life) and gondii for the host.[5]

At the same time, when Charles Nicolle and Louis Manceaux reported the discovery of *T.gondii*, Alfonso Splendore working in Sao Paulo, Brazil, identified a similar organism in the tissues of a rabbit, but he did not name it. Years later, *T.gondii* was isolated from other animals and also human beings.[7]

The rodent gundi was found in the mountains and foothills of Tunisia, Africa. In 1917, Chatton and Blank discovered that gundis were not naturally infected by the parasite. The gundi acquired infection when they were kept in cage for research work. The first viable isolates of *T.gondii* were demonstrated by Sabin and Olitsky in 1937, which were obtained from an animal. Wolf et al in 1939 demonstrated congenital transmission in humans and isolated the first human isolate of Toxoplasma parasite.[5] The cysts of *T.gondii* were recognized in 1951 by Frenkel and Friedlander in cytological preparations; they also described the pathogenesis of *T.gondii*. The studies conducted during 1960s and 1970s, identified T.gondii as a coccidian parasite. Frenkel et al identified cats as definitive hosts, confirming the earlier observations by

many researchers in this area. It was only in 1970 that the mode of transmission and the complete lifecycle of T.gondii could be clearly understood.[1, 7, 8]

Figure 1 shows Charles Nicolle, the discoverer of *T.gondii*.

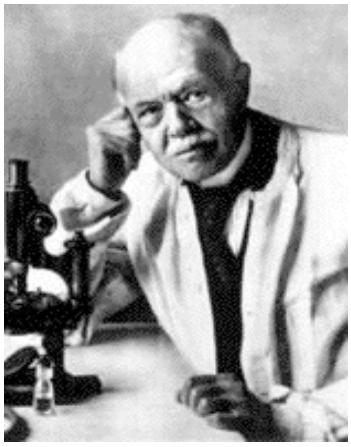


Figure 1: Charles Nicolle, courtesy[9]

Figure 2 shows *Ctenodactylus gundi*, the rodent in which toxoplasma was discovered.

Figure 2: *Ctenodactylus gundi*, courtesy[10]

Figure 3 shows the crescent shaped parasite named by the discoverers as *T.gondii*.

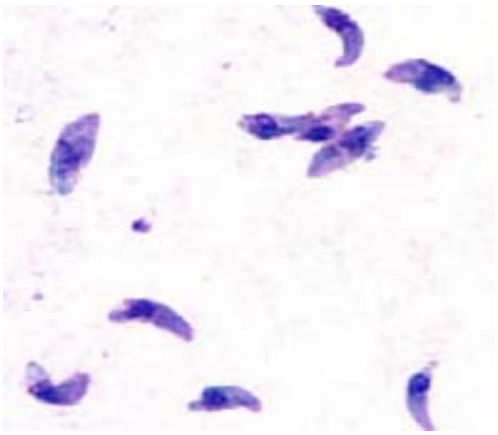


Figure 3: Tachyzoite, Courtesy[11]

Figure 4 shows Alfonso Splendore, who identified the T.gondii parasite, but he did not name it.

Figure 4: Alfonso Splendore, courtesy[12]

Table 1 presents the historical milestones in our understanding of toxoplasmosis.

Table1: Historical milestones of toxoplasmosis.

Year	Milestones
1908	Charles Nicolle and Louis Manceaux discovered *Toxoplasma gondii* in the tissues of the rodent, *Ctenodactylus gundi* in Tunisia, Africa.
1908	Alfonso Splendore found the parasite in the tissues of a rabbit in Brazil.
1910	Mello described infection in a dog.
1923	Janku from Prague, described encephalomyelitis and chorioretinitis in a boy, who lost his vision at 3 months of age and succumbed to the illness at 16 months of age. The child had hydrocephalus and convulsive seizures.[13]
1937	Sabin and Olitsky isolated the first viable isolate of *T.gondii* from an animal.
1939	Wolf et al demonstrated Congenital transmission in humans and isolated the first viable human isolate of *T.gondii* parasite from post mortem of a full term newborn girl baby born in 1938 by caesarean section at a hospital in New York. At the 3 rd day of life, baby had seizures, and on ophthalmoscopy macular lesions were observed in both the eyes. The child died at 1 month of age. On post-mortem *T.gondii* parasite was found in the brain and right eye, which was inoculated intracerebrally to mice and rabbits.[14]
1942	Sabin and Ruchman recognized the development of neutralising antibodies against *T.gondii*.
1942	Sabin and Warren found sulfonamides to be effective against toxoplasmosis.

Table1 contd: Historical milestones of toxoplasmosis.

Year	Milestones
1948	Sabin and Feldman described the dye test for diagnosis of toxoplasmosis.
1951	Frenkel and Friedlander described the pathogenesis of toxoplasmosis.
1953	Eyles and Coleman identified synergistic effect of Pyrimethamine and Sulphonamides against dividing form of the parasite.
1968, 1981	Remington and Desmonts developed tests to detect IgM in cord blood of newborns.
1970, 1972	Frenkel et al, Miller et al, and Jewell et al: described the definitive and intermediate hosts, and the shedding of oocysts only by feline species such as cat, leopard, cheetah, lion, and wild cat.
1979	Teutsch et al described the first human toxoplasmosis outbreak, as a result of ingestion of oocysts.
1983	Waldeland and Frenkel developed Ts-4 vaccines for intermediate hosts.
1986	Dubey et al constructed thermal curves to kill *T.gondii* cysts in meat by cooking, freezing and irradiation.
1989	Burg et al developed a PCR test to detect T.gondii using B1 gene as a target.
1991	Frenkel et al develop T-263 vaccine to prevent oocyst shedding in cats.

Courtesy: modified from[14, 15]

Taxonomy of parasite:

T. gondii is an apicomplexan protozoan parasite. It belongs to the family of the Sarcocystidae, and is the only species in the *Toxoplasma* genus.

T.gondii is classified as follows, courtesy[16]

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Apicomplexa
Class	Sporozoasida
Order	Eucoccidiorida
Family	Sarcocystidae
Genus	*Toxoplasma*
Species	*gondii*

Morphology

There are three morphological forms of *T.gondii*:

a) Tachyzoites

b) Bradyzoites / Cryptozoites

c) Oocysts

a) Tachyzoites:

In 1973, Frenkel coined the term tachyzoite (rapidly dividing trophozoites in acute infection) and bradyzoites (slowly dividing forms present in true cysts).

Tachyzoites are crescent shaped, measuring about 6µm x 2µm in size. They have an anterior pointed end and a posterior rounded end. When observed under electron microscope, an outer covering called pellicle, and its invagination resulting in a rod shaped structure called micropore, inclusion bodies, and many organelles are seen. The organelles seen are apical rings, polar rings, conoid, rhoptries, micronemes, micropore, mitochondrion, subpellicular microtubules, endoplasmic reticulum, golgi complex, ribosomes, rough and smooth endoplasmic reticulum, nucleus, multiple membrane bound organelles called apicoplasts, amylopectin granules and dense granules. The nucleus is present in the centre.[17]

The electron microscopic picture of tachyzoites and bradyzoites is presented in figure 5.

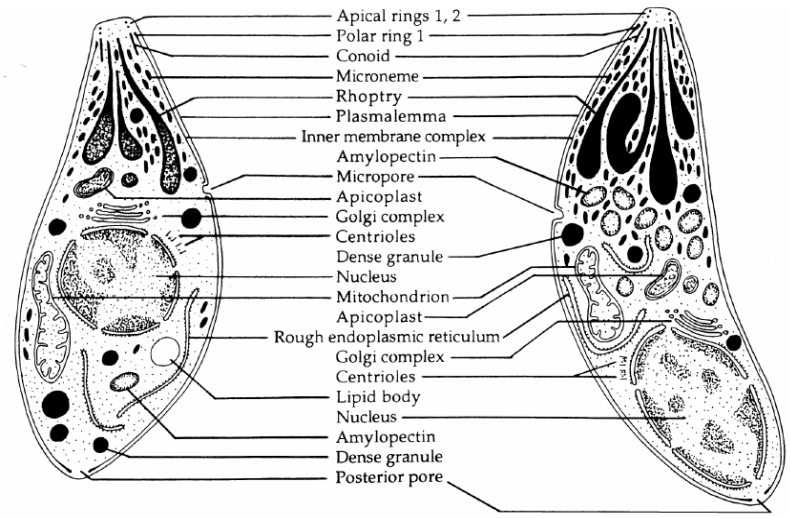


Figure 5: Schematic diagram of a tachyzoite (left) and a bradyzoite (right) of *T.gondii*. The diagrams are composites of electron micrographs, courtesy[17]

The pellicle is made up of three membranes. There is an outer plasmalamella, and an inner membrane complex composed of two membranes which are attached to one another. The inner membrane is absent at certain sites: the anterior end above the polar rings, laterally at the place where micropores are present, and at the posterior end near the posterior pore.

Polar ring 1 is a part of the inner membrane complex, surrounding a cylindrical cone called conoid. The conoid consists of six to eight (6-8) microtubules. Polar ring 2 is situated below the polar ring 1. Twenty two (22) subpellicular microtubules arise from the polar ring 2, and encircle the whole cell. These microtubules are spirally arranged like a rib cage. Two inner microtubules end inside the conoid.

Rhoptries are club shaped organelles, eight to ten (8-10) in number, located between the anterior tip and the nucleus. They have a narrow anterior neck, which passes through the conoid, and ends in a sac like structure. Rhoptries have excretory function.

The conoid, rhoptries, micropores and micronemes, probably help in penetration into the host cell, providing a suitable intracellular environment, thus helping in the growth and development of the parasite.

The conoid has the ability to rotate, tilt, retract, and extend, when the parasite comes in contact with host cell. Migration of parasite across biological barriers occurs by an actin based mechanism called gliding motility. Rhoptries excretes their contents, which have a proteolytic action.[15, 17]

A schematic representation of apical complex of *T.gondii* is presented in figure 6.

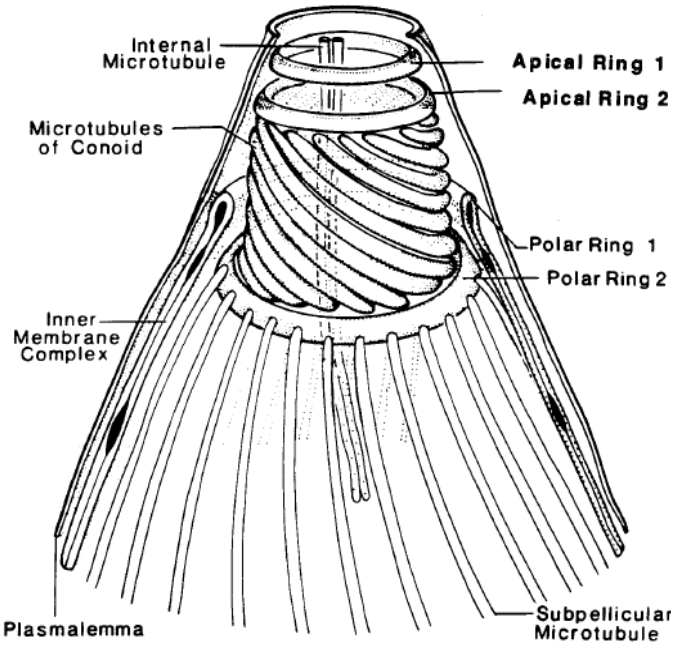


Figure 6: Schematic representation of the apical complex of *T.gondii*, courtesy[17]

The tachyzoites, on coming in contact with the host cell, can enter into it either by phagocytosis or by penetrating actively. Upon entry into the host cell, the tachyzoite becomes oval in shape. A parasitophorous vacuole is formed around the tachyzoite, which is derived from both the host cell and the parasite.

Tachyzoites start multiplying asexually by a process called endodyogeny every 6 to 8 hours. In this, the golgi complex divides first near the anterior end of nucleus. It is a kind of division, in which within a parent cell, two daughter cells are formed (endo = inside). The daughter cells grow in size within the parent cell and are released by rupturing the parent cell. In few strains tachyzoites may divide by binary fission. Eventually the host cell enlarges in size. During acute infection, the tachyzoites within the host cell multiply rapidly, stretch the host cell, and form a pseudocyst. In these pseudocysts, the tachyzoites are slightly PAS positive, and the cyst membrane is neither PAS positive nor argyrophilic.[17, 18]

In Giemsa stained films the parasitic cytoplasm is bluish, while the nuclear chromatin takes up reddish purple color, as found in other protozoan parasites like malaria and leishmania. The morphology varies to some extent with the fixatives and tissue staining techniques used.[8, 18]

b) Bradyzoites

During chronic infection, bradyzoites gather together intracellularly in muscles and a variety of host tissues. A membrane, around the gathered cluster of bradyzoites develops, forming an immune – protective cyst. It measures anywhere between 12 to 100 µm in diameter, and contains numerous bradyzoites.

Such tissue cysts may be found in organs like brain, skeletal muscle, heart, kidney, lung, and liver of the intermediate hosts such as goats, sheep, pigs, cow, buffalo, chicken, dog, beavers, bear, fox, as also man.

The organisms found in the tissue cysts are strongly PAS positive, the cyst wall is eosinophilic, argyrophilic and weakly PAS positive. It is thin and elastic. The cyst wall can be stained by Bodian protoargol and palmgren silver. It remains unstained with methenamine silver stain, implying that cyst wall lacks glycogen and polysaccharides.

When the tissue cysts are intact, they can persist for the host's lifetime, without causing any inflammatory response. However, frequently, these tissue cysts can undergo calcifications. The bradyzoites within the tissue cyst measure around 7 µm x 1.5 µm in size.[15, 18, 19]

Figure 7, shows a brain tissue cyst of an infected rat, containing numerous bradyzoites.

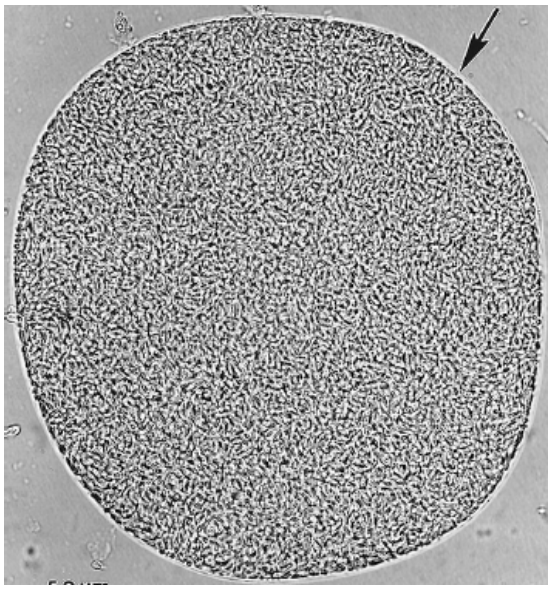


Figure 7: A tissue cyst from the impression smear of infected rat's brain containing numerous (more than 1,000) bradyzoiytes. Arrow indicates the cyst wall, courtesy.[17]

The cyst wall is made up of both the host cell and the parasite components. It is separated from bradyzoites by a granular material. In case of older tissue cysts, there may be degeneration of a few bradyzoites.[17, 18]

Tachyzoites differ from bradyzoites only in a few structural details, the below table 2, shows the differences between them.

Table 2: Differences between tachyzoites and bradyzoites. Courtesy: condensed from[17]

Parameters	**Tachyzoites**	**Bradyzoites**
Size	6 x 2 µm	7 x 1.5 µm
Location of nucleus	Central	Posterior end
Contents of Rhoptries	Labyrinthine	Usually electron dense
Amylopectin granules	Absent or in very few in number	Present
PAS stain	Negative	Positive (red)
Susceptibility to prokaryotic enzymes	Susceptible	Less susceptible
Susceptibility to acid pepsin digestion	Destroyed within minutes	Survived for 2 hours

c) Oocysts: It is the infective form of *Toxoplasma gondii*, produced after sexual reproduction in definitive hosts mainly cats and other feline species. The following 16 feline hosts (shown in table 3), have been either confirmed by bioassays of the oocysts or immunohistochemical post-mortem examination to be definitive hosts of *T.gondii.*

Table 3: List of feline species which can act as definitive hosts for *T.gondii*. Courtesy[14]

Common name	Scientific name
African wild cat	Felis lybica
Amur leopard cat	Felis euptilurus
Asian leopard	Felis bengalensis
Bobcat	Lynx rufus
Cheetah	Acinonyxjubatus
Cougar	Felis concolor vancouverensis
Geoffroy's cat	Oncifelis geoffroyi
Iriomote cat	Felis iriomotensis
Jaguarundi	Felis yagouaroundi
Lion	Panthera leo
Mountain lion	Felis concolar
Ocelot	Felis pordolis
Pallas cat	Felis manul
Pampas cat	Oncifelis colocolo
Siberian tiger	Panthera tigrisaltaica
Wild cat	Felis silvestris

Ingestion by cats of any of the infectious forms of T. gondii, namely tachyzoites, bradyzoites or sporozoites, results in shedding of the oocysts in cat feces. The frequency of oocyst shedding depends on the stage of T.gondii ingested. Nearly 30% of cats shed oocysts in their feces after consumption of tachyzoites or oocysts, while almost all cats shed oocysts after consuming tissue cysts.

The time duration between initial infection and shedding of oocysts varies according to the stage of T.gondii ingested by the cat. This is known as 'pre-patent' time, as shown in table 4.

Table 4: Prepatent time. Courtesy: condensed from[17]

Ingestion of	**Prepatent time**
Tissue cyst	3-10 days
Oocysts	≥ 18 days
Tachyzoites	≥ 13 days

Within the cat's small intestine, asexual reproduction (schizogony) and sexual reproduction (gametogony) takes place, resulting in production of unsporulated oocysts, which are shed in the cat feces. Cat has the habit to defecate and cover it with soil. This might help in the survival of oocysts for a longer duration (one year) in the environment.

Unsporulated oocysts are spherical to subspherical measuring 10 x 12 µm in diameter. When observed under light microscope, the oocyst wall is made up of 2 colourless layers. The sporont present in the oocyst fills it almost entirely. Sporulation occurs in the external environment outside the cat after about 1-5 days, depending on the temperature of soil and aeration.[15, 17, 18] Figure 8 shows unsporulated and sporulated oocysts.

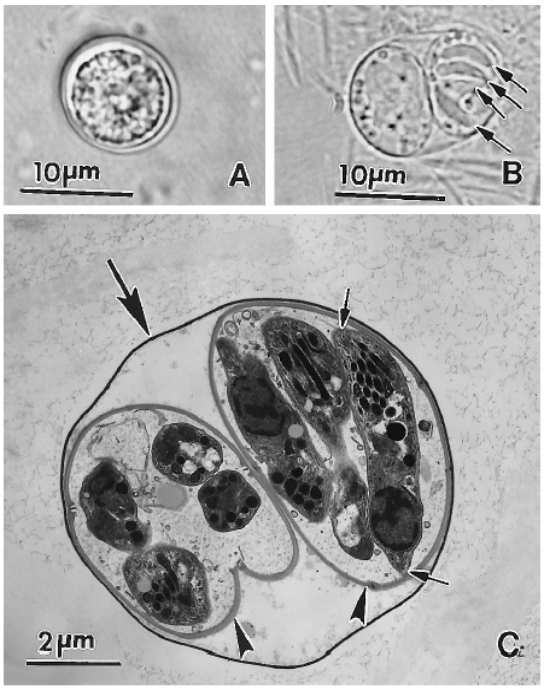


Figure 8: Oocysts of *T.gondii*. (A) Unsporulated oocyst containing central mass (sporont) occupying most of the oocyst. (B) Sporulated oocyst with two sporocysts, the arrows show four sporozoites in one of the sporocysts. (C) Transmission electron micrograph of a sporulated oocyst. Large arrow shows the thin oocyst wall, arrowheads show the two sporocysts, and small arrow shows the sporozoites, one of which is cut longitudinally, courtesy[17]

The nucleus of sporont divides twice giving rise to four nuclei, followed by division of cytoplasm. This results in formation of two spherical sporoblasts each containing two nuclei within the oocyst wall. Sporoblasts elongate and form sporocysts. Each sporocyst measures about 6 x 8 µm. The sporocyst wall is made up of two layers, an outer thin electron dense and an inner moderately electron dense layer.

The two nuclei within the sporocysts divide and produce four sporozoites. Thus finally the oocyst consists of 2 sporocysts, each containing 4 sporozoites, and subsequently 8 sporozoites are released from one oocyst. Sporulated oocysts are ellipsoidal to subspherical, measuring about 11 x 13 μm in diameter.

When observed under electron microscope, sporulated oocysts consist of 3 layers, an outer electron dense, middle electron-lucent, and an inner moderately electron dense layer. The outer layer is lost on exposure to 1.3% sodium hypochlorite. The oocyst wall has a single micropyle, which may be located anywhere on it.

The micropyle measures around 350 nm in diameter, and is made up of 3 layers in continuity with the three layers of the cyst wall. However, there are certain differences, the outer layer of micropyle is thin and moderately electron dense and the inner layer is electron dense and is slightly thicker than the inner layer of the oocyst.

The micropyle functionally may represent a permeable site in the oocyst wall, which is susceptible to the actions of different enzymes, which allow the entry of bile salts, trypsin and carbon dioxide. The rupture of sporocyst releases sporozoites from it. Ultimately the sporocyst residual consist of amylopectin granules and lipid bodies.

When observed under electron microscope, sporozoites are structurally similar to tachyzoites. But they differ in the composition of micronemes, rhoptries and amylopectin granules. Sporozoites measure 6-8 x 2 μm in size and have a sub-terminal nucleus.[17]

The electron microscopic picture of a sporozoite is presented in figure 9.

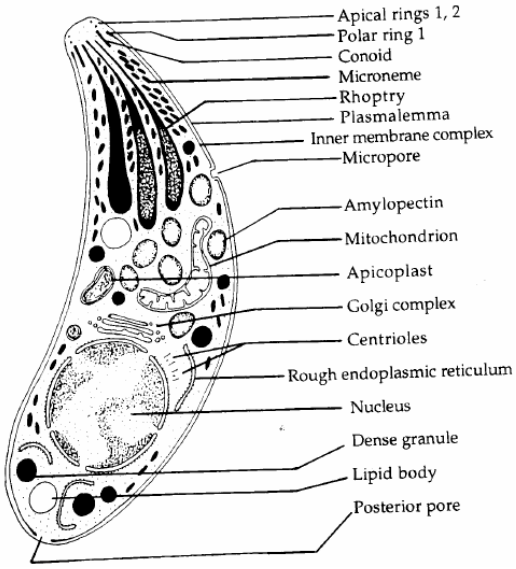


Figure 9: Schematic diagram of a T.gondii sporozoite, courtesy[17]

Ultra structural comparison of Tachyzoites, Bradyzoites and Sporozoites: All these morphological forms are ultrastructurally similar, but differ in the amount of certain organelles and inclusion bodies present in them, as shown in table 5.

Table 5: Ultrastructural differences between tachyzoite, bradyzoite, and sporozoite. Courtesy:condensed from[17]

Stage	**Electron dense granules**	**Amylopectin granules**	**Lipid Bodies**	**Micronemes**	**Rhoptries**
Tachyzoite	Numerous	Few, small or absent	Rare	Few	Uniformly labyrinthine
Bradyzoite	Few	Numerous, large	Absent	Many	Uniformly electron dense
Sporozoite	Numerous	Numerous, large	Numerous	Intermediate	Both labyrinthine and uniformly electron dense

Genetics

The nucleus of *T.gondii* is haploid, except for the zygote in the intestine of the cat. Sporozoites are the result of sequential meiotic and mitotic division. The genetic segregation follows the Mendelian laws. The haploid genome has been organized as 11 chromosomes, containing around 8 x 10^7 basepairs. There is also a circular mitochondrial DNA. [15]

Classification of *T.gondii* based on the genetic studies.

T.gondii is present worldwide. However, it shows limited genetic diversity. This is because the strains isolated in particular region show clonality: a single set of genes are inherited by *T.gondii* in a particular geographic zone, and the offsprings belonging to a particular clonality show similar phenotypic characteristics. Thus, the strains of *T.gondii* can be typed using a single locus. *T.gondii* has been classified into 3 genotypes I, II and III, based on restriction fragment length polymorphism.[15]

However, the majority of isolates from South America, Africa and Asia, do not fit into these three major genotypes. These clusters of genotypes have been grouped, into new haplogroups. These have also been considered successful clonal lineages as they have been distributed over large areas of continents. To date, 12 haplogroups have been identified, including the three major genotypes.

The virulence of *T.gondii* is related to genotypes in animals. However, what is true with animal infections is not true with human infections.[15, 20]

In table 6, the genotype of *T.gondii*, distributed in different geographical areas of world is presented.

Table 6: Geographical distribution of T.gondii genotypes. Courtesy: modified from[21, 22, 23, 24]

Geographical area	Genotypes
Europe	Type II (haplogroup 2), Type III
North America	Type II (haplogroup 2), haplogroup 12, Type III (haplogroup 3)
South and central America	Type II, Type I, haplogroup 6, atypical genotypes
Africa	African 1,2,3 (haplogroup 6); Type III (haplogroup 3), Type II
Egypt[22]	Type II, Type III, atypical genotypes
Middle East[23]	Type II, Type III
Turkey[23]	Type II, Type III, African 1
Asia	Type III
India[24]	Type III

Antigens

All infective stages of T.gondii share common proteins, although there are stage specific proteins. Tachyzoites of T.gondii have four major surface proteins, of 22, 30, 35 and 43 kDa size, of which p30 is the major type.[15]

Cultivation

T.gondii can be cultivated in laboratory animals such as mice, hamsters, guinea pigs and rabbits, and also in chick embryos and cell cultures. Intraperitoneal inoculation in mice results in ascites and after 8 weeks tissue cysts can be seen in the

brain of infected mice. Virulent strains can cause illness in the mice and can also kill them. Most strains do not kill mice.

Within 3 days of inoculation of tachyzoites in cell cultures, tissue cysts are formed, although the yield is lower than that produced in mice. Virulent mouse strains grow rapidly destroying the cells; while avirulent strains grow slowly and cause less damage.The mean generation time of tachyzoites of the virulent RH strain is 5 hours. The oocysts can be obtained by feeding cats with tissue cysts from infected mice from the cat's faeces. Feline enteroepithelial stages of *T.gondii* have not been cultivated in vitro so far.[15]

Figure 10 shows life cycle of *Toxoplasma gondii.*

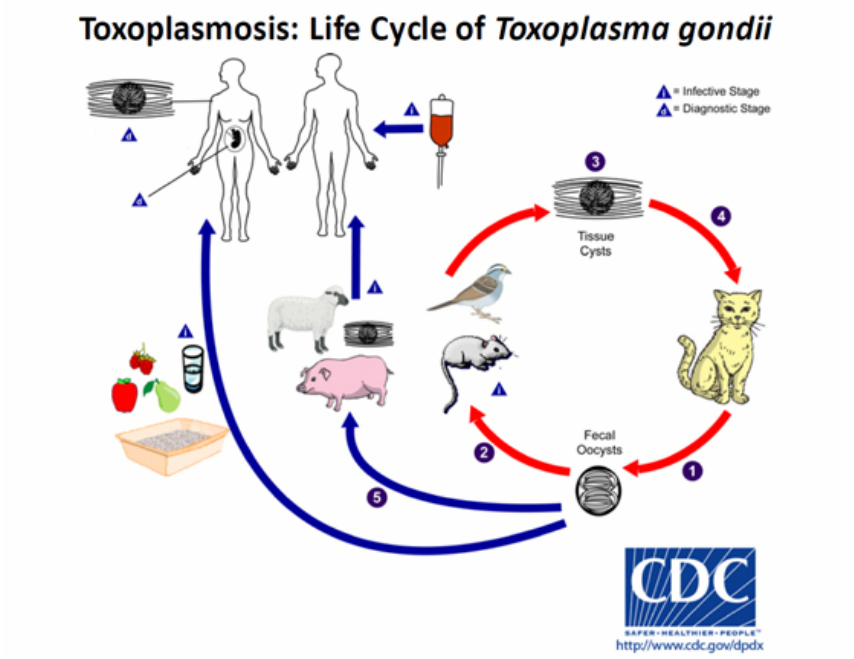


Figure10: **Life cycle of *T.gondii*. Courtesy**[25]

1. Large numbers of unsporulated oocysts are shed for 1-2 weeks in the infected cat's faeces. Once shed, the oocyst sporulates in 1 to 5 days, becoming infective and remains infective for more than a year in the moist soil.

2. Intermediate hosts (all non – feline vertebrates such as humans, birds, rodents, and beavers) become infected after ingestion of infective oocysts present in contaminated food and water.[19]
3. After ingestion by intermediate hosts, oocysts release sporozoites which readily convert to tachyzoites. Antibodies are produced in response to *T.gondii* infection. They limit the tachyzoite replication in mice, especially in lungs and brain.These tachyzoites localize in muscle and neural tissue and develop into tissue cyst containing bradyzoites.[26]
4. Cats acquire toxoplasmosis by eating infected rodents resulting in an enteroepithelial cycle, sexual reproduction and production of oocysts.[26]
5. Cats may also become infected directly by ingestion of sporulated oocysts. Thus infection in cats can occur due to ingestion of bradyzoites, tachyzoites or oocysts.
6. In infected rodents like mice, the parasitic cysts are found in the amygdala and hippocampus of brain. There is behavioural alteration in mice and this increases the chances of predation by the cats. Thus the location of the cysts in the brain of the rodents may facilitate the completion of the cycle, because the behavior of the rodent is altered and made convenient for the feline host to catch and feed on the predator.[27]
7. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment. Courtesy: modified from[28]

Modes of Infection: Humans can become infected by any of the several routes. Figure 11, shows the sources of *T.gondii* infection in humans.

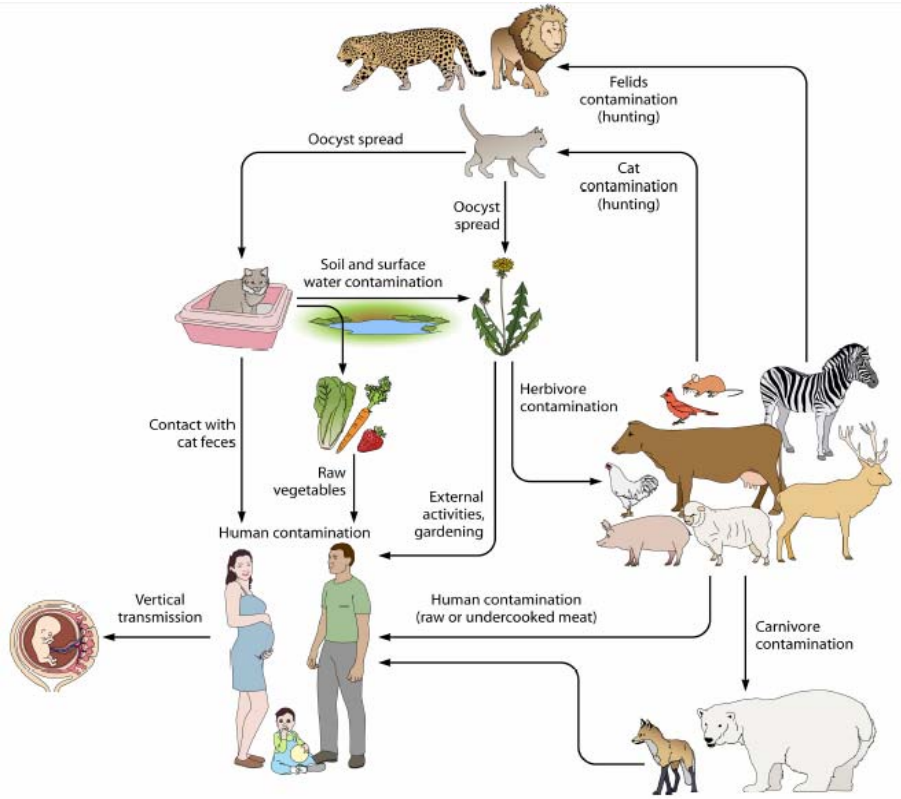


Figure 11 : Sources of *T.gondii* infection in humans. The various sources of food-borne and environmental contamination of humans are represented. Courtesy[21]

The following are the common modes of transmission of *T.gondii* infection in man:

a. Consumption of food or water contaminated with cat feces or by contaminated environmental samples such as fecal-contaminated soil or during changing the litter box of a pet cat

b. Blood transfusion or organ transplantation of solid organs such as heart, kidney, lung and liver

c. Transplacentally from mother to fetus, by means of tissue cysts formed in placenta.

d. Eating undercooked meat of animals containing tissue cysts. Carnivorism between intermediate hosts can transmit *T.gondii* to the uninfected host. There is no need of definitive hosts such as cats in this scenario. Man can be considered as a dead end host, unlike other mammals, which may contribute in the completion of lifecycle as part of the food chain.

e. Accidental inoculation of Toxoplasma among laboratory workers handling live toxoplasma.

f. Abattoir workers handling raw meat may get infected on exposure to tissue cysts, of infected animals. [6,29]

Pathogenesis and Pathology in Man:

The course of toxoplasmosis in human beings depends on the immunological status of the individual, genetic background, inoculum size and the virulence of the parasite.

T.gondii is an obligate intracellular coccidian parasite. Infected cats do not manifest any disease, but they shed millions of oocysts in their faeces. The oocysts are highly resistant to environmental changes.[2] Humans acquire infection by the ingestion of food or water contaminated with oocysts or by eating tissue cysts containing bradyzoites in infected meat of an intermediate host. The infective forms, bradyzoites or sporozoites are released from the tissue cysts and oocysts respectively by the action of proteolytic enzymes in the stomach which digests the cyst wall**.** The bradyzoites, being resistant to proteolytic digestion, initiate infection in the small intestine. These bradyzoites or sporozoites penetrate the intestinal epithelial cells and convert to tachyzoites. After invading the enterocyte the parasite replicates rapidly.

As a result the host cell lyses and releases actively dividing tachyzoites. The parasite can also traverse the epithelium by gliding motility, without disrupting the endothelial layer and infect the lamina propria of the intestine. This initiates the recruitment of dendritic cells, which are induced with enhanced migratory response, and thus facilitates parasitic dissemination.

Dissemination via dendritic cells is more efficient by Type 2 avirulent strains as compared to virulent Type 1 strains of the parasite. Eventually tachyzoites spread to mesenteric lymph nodes and multiply enormously and disseminate via the bloodstream throughout the host.

The parasite can lodge in skeletal muscle, heart, kidney, adrenals, eyes and myocardium and form tissue cysts.These cysts may remain throughout the host's life and pose a threat of reactivation of latent toxoplasmosis, when the host's immunity is low.

T.gondii has the ability to cross the blood brain barrier, and may result in the formation of cysts in brain. The placental barrier can also be penetrated by T.gondii parasite and can infect the fetus in about 30-50% of infections, which are acquired during antenatal period. Congenital infection is rare if mother had been infected before pregnancy.

The clinical features depend upon the extent of dissemination of the parasite to various organs of the host. Focal areas of necrosis are formed in these organs. The intracellular growth of tachyzoites causes necrosis.

Infections in the brain show foci of necrosis, microglial nodules and perivascular mononuclear infiltration, along with free and intracellular tachyzoites. Areas of coagulation necrosis are formed as a result of vascular thrombosis. The formation of necrosis around the ventricles or aqueduct of Sylvius or the foramen of

Monro may result in hydrocephalus. There may be calcification of necrotic areas of brain.

Involvement of central nervous system can be seen in addition to involvement of the eyes in toxoplasmosis. Necrosis of retina resulting in bilateral retinochoroiditis and granulomatous inflammation of choroid can be seen.

The lesions in toxoplasmosis vary in size from microscopic to macroscopic. Microscopic lesions are seen in the eye, while macroscopic lesions are seen in the other infected organs. Necrotic lesions in the brain may lead to encephalitis in immunosuppressed, as well as few immunocompetent individuals. Necrosis is followed by inflammation by the infiltration of mononuclear cells.

The inflammation gets subsided when the tissue cysts start to develop, excepting a few cases where inflammation might persist for months or years after primary infection.[15, 30, 31]

Host responses

Innate immune response:

1. Enterocytes infected with parasites secrete chemokine which recruit dendritic cells in the lamina propria. The infected dendritic cells produce interleukin 12 (IL12), which stimulates synthesis of Interferon γ (IFN γ) by Natural Killer (NK), NKT and T cells.
2. Inflammatory monocytes are recruited from bone marrow to the site of infection in the gut. They produce Nitric oxide (NO), which restricts the growth and proliferation of T.gondii.
3. Neutrophils are the first immune mediating cells reaching the site of infection. They also secrete IL12, and kill the parasite by oxygen dependent and oxygen independent mechanisms. Hence they play an important role in mucosal immunity.

4. Dendritic cells are the initial antigen presenting cells. They are either directly infected by the parasite, wherein it multiplies and processed for antigen presentation or the dendritic cells ingest apoptotic enterocytes which are then digested and processed for antigen presentation. Both conventional and plasmacytoid dendritic subsets are activated by *T.gondii* infection.
5. The plasmacytoid dendritic cells stimulate release of high levels of IL12 and upregulate the expression of Major Histocompatibility complexes, MHC1, MHC2 and other accessory molecules like CD86. This helps in priming of CD4+ T cells.
6. In addition the bystander dendritic cells (and not infected DCs) interact with CD8+ T cell in the draining lymph nodes in presence of the antigen. This is a part of the priming of CD8+ as an "Early T cell response"
7. Toll like receptors (TLR):
    a) TLR-11 is required by dendritic cells, for IL12 production. The TLR-11 recognizes the actin binding "profilin" protein on the parasite which is used for gliding motility.
    b) The TLR -11 also helps in prevention of immunopathology by regulation of secretion of IFN-γ by natural killer cells
    c) TLR-2 when stimulated by parasitic ligand Glyco-phosphatidyl-inositol (GPI) induces macrophage activation and Nitric oxide production. It also helps in secretion of chemokine CCL2.

Adaptive immune response:

This is initiated by antigen presenting cells (APC). Through Toll like receptors, they recognize that host is infected and stimulate the secretion of IL-12, which induces production of IFN-γ by NK cell, CD 4+ and CD8+ cells.

Activated CD4+ cells produce IL-2 (T cell mitogen), which along with IFN-γ, regulate the production of parasite specific CD4+ and CD8+ T cells, releasing abundant IFN-γ at the site of parasitic infection. The IFN-γ induces the generation of Nitric oxide and reactive oxygen intermediates (ROI).

The CD8+ T cells also cause direct killing of parasite via "perforin" dependent mechanisms. It is the principle T lymphocyte population associated with control of chronic infection and prevention of reactivated infection in brain. They are activated via MHC-1 bound parasitic antigen. This activation is via direct interaction with infected host cell as opposed to cross presentation by dendritic cells in innate immunity.

The antigen presenting cells for CD8+ not only include cells such as dendritic cells and macrophages, but also cells like endothelial cells, epithelial cells, astrocytes. The effector mechanisms of activated CD8+ T cells are:

a. Production of cytokines, especially IFN-γ, leading to anti-parasitic effectors in macrophages, microglia and astrocytes.
b. Production of anti-inflammatory cytokines IL-17 and IL-27, which down regulate the inflammatory response to *T.gondii* during infection.
c. Cytotoxic activity (CTL) against toxoplasma infected cells, by perforin mediated lysis.

Interferons:

Macrophages activated by IFN-γ acquire anti-parasitic activity. It is dependent on synthesis of reactive nitrogen intermediates.

The Interferon response genes (IRGs) belong to a class of IFN-γ induced proteins, which target vacuolated pathogens such as Toxoplasma gondii. They load

GTPases and induce the disruption of the Toxoplasma vacuole, leading to degradation of parasite via auto phagosomal delivery to the lysosomes.

Virulent strains however, evade vacuolar disruption via phosphorylation of several IRG proteins through parasite kinase ROP18. IFN-γ can also induce damage to *T.gondii* vacuole via an autophagosome independent mechanism involving autophagy protein Atg5.

Immunoregulation:

Anti-inflammatory cytokine, IL-10 inhibits the production of IL-12, IFN-γ, TNF-γ and IL-6 from macrophages and microglia in the brain. Depletion of IL-10 results in increased CD4+ T cells and macrophages and a lethal inflammatory response.However, the down regulation of Type 1 immune response in brain may facilitate persistence of parasite in the brain resulting in chronic infection.[26]

Clinical features

Primary disease:

Most cases of toxoplasmosis are asymptomatic or have mild flu like illness, with low grade fever, and painless lymphadenopathy. Approximately 90% are asymptomatic and remaining 10% have nonspecific self –limited illness.[32]

After an incubation period of 5-20 days, a syndrome consisting of fever, headache, malaise, myalgia, lymphadenopathy, hepatosplenomegaly, retinochoroiditis and atypical lymphocytosis may develop. In majority of the cases, a single cervical lymphnode is enlarged, but in a few cases, there may be generalized lymphadenopathy. The lymphadenopathy persists for duration of 4-6 weeks. The rare

complications are development of a maculopapular rash, hepatitis, encephalomyelitis or myocarditis.[31]

Congenital disease:

Primary infection of a sero-negative pregnant woman by *T.gondii*, can cause placental infection resulting in congenital infection of the fetus. The other reasons for congenital infection of the fetus are when the mother is immunocompromised (reactivation of latent toxoplasma infection), immunocompetent mother with reinfection by a different strain of parasite. In congenital toxoplasmosis the tachyzoites present in the blood may cross the placenta and infect the fetus.[20]

The chances of fetal infection by T. gondii, increases with the stage of pregnancy from 5 to 15 % in the first half to 60 to 80% in the second half of gestation. Conversely the chances of serious lesions and death decrease from 70 to 80% in the first half to less than 10% in the second half of gestation.[33]

Most of the children afflicted by congenital toxoplasmosis are asymptomatic at birth. The severity of complications is highest in the earlier part of pregnancy, resulting in spontaneous abortions, hydrocephaly, premature births, still births and mental retardation. Fetal infection during last trimester, are usually asymptomatic, subclinical or can present as recurrent chorioretinitis, impaired psychomotor development throughout early adulthood.[26, 34, 35, 36, 37]

The classic triad associated with congenital toxoplasmosis includes hydrocephalus, intracranial calcifications, and chorioretinitis. Prenatal ultrasound may reveal intracranial calcifications, ventricular dilatation, increased placental thickness, and enlarged liver.[32, 38]

Figure 12, shows congenital toxoplasmosis scar, of an eye.

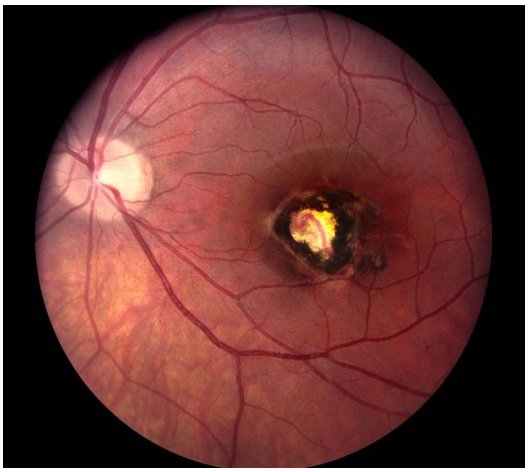


Figure 12: Congenital toxoplasmosis scar. Courtesy[39]

In the fetus, initially there will be generalized infection, later the parasite clears from the visceral organs. Overall about 15% of infants with congenital toxplasmosis have involvement of brain or eyes. But eventually 85% of those with asymptomatic infection will develop late complications such as hydrocephalus, mental retardation, and eye diseases.

The systemic manifestations seen are fever, jaundice, hypothermia, vomiting, diarrhea, lymphadenoapthy, hepatosplenomegaly, pneumonitis, myocarditis and rash in the postnatal period of infected newborns. Blood examination may show anemia, thrombocytopenia. CSF analysis may show CSF pleocytosis, and high CSF protein.

Neurologic complications of toxoplasmosis are seizures, psychomotor retardation, deafness, hydrocephalus, microcephaly, and prominent intracerebral calcifications. The ocular involvement in congenital toxoplasmsosis may be in the form of bilateral retinochoroiditis, granulomatous inflammation of choroid, uveitis and vitritis.

Congenital transmission may be reduced by antenatal treatment by giving a combination of pyrimethamine and sulphadiazine in case of suspected fetal infection.[40]

Infected mothers usually have no symptoms, but there will be temporary parasitemia in the mother. The toxoplasma parasite will reach placenta and form focal lesions in the placental tissue in the form of tissue cysts with bradyzoites. Since the fetus is entirely dependent on placenta for survival, the fetus may get infected from these infective foci.

In Austria and France, it is compulsory by law to test all pregnant women for T.gondii by serological tests, because of very high (70-90%) prevalence of infection. This higher prevalence of infection in France is attributed to eating of undercooked or raw meat.[8]

Ocular disease

Acute infection or reactivation of latent infection (as a result of immunosuppression, HIV, cancer chemotherapy) results in chorioretinitis. The characteristic findings are vitreal inflammation with white focal lesions described as "headlight in the fog".[32]

Worldwide toxoplasmosis accounts for about one third of cases of retinochoroiditis. In the past it was thought as late sequelae of congenital toxoplasmosis, but now it is proved that majority of cases of retinochoroiditis is acquired postnatally.

Ocular involvement may be delayed several months after primary infection and is not usually present in cases of recent primary infection

The major clinical presentation of chorioretinitis is a focally necrotic retinal lesion with hazy outlines. The symptoms of active eye disease are pain, photophobia, and blurred vision. The characteristic features in ocular toxoplasmosis are recurrent episodes of chorioretinitis at the borders of the scars and this may result in blindness.

There is a probability of certain *T.gondii* strains having a tropism for the tissues of the eye. The host factors such as immune status and certain *T.gondii* strains having tropism for the ophthalmic tissues are the factors determining the development of ocular toxoplasmosis.

In South America and Africa, a high prevalence of ocular toxoplasmosis is present. In the United Kingdom, the risk of ocular toxoplasmosis among black people born in West Africa is hundred times more than those who are born in Britain.

In Brazil and Alabama (USA) the incidence of Ocular toxoplasmosis are 17.7% and 0.6% respectively. Ocular toxoplasmosis in Brazil is more common and recurrent as compared to Europe.[20, 30, 31]

In India, Ocular toxoplasmosis cases have been reported from Coimbatore, TamilNadu as an outbreak in September 2004. It was hypothesized that municipal water could be the source of infection, since chlorination alone cannot destroy the oocyst of *T.gondii*.[41]

Toxoplasmosis in persons with AIDS and other causes of Immunodeficiency

Immunosuppression promotes both primary infection and reactivation of latent toxoplasmosis. Patients receiving cancer chemotherapy or who underwent organ transplantation are also susceptible. Reactivation of cerebral toxoplasmosis is a major cause of encephalitis in patients with AIDS. Toxoplasma encephalitis occurs in 10%

to 50% of AIDS patients, who are seropositive for Toxoplasma and have a CD4 count less than 100/µl.[20]

In the past about one third or more of AIDS patients with anti-toxoplasma antibodies had reactivation of their latent infection. Bradyzoites which are dormant in tissue cysts transform to tachyzoites and cause disseminated infection.

Off late the mortality due to Toxoplasmosis has reduced due to widespread use of Trimethoprim/Sulfamethoxazole prophylaxis for pneumocystis jerovecii pneumonia and the use of highly active anti retroviral therapy (HAART).[30]

Clinical features include headache, confusion, hemiparesis and retinochoroiditis. Cerebrospinal fluid shows lymphocytic pleocytosis, detectable toxoplasma specific IgG in serum and CT scan revealing lesions in the brain are suggestive of toxoplasmosis. Necrotic foci, with Toxoplasma organisms in brain biopsy or organisms demonstrable in spinal fluid are diagnostic.[8, 32, 42,]

The most commonly involved organs are brain, lungs and heart. There may be generalized toxoplasmosis in immunocompromised patients with involvement of liver, spleen, lymph nodes, testes, pancreas, intestines, thyroid, peritoneum, retina and spinal cord.[20, 30]

EPIDEMIOLOGY

Wildlife: Toxoplasma can infect more than 350 varieties of mammals and birds. Shedding of oocyst by felids, has a direct influence on contamination of environment, and hence of intermediate wild host. Infection in raccoons and bears, which are scavenging animals, is a good indicator of prevalence of *T.gondii* in the wild environment.

Factors which affect infection in wildlife include:

a. Climate: Areas which are dry and hot are unfavourable for the survival of oocysts, whereas tropical and humid conditions, promotes the viability of oocyst and their spread.
b. Susceptibility of host species to Toxoplasma infection
c. Size and weight of animal species: The prevalence is correlated with duration of life, which increases the chances of infection. This may explain the low infection rates (1-5%) among small rodents, such as *Mus musculus* (House mouse).
d. Diet and feeding behavior of host: Studies among wildlife of Amazon forest have revealed, higher prevalence of toxoplasma infection in terrestrial (ground dwellers) as opposed to arboreal (on trees) mammals, pointing towards possible environmental contact. This is also supported by the fact that, the infection is more common among black bears and red foxes in the Northern hemispheres. These observations suggested that among carnivores and omnivores the prevalence of infection was higher, when compared to herbivores. This is the result of cumulative effect, since the carnivores and omnivores are present at the top of the food pyramid.
e. Exposure of domestic animals: Pigs, cattle, sheep and poultry reared in secure farms, with clean sanitation facilities had lower prevalence of infection as opposed to those allowed to graze on outdoor farms.[21]

Prevalence in animals

Detection of chronic infection with *T.gondii* in animals relies mainly on serological assays. There is no clear reference or gold standard method for screening of the vast diversity of animals. Serological tests are widely used test for screening of

large number of species. In circumstances where sera are not available, meat juices are analyzed to determine the presence of toxoplasmosis.

Toxoplasma strains have been isolated from seronegatives, and the direct detection of parasite is usually absent in seropositives. Bio assays are one of the most sensitive means of detecting cyst in animal tissues. Tissues digested invitro with pepsin and trypsin is inoculated into mice, and the animal is observed for development of illness and serological findings. Bio assays done in cats are more sensitive, but expensive. Experimental cats are fed with tissue samples and after 3-14 days post feeding, the faeces are examined for the shedding of oocysts. These techniques are laborious and time consuming. They have now been replaced with molecular methods to detect DNA in meat.[21]

Prevalence of infection in Humans:

It has been estimated that 25 -30% of people around the world are infected by toxoplasmosis. The prevalence varies among various countries. Low prevalence (10-30%) is seen in North America, South East Asia, Northern Europe; moderate prevalence (30-50%) is seen in Central and Southern Europe, while high prevalence (> 50%) is seen in Latin America, France, Austria, and Tropical Africa.

Factors affecting seroprevalence in humans include:

a. Climate: The seroprevalence decreases in dry and arid regions, at high elevations, whereas humid and warm climates favour oocyst survival and hence their spread.
b. Infection rate in meat producing animals such as cattle, pigs and sheep.
c. Dietary habits: method of cooking food, hand washing, kind of meat or vegetables consumed, method of cleaning vegetables.

T.gondii tissue cysts are found in edible tissues of chicken and poultry products. They may not form a potential source of infection, because they are frozen for storage and cooked thoroughly which kills the parasite. The parasite has also been isolated from chicken eggs.

d. Economic, social and cultural behavior: In some countries like France, it was a tradition to consume raw meat extracts. Following increased awareness, such practices are reduced.

e. Water quality and sanitation coverage: Water is a potential source for *T.gondii* infection, in both animals and humans. Studies have shown oocysts can sporulate by 1-3 days in sea water and can survive upto 6 months in water treated with sodium hypochlorite or ozone, but not Ultraviolet radiation. Water borne toxoplasmosis outbreaks have been documented in Brazil, [43] Western Canadian province of British Columbia, and also from Coimbatore in India.

f. Seroprevalence increases with increasing age: The incidence of *T.gondii* antibodies increase with increasing age in humans. The incidence does not vary significantly between sexes.

g. The prevalence of antibody titres varies over different geographic areas and also among individuals in the same population. Abattoir workers are at increased risk.[21]

Genetic susceptibility:

It has been observed that all those infected will not end up in complications like toxoplasma encephalitis or congenital toxoplasmosis. This has been investigated by animal models using mice and North American white AIDS patients. There is a significant association among human beings with Class II MHC genes (HLA-Dq3).

Those who possess this HLA-Dq3, show a higher frequency of association with development of Toxoplasma encephalitis or congenital toxoplasmosis.[44]

Seasonal variation:

Prevalence of Toxoplasmosis depends on the environmental conditions. Climatic conditions in South India are thought to favour the sustenance and development of Toxoplasma Oocysts. A highly significant number of houses in this region own cats and other feline species. These factors might increase the chances of transferring *T.gondii* oocysts from soil and water to their food. On the contrary, West India is a dry, arid climate, which is not suitable for *T.gondii* parasites to maintain its life cycle.[4, 45] A higher risk of acquiring toxoplasmosis, may be present in the early spring.[46]

Prevalence of toxoplasmosis worldwide:

Prevalence of toxoplasmosis varies worldwide. Some of the studies have been included in table 7, table 8, and table 9.

Table 7: Prevalence of toxoplasmosis worldwide.

Year of study	Country	Study group	Mother's IgG (%)	Mother's IgM (%)
Low Prevalence countries				
2012	Kyushu, Japan[47]	Pregnant women	10.3	0.25
2015	Eastern China[48]	Pregnant women	15.2	2.9
2015	Lages, Brazil[49]	Pregnant women	16	1
2014	Southern Thailand[50]	Pregnant women	25	3
2016	Urmia, Iran[51]	Pregnant women	28.2	2.56
High Prevalence Countries				
2016	Accra, Ghana[52]	Preganant women	51.2	0
2014	Mansoura, Egypt[37]	Pregnant women, and Infertility ladies	57.52	NT
2011	Sao Paulo, Brazil[53]	Pregnant women	64.4	2.3
2015	Gurupi, Brazil[54]	Pregnant women	68.37	5.33
2014	Kinshasa, Democratic Republic of Congo[3]	Pregnant women	80.3	4.4

Note: NT = not tested

Table 8: Prevalence of IgG and IgM antibodies among newborns, worldwide

Year	Country	Study group	Babies' IgG (%)	Babies' IgM (%)
2015	Kufa, Iraq[55]	Newborns cord blood	35	0.33
2007	Trinidad and Tobago, West Indies[56]	Newborns cord blood	43.7	0.4
2012	Tehran, Iran[29]	Infants less than 1 year	60	0
2011	Sao Paulo, Brazil[53]	Newborns cord blood	64.4	0

Prevalence of toxoplasmosis in India:

The knowledge about human infections of toxoplasmosis in India is available to us from 4 types of studies.

1. Prevalence studies: where women of the reproductive age or pregnant women, have been screened for toxoplasma IgG antibodies. The seroprevalence ranges from 4.4% to 77% as shown in table 9.
2. Serological prevalence in women with bad obstetric history such as abortion, still birth, premature birth, and prenatal ultrasound scan showing intrauterine fetal malformations (ventriculomegaly, ascites, intracranial or intrahepatic calcifications) varies from 7.72 % to 49.52%, as shown in table 10 .
3. Case reports of congenital toxoplasmosis. Some of the studies have been included in table 11.
4. Prevalence among HIV patients. The seroprevalence of toxoplasma IgG antibodies in HIV patients varies from as low as 4 % to as high as 67.8 %, as shown in table 12.

Table 9: Prevalence of toxoplasmosis in India among women of child bearing age and pregnant women

Year	Place	Study group	IgG (%)	IgM (%)
2014	Wayanad, Kerala[57]	Young women in reproductive age group	4.54	1.01
2012	Salem, Tamil Nadu[33]	Pregnant women	9.9	3.9
2010	Chandigarh[58]	Pregnant women	15.33	3
2014	Different parts of India[4]	Women in reproductive age group	22.4	1.43
1997	Bombay, India[59]	Voluntary blood donors	30.9%	0
2016	North east states of India[60]	Antenatal women	48	NT
1991	Kumaon region[61]	Women	77%	NT
2015	Delhi[62]	Antenatal women	NT	1.8

Note: NT = not tested

Table 10: Prevalence of toxoplasmosis in India among women with bad obstetric history.

Year	Place	Study group	IgG (%)	IgM (%)
1995	Delhi[63]	Women with BOH	7.72	NT
2013	Tirunelveli[64]	Pregnant women with BOH	23	3.8
2015	Hyderabad[65]	Women with High risk pregnancy	28	0
2007	Assam[66]	Antenatal cases with BOH	44.6	8.9
2013	Vishakhapatnam[67]	Pregnant women with BOH	45	20
2009	Gujarat[68]	Women with BOH	46.7	41.3
2012	West Bengal and Andhra Pradesh[35]	Antenatal women with BOH	49.52	NT
2011	Lucknow[69]	Women with high risk pregnancy	NT	8.3

Note: NT = not tested

Table 11: Case reports of toxoplamosis in India

Year	Place	Case report / outbreak
1953	Bombay, India[13]	5 cases of toxoplasmosis, presenting with visual complaints.
1998	India[70]	Congenital toxoplasmosis in a 4 month old child, manifesting as hapatosplenomegaly and cholestatic jaundice. The child was successfully treated with a combination of sulphadiazine and pyrimetahmine
2006	Vellore, India[71]	A preterm baby boy presented with respiratory distress, on evaluation had pneumonitis.He progressed to have 2 episodes of convulsions. On skull radiography there were multiple calcifications in cerebral cortex and left orbit. Anti-toxoplasma IgM antibodies were seen in both mother and infant. The child was treated with sulfadoxine, pyrimethamine and folinic acid.
2008	Manipal, India[72]	A 27 year immunocompetent person, presented with sudden onset of headache associated with left sided weakness and vomiting. He had multiple cranial nerve palsies due to brainstem lesions, which was because of a toxoplasma granuloma. He was treated successfully with a combination of sulphadiazine and pyrimetahmine
2012	Mumbai, India[73]	A 2 month old girl, presented with neonatal hepatitis. IgG positive for toxoplasma, CMV, rubella. Repeat titres after 3 weeks, revealed increased IgG for Toxoplasma, and a positive *T.gondii* IgM. The child was successfully treated with a combination of Sulphadoxine and Pyrimethamine for 1 year.
2014	Pune, India[74]	A 35 year old male presented with diminished vision in the right eye, since 10 years. Fundus examination showed well defined pigmented scar on the macula of right eye and 2 small peripheral pigmented scars in the left eye. IgG anti-toxoplasma antibodies showed high titres. HIV test was negative. He was diagnosed as inactive toxoplasmosis and advised yearly follow up.

Table 12: Prevalence of Toxoplasmosis among HIV patients in India

Year	Place	Study group	IgG (%)	IgM (%)
2014	Bellary, Karnataka[75]	HIV patients	4	NT
2013	Tamil Nadu[76]	HIV patients	15	NT
2014	Khammam, Telangana state[77]	HIV patients	34.78	NT
1997	Bombay, India[59]	HIV patients	67.8	0

Note: NT = not tested

Table 13: Study of reports of toxoplasmosis from different parts of Karnataka.

Year	Place	Study group	IgG (%)	IgM (%)
2007	Bangalore[78]	Healthy voluntary blood donors	20.3	3.6
2014	Coastal Karnataka[4]	Women of child bearing age	37.3	2.9

The pan-India study on seroprevalence of toxoplasmosis among women of child bearing age reported an overall IgG antibody prevalence rate of 22.4%. About 70% of the women in child bearing age run the risk of infection during pregnancy. The prevalence rates varied widely: highest rates of 37.3% were found in coastal Karnataka and lowest rates of 8.8% were found in Gujarat.[4]

Laboratory diagnosis of Toxoplamosis

Direct evidence: *T.gondii* infection can be demonstrated by the following methods.

1. **Microscopy:** Microscopic examination of stained smears of materials obtained from bone marrow puncture, splenic puncture or centrifuged deposits of CSF, and smears made from biopsy tissues (preferably lymph node or muscle) or autopsy.

   Staining Reaction: Alkaline methylene blue and Giemsa stain have been used for this purpose. When stained with Giemsa stain, the cytoplasm appears blue and the nucleus reddish purple, as found in other protozoan parasites like malaria and leishmania.

   Histological diagnosis: Immunohistochemistry using immunoperoxidase reaction has been used to demonstrate *T.gondii* in tissue sections, or smears prepared from body fluids such as CSF, BAL and amniotic fluid. Detection of tissue cysts near

inflammatory necrotic lesions is useful to establish the diagnosis in acute infection or reactivation of latent infection. Multiple cysts are present around a necrotic area.[79]

2. **Cultivation:** Cultivation in systems such as animals: mice, guinea pigs and hamsters, cultivation in cell cultures such as Vero and MRC 5 cell lines, and fertilized hen's egg have been used to demonstrate live *T.gondii* from tissue and body fluids of clinically suspected patients.[6, 44, 80]
3. **Molecular methods:** Molecular methods to detect active infection by demonstrating specific DNA using PCR technique have been used. For this purpose, amplification of the B1 gene of the parasite is carried out. PCR technique can be used on amniotic fluid for prenatal diagnosis.

The sensitivity of PCR is reported to be higher, when maternal infection has occurred between 4th and 5th month of pregnancy. However, the reliability of PCR test in the first trimester of pregnancy is not known. Thus, a negative PCR test, does not rule out congenital infection. [40, 68]

Indirect evidence:

Serologic Tests: Serological tests to measure different antibodies and their profile during infection have been used to diagnose *T.gondii* infection. The initial serological tests done usually are detection of IgG and IgM antibodies to *T.gondii*. No single test, is of help in arriving at diagnosis

The serological tests are based on different antigen – antibody reactions such as neutralization (Sabin - Feldman dye test), indirect immunoflourescence test, differential agglutination test, immunosorbent agglutination assay, and enzyme linked immunosorbent assays.

Sabin - Feldman dye test:

The Sabin – Feldman neutralization test has been used as a gold standard in evaluating other tests. It is a sensitive and specific neutralization test, in which living organisms are lysed in the presence of complement and IgG anti-toxoplasma antibodies.

Methylene blue dye is used for detecting *T.gondii*, which is not killed by the membrane attack complex formed by the antibody and complement.[81]

The method assists in finding out a cytoplasm-modifying antibody in the patient's serum. If the patient's serum contains the specific antibody, more than 50 percent of free toxoplasma do not accept the stain and the cytoplasm remains colourless (those in intact cells however accept stain). In a negative serum, 90 to 100 percent of the free toxoplasma accepts the stain.

A positive dye test indicates the patient has been previously exposed to the parasite. A negative dye test rules out previous exposure to *T.gondii*, except in patients who have been infected very recently (within 2 weeks after exposure), immunosuppressed or having congenital agammaglobulinemia.

Technique:

Equal amounts (0.1 ml) of diluted patient's serum (1:16, 1:64, 1:128 and 1:256 dilutions), toxoplasma suspension obtained from the peritoneal exudate of infected mice and normal human serum (for "accessory factor") are incubated for 1 hour at 37^{o}C in a water bath.

To each tube is then added one drop of saturated alcoholic solution of methylene blue at pH 11. A drop of mixture is then put on a slide, covered with a coverslip and examined under the high power lens of the microscope.

The number of extracellular (free) toxoplasma with stained and unstained cytoplasm is counted. The highest dilution of the serum in which 50 percent or more of the organism have unstained cytoplasm is taken as the titre.

The dye test (due to persistent antibody) is positive early, persists longer and does not disappear completely.

The dye test gives false positive reactions with sarcocystis, trichomonas vaginalis, trypanosoma lewisi and other parasites. Hence, a dye test positive with a titre of 1:128 should be taken as diagnostic of active toxoplasmosis

The Sabin – Feldman dye test measures IgG antibodies. The IgG antibodies appear 1-2 weeks after infection; reach peak levels at 6-8 weeks, then decrease over 1-2 weeks. Low level titres, usually persist for lifelong.[8, 44, 79]

Indirect Fluorescent Antibodies Test (IFAT):

Indirect fluorescent antibody test is a simple, sensitive method for detection of *T.gondii* antibodies. The test uses whole, killed toxoplasma tachyzoites. They are incubated with diluted serum, then the appropriate fluorescein labeled anti- species serum is added and the results are viewed under a fluorescent microscope.

Sera that have anti-nuclear antibodies gives false positive results and the test may not detect low levels of IgG antibodies.

Pappas et al improvised the test by using by using 1.5% formalin fixed tachyzoites as antigen. The test has been modified by Remington et al to detect IgM antibodies in congenitally infected children.[82]

Differential Agglutination test:

In this test, suspensions of acetone treated tachyzoites and formalin treated tachyzoites are incubated parallely with the patients sera. Agglutination is seen with both kinds of antigens in patients who have recent infections. In patients who have past infections, agglutination at higher titres is seen with formalin fixed parasites and lower or negative results are seen with acetone fixed parasites.

The ratio of titres with acetone treated parasites and formalin treated parasites is used to interpret acute, non-acute, equivocal or non-reactive patterns. The ratio of titres with acetone treated parasites and formalin treated parasites, if it is low, excludes infection in the prior 13 months.

The words as acute and non-acute patterns may not reflect whether the patient had a recently acquired infection or not. It is thought that the behavior of the parasitic antigens in this test, when fixed with two different fixatives may be due to variation in the surface antigens of the parasite, as infection proceeds from an acute to a more chronic stage.[79]

Enzyme Linked Immunosorbent Assay (ELISA):

Different varieties of ELISA: IgG ELISA, IgG avidity test, IgM ELISA, IgA ELISA, and IgE ELISA have all been employed to diagnose *T.gondii* infection. However, a single test cannot be used for this purpose.

IgG ELISA:

IgG antibodies to *T.gondii* indicate, past infection.

IgG Avidity Test:

The anti-toxoplasma IgG antibodies after a recent infection bind weakly (low avidity) with the antigen, while IgG from the sera of chronically infected patients bind more strongly (high avidity). The antigen-antibody complexes dissociate readily in the presence of a protein denaturating agent such as urea, if the antibodies are of low avidity.

This test can be done to differentiate between recent and past infections. The time of conversion from low to high avidity is variable among different patients. If the avidity is high, it tells us that the patient is infected atleast 3-4 months earlier. Low avidity antibodies in pregnant women suggest recent infection.

IgM ELISA:

IgM ELISA, in the μ capture format (double-Sandwich format) is commonly used for demonstrating IgM class of antibodies to *T.gondii*. This test has been used to detect recent infection in adults and congenital infection in newborns.

IgM ELISA is more sensitive than indirect immunoflourescence to detect IgM in diagnosis of toxoplasmosis. Sera containing antinuclear antibodies and rheumatoid factor give false positives in indirect immunoflourescense test and no such false positivity is seen in μ capture ELISA. However, recently it has been found that mere detection of IgM antibodies does not provide evidence for vertical transmission or recent infection, as many of them are false positives.[83]

Immunoglobulin M immunosorbent agglutination assay (ISAGA)

In this test the IgM antibodies from the patient's serum bind to killed tachyzoites on a solid surface. The test does not use enzyme conjugate, and is read similar to an agglutination test.

The test is more sensitive and specific. In IgM positive result in ISAGA test in first 10 days of life of a neonate, needs to be repeated after 10 days, to rule out maternal contamination of IgM.

IgA ELISA:

The IgA antibodies to *T.gondii* can be detected in acutely infected adults or congenitally infected newborns using ELISA. The IgA antibodies may persist for many months or more than a year as are the IgM antitoxoplasma antibodies.

IgA assays are reported to be more sensitive than IgM assays for detecting congenital toxoplasmosis

IgE ELISA:

The duration of IgE anti-toxoplasma antibody seropositivity is briefer than IgM or IgA antibodies and thus can be used to detect recent infections. For neonatal diagnosis of congenital toxoplasmosis, IgE antibodies are less sensitive than IgM or IgA. A strategy to detect IgM, IgA and IgE to diagnose congenital toxoplasmosis is known to improve the diagnostic yield.[15, 44, 79]

Though, it is difficult to collect blood from neonates, serological tests on the venous blood is preferable to cord blood in the diagnosis of congenital infections. But, majority of the studies have employed cord blood for the reasons of feasibility.

Table 14, shows the clinical interpretation of anti-toxoplasma IgG and IgM test results in pregnant women.

Table 14: Interpretation of serological tests results for toxoplasmosis for pregnant women, performed at clinical (non-reference laboratories).

IgG test result	IgM test result	Clinical Interpretation
Negative	Negative	Woman has not been infected with Toxoplasma gondii. She is still at risk of acquiring primary infection during gestation and transferring to fetus
Positive	Negative	During first or second trimester, most probably due to an infection acquired before present pregnancy*
Negative	Positive or equivocal	Indicates acute infection. IgM can persist for prolonged duration, possibly due to infection prior to gestation. Confirm with another test.
Positive	Positive or equivocal	Indicates acute infection. IgM can persist for prolonged duration, possibly due to infection prior to gestation. Confirm with another test.

* In the third trimester, this result is most consistent with an infection acquired before pregnancy, courtesy.[84]

Guidelines for serological testing and management of toxoplasmosis during pregnancy, is shown in figure 13 (Algorithm A).

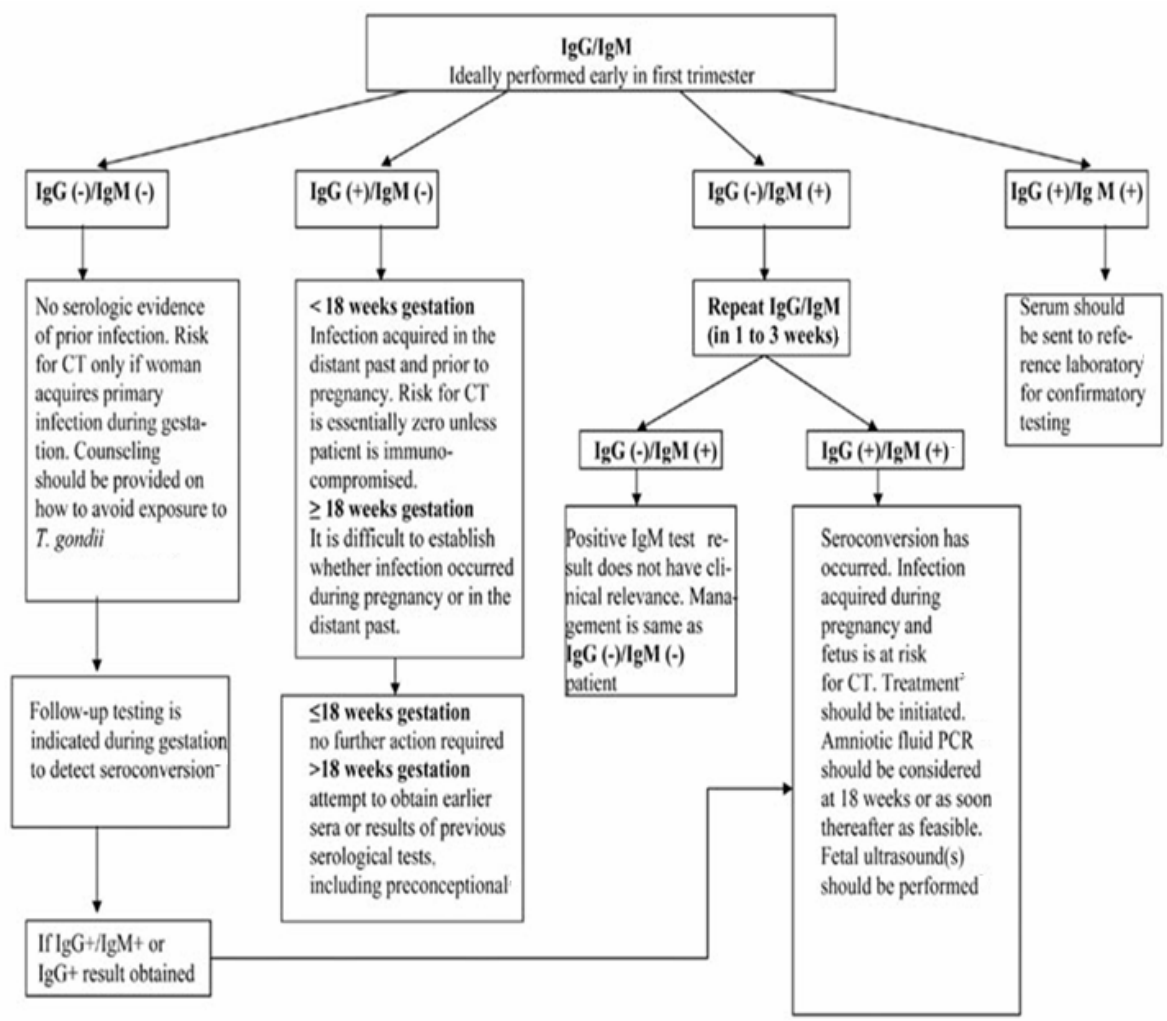


Figure 13: Algorithm A. Guidelines for serological testing and management of toxoplasmosis during pregnancy, courtesy[84]

A diagnostic approach for pregnant women, who are suspected or confirmed to have toxoplasmosis acquired during gestation, is shown in figure 13 (Algorithm B).

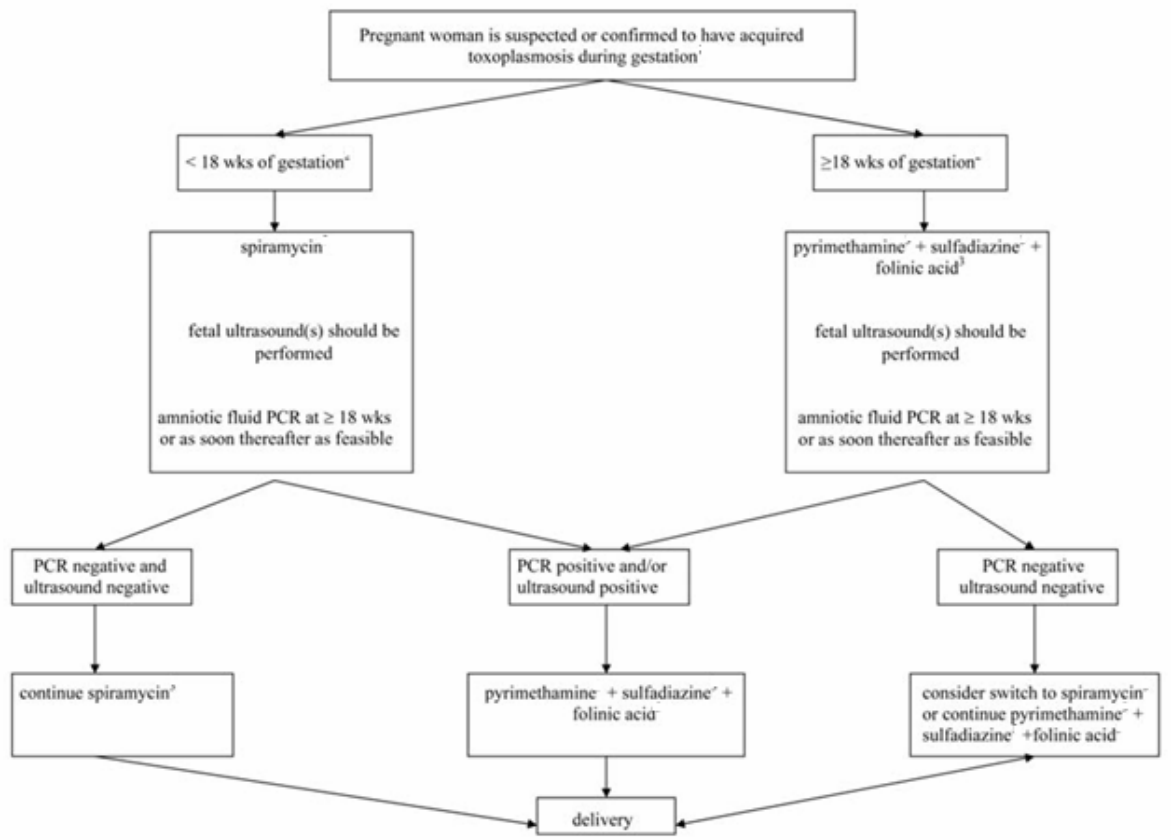


Figure 13: Algorithm B. Approach for pregnant women who are suspected or confirmed to have toxoplasmosis acquired during gestation, courtesy[84]

Radiological diagnosis

Plain Computed Tomography (CT) scan of Brain: Abscesses due to cerebral toxoplasmosis usually appears as multiple hypoattenuating or isoattenuating lesions with surrounding vasogenic edema and mass effect. Solitary lesions have also been less frequently reported. Calcifications are rare and usually seen after therapy with antitoxoplasmic agents. However, calcifications are commonly seen in congenital toxoplasmosis.

Contrast enhanced CT reveals lesions as either a thin, smooth or ill-defined rim of enhancement or a solid, eccentric nodular enhancement.

Magnetic resonance imaging (MRI): The imaging patterns may vary in encephalitis due to toxoplasmosis. These are usually depicted as hypointense lesions on T1 weighted images along with peripheral hyperintensity. On enhancement of T1 weighted images with contrast, the lesions appear as rim-like enhancement with surrounding hypointense edema.[85]

Ultrasonography: USG can be used to determine intrauterine fetal malformations such as ventriculomegaly, ascites, intracranial or intrahepatic calcifications.[40]

Treatment of Toxoplasmosis:

Toxoplasmosis is treated with a combination of pyrimethamine and sulfadiazine. This drug combination acts synergistically, to inhibit the production of the enzyme dihydro-folate reductase by the parasite. Thus it inhibits the synthesis of DNA, RNA, and proteins.

Folinic acid (Leucovorin) is given additionally, in order to counteract the bone marrow suppression caused by pyrimethamine.

During pregnancy spiramycin is preferred over pyrimethamine, as spiramycin gets concentrated in placental tissue, hence used in treatment of acute acquired toxoplasmosis in pregnancy to prevent fetal transmission of parasite.

Alternative regimens used for treating toxoplasmosis are azithromycin, clarithromycin, atovaquone or dapsone with cotrimoxazole /pyrimethamine plus folinic acid. These alternative regimens are less toxic and also less effective.[42, 86]

Table 15, shows the list of drugs used for treating toxoplasmosis and their mechanism of action.

Table 15: List of drugs used for treating toxoplasmosis with, their mechanism of action. Courtesy[87]

Drug	**Mechanism of action**
Pyrimethamine	Antifolate
Dapsone, sulfadiazine, sulfadoxine, sulfamethoxazole	Antifolate
Clindamycin	Protein synthesis inhibitor, acts on non-photosynthetic chloroplast like organelles called 'plastids'
Trimethoprim (usually combined with sulfamethoxazole)	Antifolate
Atovaquone	Mitochondrial electron transfer chain inhibitor
Doxycycline, minocycline	Apicoplast division inhibitor
Spiramycin, azithromycin, clarithromycin, erythromycin	Protein synthesis inhibitor

a) **Acute asymptomatic infection:** no treatment needed.

b) **Acute toxoplasmosis in pregnancy:** Spiramycin 1 gram orally thrice daily on empty stomach, until term or until fetal infection is documented.

c) **Fetal infection:** more than 12 to 18 weeks of gestation, a combination of Pyrimethamine 50 mg twice daily for 2 days, then 50 mg QID plus Sulfadiazine 25 mg per kg per day(in two doses) upto 4 gram per day, then 50 mg per kg BID (maximum 4 gram per day), plus Leucovorin 5 to 20 mg per day. Treatment is given until term and leucovorin is continued for another week.

d) **Chorioretinitis:** Pyrimethamine 200 mg single dose, then 50 - 75 mg per day plus Sulfadiazine 1 to 1.5 mg QID plus Leucovorin 5-20 mg is given three times per week. Treatment is continued for 1 to 2 weeks, even after resolution of symptoms. Leucovorin is continued for another one week.

e) Encephalitis in immune-compromised host:

Pyrimethamine 200 mg single dose is given, then 50 - 75 mg per day plus sulfadiazine 1 to 1.5 grams per day. Alternatively, clindamycin 600 mg every six hours per oral, or clindamycin 600 mg every 12 hours is given intravenously. Leucovorin is also given 10 to 20 mg per day per oral, intravenously, or intramuscularly. Treat until 4 to 6 weeks after resolution of clinical signs and symtoms. Leucovorin is continued for another one week.[32, 42]

Off late the mortality due to Toxoplasmosis has reduced due to widespread use of Trimethoprim/Sulfamethoxazole prophylaxis for pneumocystis jerovecii pneumonia and the use of highly active anti- retroviral therapy (HAART).[30]

Earlier studies have reported favourable outcome by using cotrimoxazole or clindamycin in cases of cerebral toxoplasmosis who are retropositive.[88,89]

Immunocompetent patients recover from toxoplasmosis without treatment, unless symptoms are severe or prolonged beyond a few weeks. Such patients can be treated by a combination of drugs such as pyrimethamine and sulfadiazine.

An acute infection in pregnant women is generally treated with the macrolide spiramycin, to prevent the infection of their child. However the ability of this drug to reduce the incidence of vertical transmission is controversial.[1]

Prevention:

Primary prevention:

Educating and creating awareness among general public, and especially women of child bearing age, regarding modes of transmission, clinical consequences, need for prompt diagnosis and treatment.

The following steps can be emphasized:

a. Fruits and vegetables should be washed properly before consumption.
b. Drinking untreated water should be avoided, which may be contaminated with oocysts
c. Avoiding contact with materials potentially contaminated with cat feces. Wearing gloves while handling cat litter box, or while gardening.
d. Treating cat litter box with near boiling water for 5 minutes before refilling.
e. Avoiding contact with mucous membranes while handling raw meat which may contain tissue cysts.
f. Washing hands thoroughly after handling of raw meat is necessary.
g. Smoked and dried meat can still be infectious. Cooking of meat "thoroughly" to 67^{o} C (153^{o} F). Meat should not be pink in the centre.
h. Washing of kitchen surfaces and utensils which come in contact with raw meat, by wearing gloves.

Secondary prevention:

a) Serological screening to identify women who have acquired *T.gondii* infection during gestation, and prenatal screening for fetal infection should be done.

b) Initiation of prompt treatment on diagnosis. Discussing with the patient regarding antimicrobial treatment in utero and medical termination of pregnancy.

c) Molecular methods for post natal screening of newborns to identify those infected late in pregnancy, and have not formed antibodies.[84]

Development of *T. gondii* Vaccines

1. The S48 strain Toxovax is a live vaccine, originally developed for use in sheep, to reduce the tissue cyst development. When used in cats, it inhibited sexual development of *T.gondii*.
2. The T-263 strain of *T.gondii* is a live mutant strain, designed to develop only partial infection in the feline intestinal tract, thereby reduces and prevents oocyst shedding by cats. Field trials done on US pig farms have shown reduced environmental contamination and less infection risk for the pigs.
3. Strains are being modified by irradiation, chemical treatment, and usage of selected recombinant antigens and new delivery systems like feline herpes virus type 1 vehicle for delivery.
4. Liposomal embedded antigens like soluble tachyzoite antigen, Tissue cyst antigen, tachyzoites plus tissue cyst and purified tachyzoite antigen are being evaluated.
5. Beauvillain et al. prepared a vaccine using a vesicle secreted by *T.gondii*, which is cell free and contains antigenic properties. He found a protective response against challenge with *T.gondii*, raising hopes of a vaccine production for human use.[90]

MATERIALS AND METHODS

Sample size calculation:

The sample size was 250. The sample size of 250 was calculated based on 22.4% seroprevalence of *T.gondii* antibodies in pregnant women found across the country in a previous study from India.[4] Based on this data with 95% confidence level and 5% absolute error, there was 95% chances of prevalence falling in the range of 17.4 to 27.4 (22.4±5).The sample size was calculated using the formula,

$$n=Z_{\alpha}^{2}pq / d^{2}$$

$$n= (1.96)^{2}\times22.4\times(100-22.4) / 5^{2}$$

$$n= 250$$

Where n = Sample size

Z_{α} =1.96, which is a constant

p = Prevalence

q = 100-p

d = 5% absolute error at 95% confidence interval

Sample Source & Method of Collection:

In this observational cross sectional study a convenient sample of 251 venous blood samples from pregnant women admitted for labour at R. L. Jalappa Hospital, Kolar, from December 2014 to October 2016, and 251 cord blood samples from their respective newborn babies were collected. Written informed consent was taken from the pregnant women, after duly informing them with the help of the information sheet. Institutional ethical committee clearance was obtained prior to the start of study.

Under aseptic precautions, 3 ml of peripheral venous blood sample was drawn from ante-cubital vein of pregnant women using 5 ml disposable syringe with 23 gauge needle (as shown in figure 14). Three ml cord blood sample was collected from the placental end of the severed umbilical cord of newborn babies, after the delivery by releasing the clamp.

The above samples were collected in separate sterile disposable tubes of 4 ml capacity with dimensions of 7.5 cm in length and 1.1 cm in diameter. The test tubes after collection were capped and labelled appropriately (as shown in figure 15).

The blood was allowed to clot at room temperature. The clot was separated from the walls of test tube using sterile disposable sticks. The tubes with clotted blood were centrifuged at 3000 rpm for 10 minutes in a REMI / ROTEK centrifuge.

Serum was separated from the blood using a 100 µl pipette into sterile screw capped vial of 2 ml capacity with dimensions of 4 cm in length and 1cm in diameter. The vials were labelled (as shown in figure 16), and stored at -20^{0}c initially and then later transferred to -80^{0}C and stored frozen until the samples were tested.

Figure 14 : Drawing blood

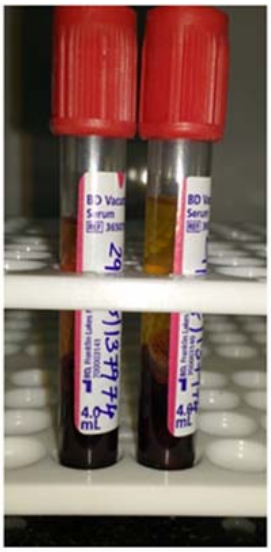


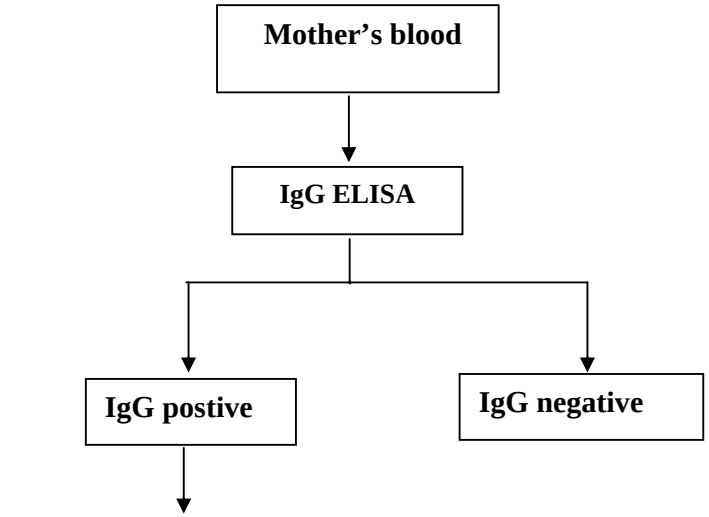
Figure 15 : Labelled sterile disposable tubes (7.5 cm x 1.1 cm, 4 ml capacity)

Figure 16 : Labelled screw capped vials (4cm x 1 cm, 2 ml capacity)

Processing of Samples:

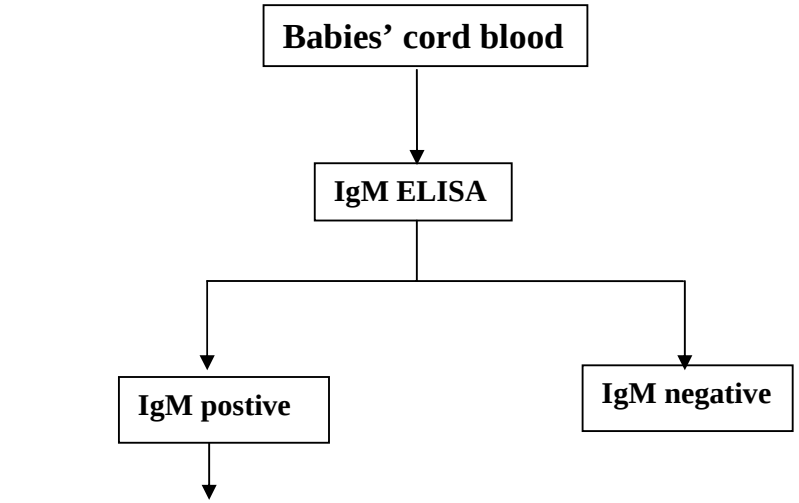
The babies' and mother's serum samples were tested for IgG and IgM anti-toxoplasma antibodies according to the following algorithms (Flowchart A and B), as shown in figure 17.

Flow chart A : Processing of mother's serum



1. Mother's sera was tested for IgM anti-toxoplasma antibodies to look for any recent infection
2. Respective babies' cord blood sample was tested for IgG anti-toxoplasma antibodies to look for any significant four-fold higher titres compared to their mothers, which may suggest intrauterine infection.

Flow chart B: Processing of babies' serum



1. Babies' sera was tested for IgG anti-toxoplasma antibodies to look for any significant four-fold higher titres compared to their mother's which may suggest intrauterine infection
2. Respective mother's sera was tested for IgM anti-toxoplasma antibodies to look for any recent infection

Figure 17: Processing of Samples (Flow Chart A and B)

Anti-toxoplasma IgG titres were also estimated in cord blood samples of newborn babies, if their respective mother's were positive for IgG antibodies against *T.gondii*. This was done to look for any significant four-fold higher titres in the cord blood compared to their mother's, which may suggest intrauterine infection. IgM ELISA was also performed on samples of mother's that were positive for IgG anti-toxoplasma antibodies to look for any recent infection.

A. IgG ELISA: IgG class of antibodies to *T.gondii* were estimated using IgG ELISA NOVATEC IMMUNDIAGNOSTICA kits. IgG ELISA is a quantitative test.

Figure 18 : IgG ELISA kit

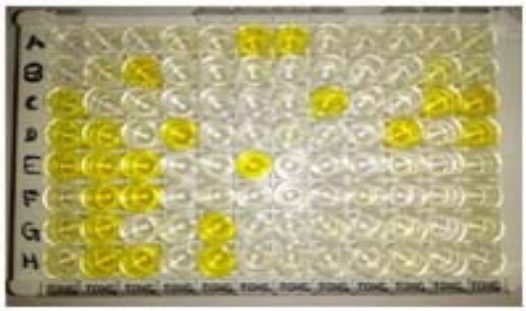


Figure 19 : IgG ELISA results

Principle:

The quantitative enzyme immunoassay is based on the principle of"Indirect ELISA" technique.

Microtitre strip wells were pre-coated with *T.gondii* antigens to bind corresponding IgG antibodies of the specimen. Serum samples and controls supplied in the kit were added to the pre-designated different microtitre wells and incubated for one (1) hour at 37^0 C. IgG antibodies to *T.gondii*, if present in the sample bound to the antigen in the well.

The plate was then washed to remove all unbound material present in the sample. Horseradish peroxidase labeled anti-human IgG conjugate was added to each well, as a detector antibody and the plate was incubated for thirty (30) minutes at room temperature. This conjugate bound to the toxoplasma IgG antibodies if present in the well. Finally the substrate solution containing chromogen and hydrogen peroxide was added to the wells and incubated for fifteen (15) minutes at room temperature. A blue colour developed in proportion to the amount of *T. gondii*-specific IgG antibodies present in the specimen.

The colour reaction was stopped by a stop solution consisting of 0.2 mol/L sulphuric acid producing a yellow colour, which was the end point. The enzyme substrate reaction was read by an ELISA micro well plate reader for absorbance at a wavelength of 450 nm. If the sample did not contain toxoplasma antibodies of IgG class, then the enzyme conjugate would not have bound and the solution in the wells would be either colourless or only a faint background colour would have developed.

Test procedure:

The procedure was completed without interruption. The strip holder was fitted with the required number of strips. All the reagents were dispensed from the tip of the pipette into the centre of the well of the microtiter plate without touching the wall of the well. The sequence of the procedure was carefully followed. Prior to commencing the assay, the distribution and identification plan for all specimens and controls was carefully established on the matrix sheet.

1) **Serum dilution:** 0.01 ml (10 µl) of serum sample was taken in a micropipette and placed at the bottom of a sterile tube (4cm x 1 cm) of 2 ml capacity. Then 1ml (1,000 µl) of the sample diluents, supplied with the kit was added and mixed. This gave a

dilution of 1 in 100. The diluted serum sample was thoroughly mixed in a vortex mixer.

2) **Addition of controls:** The first well in the row A was left as blank. Hundred micro litre (0.1ml) quantities of *T.gondii* IgG standard controls A(0 IU/ml) ,B(50 IU/ml),C(100 IU/ml) , and D(200 IU/ml), supplied with the kit were added to the first wells in the rows B,C,D, and E respectively.

3) **Addition of serum samples:** 0.1ml (100 µl) of samples diluted as above were added to the rest of the wells.

4) The wells were covered with the foil provided in the kit and the microtiter plate was incubated for 1 hour at 37^0 C.

5) **Preparation of wash buffer:** Phosphate buffer solution for washing the ELISA plate was prepared by addition of 10 ml of wash buffer concentrate provided with the kit, to 190ml of germ free redistilled water.

6) **Washing:** After completion of incubation period as above, the microtiter plate was washed 5 times with the working wash solution using an ELISA washer (Meril). At the end remaining fluid was carefully removed from the microtiter plate by tapping the strips onto the tissue paper.

7) 0.1 ml of horseradish peroxidase labelled anti-human IgG conjugate was added to all the wells except A-1 (blank)

8) The plate was covered and incubated at room temperature for 30 minutes.

9) The washing of the wells was repeated as in step 6.

10) **Addition of chromogen and substrate mixture :** 0.1 ml of tetramethylbenzidine (TMB) substrate solution was added to all the wells including the blank well (A-1)

11) The plate was covered and incubated in dark at room temperature for 15 minutes.

12) **Stopping the reaction:** After incubation at room temperature in dark, the microtitre plate was removed and 0.1ml of stop solution provided in the kit was added to all the wells.

13) When the stop solution was added, the blue colour developed in the wells turned to yellow colour.

14) **Reading the test:** The reading was taken using an ELISA reader (Meril) at 450 nmwavelength within 30 minutes.

Test validation:

In order for an assay to be considered valid, the following criteria had to be met, according to the manufacturer's instructions (as shown in table 16).

Table 16: Test validation criteria for IgG ELISA

Sl no.	In the test run	Absorbance value
1	Substrate blank	< 0.100
2	Standard A	< 0.200
3	Standard B	> 0.300
4	Standard C	>0.500
5	Standard D	>1.000

If these criteria were not met, the test was considered invalid and the test was repeated.

Calculation of results

In order to obtain quantitative results in IU/ml the (mean) absorbance values of the 4 Standards A, B, C and D were plotted on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 50, 100 and 200 IU/ml). A standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis) was obtained. Results were read from this standard curve employing the absorbance values of each patient specimen.

Interpretation of results

The following values were considered as a guideline:

- Reactive - >35 IU/ml
- Equivocal - 30-35 IU/ml
- Non- reactive - <30 IU/ml

B. IgM ELISA: IgM class of antibodies to *T.gondii* were detected using IgM μ capture ELISA NOVATEC IMMUNDIAGNOSTICA kit. IgM μ capture ELISA is a qualitative test.

Figure 20 : IgM ELISA kit

Figure 21 : IgM ELISA results

Principle:

The qualitative enzyme immunoassay is based on the principle of "Indirect ELISA" technique. Microtiter strip wells were precoated with anti-human IgM.Serum samples and controls supplied in the kit were added to the predesignated different microtiter wells and incubated for one (1) hour at 37^0 C. IgM antibodies to *T.gondii*, if present in the sample were captured by the anti-human IgM coated onto the well.

The plate was then washed to remove all unbound material present in the sample. Horseradish peroxidase labeled *T gondii* antigen conjugate, as a detector was added to each well and incubated. This conjugate bound to the captured toxoplasma IgMantibodies if present in the well. Finally the substrate solution containing chromogen and hydrogen peroxide was added to the wells and incubated. A blue colourdeveloped in proportion to the amount of *T.gondii*-specific IgM antibodies present in the specimen.

The colour reaction was stopped by a stop solution consisting of 0.2 mol/Lsulphuric acid producing a yellow colour, which was the endpoint. The enzyme substrate reaction was read by an ELISA microwell plate reader for absorbance at a wavelength of 450 nm. If the sample did not contain toxoplasma antibodies of IgM class, then the enzyme conjugate would not have bound and the solution in the wells would be either colourless or only a faint background colour would have developed.

Test procedure:

The procedure was completed without interruption. The strip holder was fitted with the required number of strips. All the reagents were dispensed from the tip of the pipette into the centre of the well of the microtiter plate without touching the wall of the well. The sequence of the procedure was carefully followed. Prior to commencing

the assay, the distribution and identification plan for all the specimens and controls was carefully established on the matrix sheet.

1) **Serum dilution:** 0.01 ml (10 µl) of serum sample was taken in a micropipette and placed at the bottom of a sterile tube (4cm x 1 cm) of 2 ml capacity. Then 1ml (1,000 µl) of the sample diluent supplied with the kit was added and mixed. This gave a dilution of 1 in 100. The diluted serum sample was thoroughly mixed in a vortex mixer.

2) **Addition of controls:** The first well in the row A was left as blank. One hundred microliter (0.1ml) quantities of negative control, cut off control (in duplicate), and positive control supplied with the kit were added to the first wells in the rows B, C, D, and E respectively.

3) **Addition of serum samples:** 0.1ml (100 µl) of samples diluted as above were added to the rest of the wells.

4) The wells were covered with the foil provided in the kit and the microtiter plate was incubated for 1 hour at 37ºC.

5) **Preperation of wash buffer:** Phosphate buffer solution for washing the ELISA plate was prepared by addition of 10 ml of wash buffer concentrate provided with the kit, to 190ml of germ free redistilled water.

6) **Washing:** After completion of incubation period as above, the microtiter plate was washed 5 times with the working wash solution using an ELISA washer (Meril). At the end remaining fluid was carefully removed from the microtiter plate by tapping the strips onto the tissue paper.

7) 0.1 ml of horseradish peroxidase labelled *T.gondii* antigen conjugate was added to all the wells except A-1 (blank).

8) The plate was covered and incubated at room temperature for 1 hour.

9) The washing of the wells was repeated as in step 6.

10) **Addition of chromogen and substrate mixture:** 0.1 ml of tetramethylbenzidine (TMB) substrate was added to all the wells including the blank well (A-1)

11) The plate was covered and incubated in dark at room temperature for 15 minutes.

12) **Stopping the reaction:** After incubation at room temperature in dark, the microtitre plate was removed and 0.1ml of stop solution provided in the kit was added to all the wells.

13) When the stop solution was added, the blue colour developed in the wells turned to yellow colour.

14) **Reading the test:** The reading was taken using an ELISA reader (Meril) at 450 nmwavelength within 30 minutes.

Test validation:

Test was considered valid if absorbance values of controls were within specified acceptance criteria as per manufacturer's recommendation (as shown in table 17).

Table 17: Test validation criteria for IgM ELISA

Sl. no	In the test run	Absorbance value
1	Blank	< 0.100
2	Cut-off control	0.150-1.300
3	Negative Control (NC)	< cut-off
4	Positive control (PC)	> cut-off

Note: Cut off value = Mean absorbance value of the cut-off control determination.

If it was not so, the test was considered invalid and the test was repeated.

Calculation of results

a. Sample O.D. ratio = Sample O.D ÷ Cut off Value

b. Calculation of Toxo IgM Nova Tec Units (NTU): Sample O.D. ratio × 10.

Interpretation of results

Toxo IgM units:

- Positive - >11 Units
- Equivocal - 9-11 Units
- Negative - < 9 Units

Collection of demographic, obstetrical and behavioural data:

On a predesigned proforma name, age, address, socio-economic status, and educational status of the pregnant mother's admitted for delivery, included in this study were recorded. Obstetrical and behavioural history, were also recorded.

Definition's used for filling the proforma:

Gestational age: Using gestational age, births can be classified into broad categories, as shown in table 18.[91]

Table 18: Classification of newborn based on gestational age

Sl.no	Gestational age in weeks	Classification
1	<37	Preterm
2	37 to <42	Term
3	≥42	Post-term

Socioeconomic status: Socioeconomic status of the pregnant mothers was calculated using B. G. Prasad's revised socioeconomic status scale (May2014) based on per capita monthly income of the family, as shown in table 19.[92]

Table 19: B.G. Prasad's socioeconomic status classification for May 2016

Sl no	Socioeconomic status class	May 2016
1	I	Rs.6,277 and above
2	II	Rs.3,139-6,276
3	III	Rs.1,883-3,138
4	1V	Rs.942-1,882
5	V	Below Rs.942

Birth weight: Based on birth weight newborn babies are classified as shown in table 20.[91]

Table 20: Classification of babies based upon birth weight

Sl.no	Classification	Birth weight in Kilograms (Kgs)
1	Normal	≥ 2.5
2	Low birth weight	< 2.5
3	Very low birth weight	< 1.5
4	Extremely low birth weight	< 1

Apgar score: The Apgar score is used to evaluate the newborn's condition. This is done at one and five minutes. Scores of 0-2 are given for each of the following: heart

rate, respiration, mucle tone, reflex irritability, and color, as shown in table 21. In case of asphyxia the score is less than six at one minute.[91]

Table 21: Apgar score

Sl.no	Sign	0	1	2
1	Heart rate	Absent	Slow (<100 beats/min)	Normal (>100 beats/min)
2	Respiration	Absent	Weak cry	Good strong cry
2	Muscle tone	Limp	Some flexion	Active movements
4	Reflex irritability	No response	Grimace	Cough/ sneeze
5	Color	Blue/pale	Body pink, extremities blue	Completely pink

Statistical Methods: To analyze the difference in proportions Chi square test, Fischer's exact test, and Odds ratio at 95 % Confidence Interval (C.I) were used. Unpaired't' test was used to analyze the differences in mean age among IgG positive and negative mothers, and mean titres of IgG positive mothers and IgG positive babies.

Mean of the anti-toxoplasma IgG antibody titre among pregnant women plus three standard deviations was used to detect recent infection. The data is presented as frequencies in tables with percentages, and as well as in bar charts, and graphs.

The IgG titres among positive mothers and babies were plotted separately in distribution curves. The IgG positive mothers and babies titres were plotted in a scatter plot. Pearson's correlation co-efficient was calculated with the line of regression to see the correlation between the titres observed in the mothers and their respective babies.

RESULTS

To screen the mothers from Kolar region for IgG class of antibodies to *T.gondii*, and to find out the prevalence of anti-toxoplasma IgG antibodies, serum samples from 251 pregnant women admitted for delivery between December 2014 to October 2016 at R.L.Jalappa Hospital, Kolar were tested for anti-toxoplasma IgG antibodies by the quantitative ELISA using NOVATEC IMMUNDIAGNOSTICA kit.

The age distribution and the gravid status of the subjects is presented in table 22.

Table 22: The age distribution and the gravid status of mothers

Age group (Years)	Number of women (n=251)		Total (Percentage)
	Primigravida	Multigravida	
18-20	38 (15 %)	6 (2 %)	44 (17.5 %)
21-25	61 (24 %)	84 (33.5 %)	145 (58 %)
26-30	14 (5.5 %)	39 (15.5 %)	53 (21 %)
31-35	0	9 (3.5 %)	9 (3.5 %)
Total	**113 (45 %)**	**138 (55 %)**	**251**

Majority of the women belonged to the age group between 21-25 years, which constituted 58 % of the population studied. However, multigravida (55 %) outnumbered primigravida (45 %).

Pie chart showing age distribution of study population (in percentage) is presented in Figure 22.

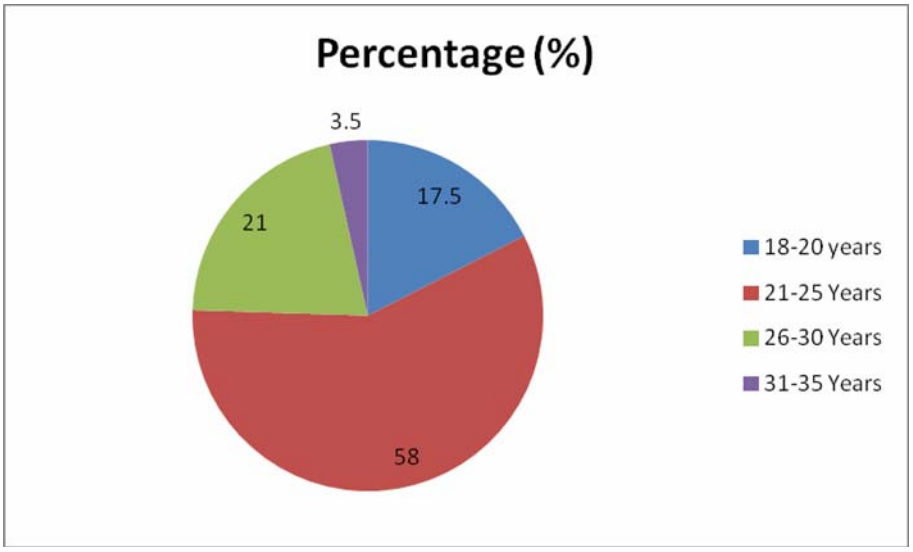


Figure 22: Pie chart showing age wise distribution of subjects

Almost all the women (95 %) were literate, and most of them worked as housewives, farmers, and manual labourers.

Socioeconomically, 57 % of the women belonged to lower middle and middle classes, and 43 % of the women belonged to lower socio-economic strata according to classification of economic status by B.G.Prasad.[92]

Prevalence of IgG anti-toxoplasma antibodies in mothers

Among the above population, antibodies to *T.gondii* could be detected in 53 subjects giving a seropositivity of 21.1 %.

Anti-toxoplasma IgG antibody titres among the positive mothers:

The prevalence of titres of anti-toxoplasma IgG antibodies, among the IgG positive mothers is presented in table 23 and figure 23. The titres in the population ranged between 35 IU/ml – 350 IU/ml. The mean titre was 167 ± 86 IU/ml, and the mode titre was 256 IU/ml. The highest titre range (301 IU/ml – 350 IU/ml), was found in only 2 subjects, accounting for 4 %. The lowest titre range (35 IU/ml – 50 IU/ml) was found in 8 subjects, accounting for 15 %.

Table 23: The prevalence of anti-toxoplasma IgG titres, among 53 IgG positive mothers.

IgG titre* (IU/ml)	Number, n=53	Percentage (%)
35-50	8	15
51-100	8	15
101-150	6	11
151-200	9	17
201-250	9	17
251-300	11	21
301-350	2	4
Total	**53**	**100**

* According to the kit manufacturer's instructions a titre of more than thirty five (35) IU/ml was considered as positive, and those below were considered as negative.

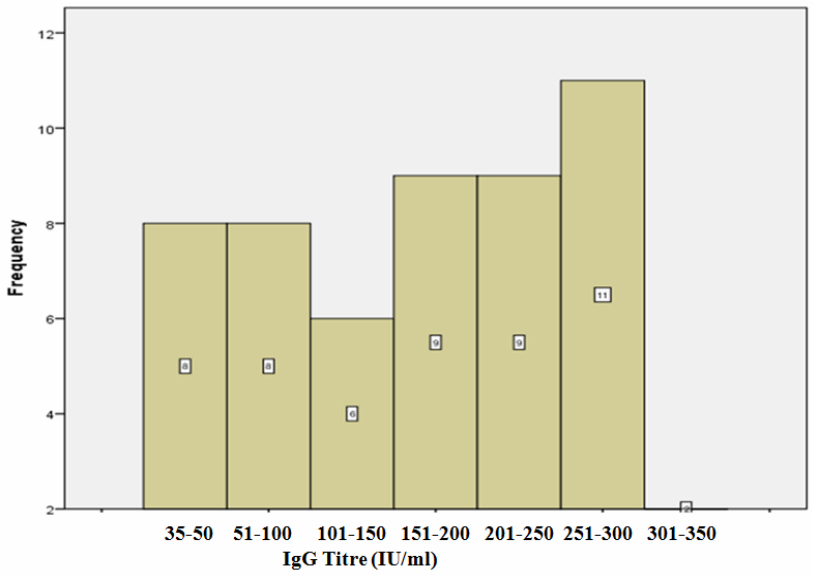


Figure 23: Histogram showing the frequency distribution among 53 IgG positive mothers.

Distribution of IgG anti-toxoplasma antibodies, among mothers who tested positive:

The distribution of anti–toxoplasma IgG titres among the 53 mothers is presented in figure 24. The distribution conforms to normal distribution pattern. The arithmetic mean ± standard deviation for the anti-toxoplasma IgG titres of 53 IgG positive mothers was 167 ± 86. Forty two (17%) of the women had an IgG titre more than the mean titre. However, none of the mothers had a titre of average plus 3 standard deviations (167+258), which amounts to 425 IU/ml.

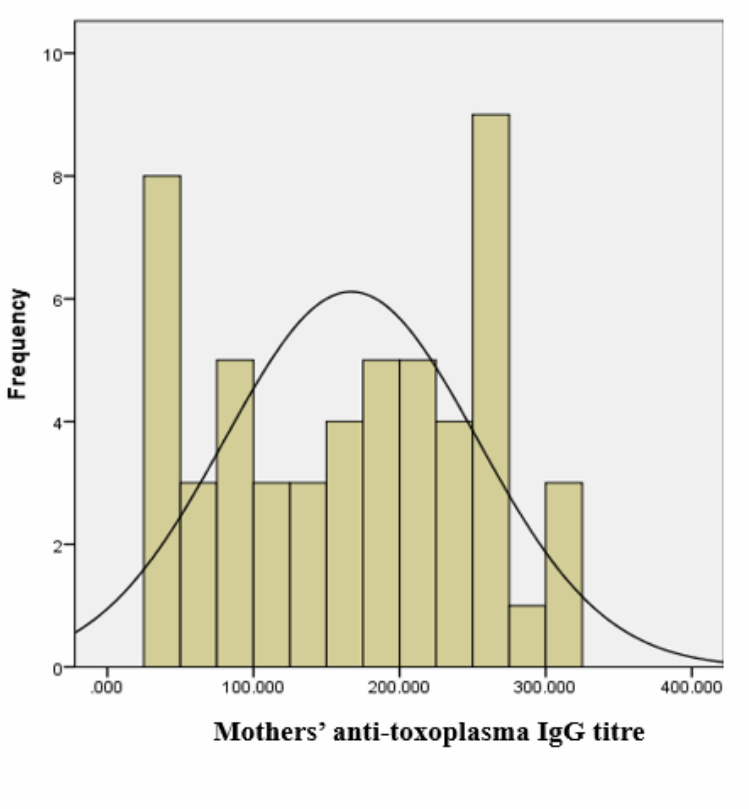


Figure 24: Distribution of anti-toxoplasma IgG titre among the 53 positive mothers

Anti-toxoplasma IgM antibodies in the sera of IgG positive mothers:

All the fifty three (53) mothers who tested positive for IgG anti-toxoplasma antibodies were also tested for IgM anti-toxoplasma antibodies. Among them, one mother tested positive for IgM. The corresponding IgG titre in her sera was 53 IU/ml. This accounted for 1.9% (1/53) prevalence of IgM among those mothers positive for IgG.

Prevalence of anti-toxoplasma antibodies in newborns:

Out of 251 cord blood samples from babies tested by using IgM μ capture ELISA NOVATEC IMMUNDIAGNOSTICA kit, 5 (2%) samples gave a positive IgM reaction, as evidenced by titres above 11 Novatec units as per the kit manufacturer's criteria (as shown in Table 24). There were no detectable anti-toxoplasma IgG antibodies in the sera of the mothers of 4 of these 5 babies, whose cord blood gave a positive IgM reaction. However, IgG anti-toxoplasma antibodies with a titre of 270 IU/ml, was detected in the mother of one of the 5 above babies.

This baby was followed up, but no IgG or IgM class of antibodies to *T.gondii* could be detected at 11 months of age, and neither there were any clinical features of congenital toxoplasmosis in this baby.

Table 24: Relationship between IgM positivity in babies, and maternal anti-toxoplasma IgG antibody.

Sl. No	Identity No	Baby's IgM (NTU) Cord blood	Baby's IgG (IU/ml) Cord blood	Mother's IgG (IU/ml)	Mother's IgM (NTU)	Observation
1	63/Bh/146738	11.71	356	270	3.22	False positive
		0.81*	7.4 *	N.R	N.R	
2	47/Ar/140996	12.94	2.7	8.72	3.91	False positive
3	56/De/77972	12.37	3.1	3.11	1.22	False positive
4	58/Pa/146336	13.7	9.6	10.2	1.69	False positive
5	65/An/146722	11.7	2.52	4.4	1.66	False positive

Note: *Venous blood sample, collected at 11 months of age (On follow up),

N.R = Not Relevant

Anti-toxoplasma IgG antibodies in the cord blood samples of newborn babies:

Cord blood samples of fifty three (53) babies, whose mothers were positive for IgG anti-toxoplasma antibodies were tested for IgG anti-toxoplasma antibodies, to find out whether there were babies with significantly higher IgG titres than the respective mothers, which would indicate congenital infection in the absence of IgM antibodies. The anti-toxoplasma IgG antibodies could be detected in 40 (76%) cord blood samples only.

Prevalence of titres of anti-toxoplasma IgG antibodies, among the IgG positive babies is presented in table 25 and figure 25. The titres in the babies ranged between 51 IU/ml – 400 IU/ml. None of the babies' cord blood sample was in the range of 35 IU/ml – 50 IU/ml. The mean titre was 193 IU/ml, and the mode titre was 232 IU/ml. The highest titre range 351 IU/ml – 400 IU/ml, was found in only 1 baby, accounting for 2.5 %. The lowest titres range (51 IU/ml – 100 IU/ml) was found in 8 babies, accounting for 20 %.

Table 25: The prevalence of anti-toxoplasma IgG titres, among the 40 IgG positive babies.

IgGtitre (IU/ml)	Number, n=53	Percentage (%)
35-50	0	0
51-100	8	20
101-150	7	17.5
151-200	5	12.5
201-250	11	27.5
251-300	2	5
301-350	6	15
351-400	1	2.5
Total	**40**	**100**

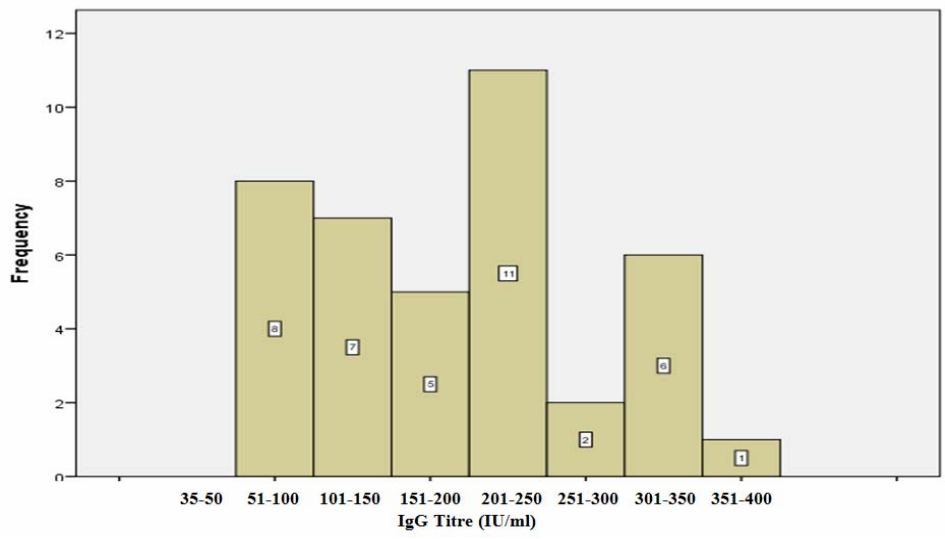


Figure 25: Histogram showing the frequency distribution among 40 IgG positive babies.

Distribution of IgG anti-toxoplasma antibodies among babies who tested positive:

The distribution of anti-toxoplasma IgG titres among the 40 IgG positive babies, is presented in figure 26. The distribution conforms to normal distribution pattern. The arithmetic mean ± standard deviation for the anti-toxoplasma IgG titres of 40 IgG positive babies was 193±88. None of the babies had a titre of average plus 3 standard deviations (167+258) of that of the mother's IgG, which amounts to 425 IU/ml, which could be a significant titre, suggestive of intrauterine infection.

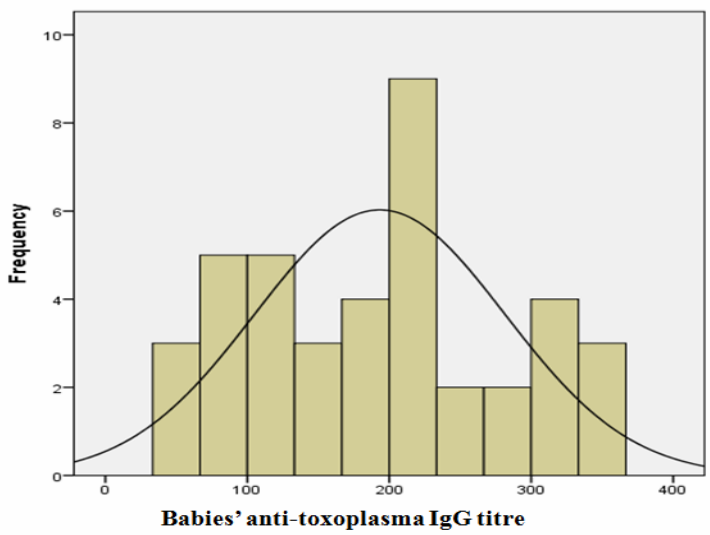


Figure 26: Distribution of anti-toxoplasma IgG titres in 40 IgG positive babies

The remaining 13 (24%) blood samples were negative for IgG anti-toxoplasma antibodies. The anti-toxoplasma IgG titres in the mothers of these negative babies ranged between 35.1 IU/ml- 274 IU/ml.

There was no statistically significant difference between the mean IgG titres of the positive mothers and the positive babies, as estimated by unpaired t test ($t=1.447$ and $p > 0.05$).

The correlation between the IgG titres in the 53 IgG positive mothers and their respective newborns was done by plotting a scatter diagram, and calculating Pearson's correlation co-efficient, with the line of regression (as shown in figure 27). There was a significantly positive correlation, with 'p' value of 0.001, and r (Pearson's correlation co-efficient) value of 0.547. This accounted for a moderate titre by titre correlation between the titres observed in the mothers and their respective babies.

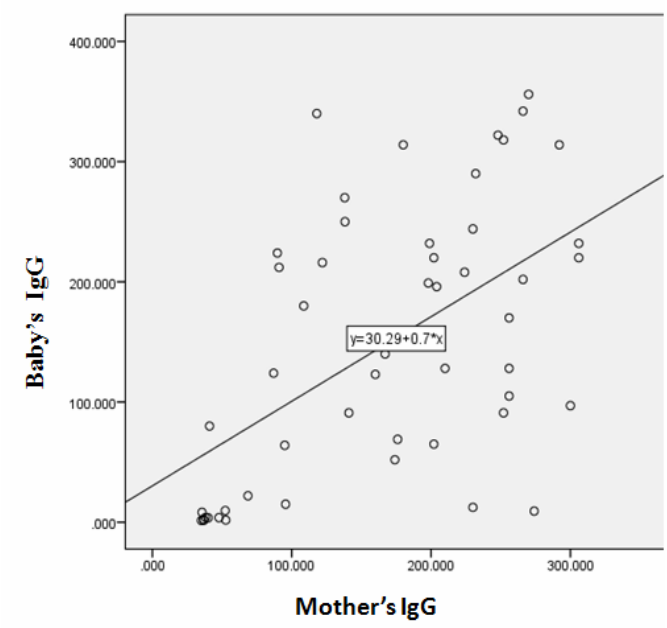


Figure 27: Scatter plot showing IgG titres in 53 mothers and their respective babies.

When the mother had an IgG titre of 100 IU/ml or more, there was almost consistent (95%) transfer of antibodies from the mother to the baby. If the mother's titre was less than 100 IU/ml, then only, 31% (5/16) of mothers transmitted antibodies passively to their babies.

The risk factors, associated with *T.gondii* infection in mothers were analyzed, and are presented in table 26.

Table 26: The risk factors associated with infection by *T.gondii* among pregnant women.

Risk factor	Sero positives n=53	Sero negatives n=198	Odds ratio (95% Confidence Intrerval)	P value
Mean age	24.2 Years	23.6 Years	*t value =1.298	>0.05
Gravida				
Primigravida	20 (38%)	93 (47%)	0.68 (0.36-1.27)	>0.05
Multigravida	33 (62%)	105 (53%)		
Previous history of abortion				
Present	10 (19%)	29 (15%)	1.36(0.61-2.99)	>0.05
Absent	43 (81%)	169 (85%)		
Gestational age				
Term	51 (96%)	179 (90%)	0.37 (0.08-1.64)	>0.05
Preterm	2 (4%)	19 (10%)		
Mode of delivery				
Normal delivery	38 (72%)	137 (69%)	0.89(0.45-1.73)	>0.05
Caesarean section	15 (28%)	61 (31%)		
Level of education				
Uneducated	5 (9%)	8 (4%)	2.47 (0.77-7.9)	>0.05
Educated	48 (91%)	190 (96%)		
Occupation				
Unskilled labour (Housewife, Farmer, manual labourer)	51 (96%)	175 (88%)	3.35 (0.76-14.69)	>0.05
Skilled labour and others (Tailor, Nurse, Teacher,I.T employee, Engineer, Clerk, Pharmacist)	2 (4%)	23 (12%)		
B.G.Prasad socioeconomic status				
Class 5	30 (56%)	77 (39%)	2.05 (1.11-3.78)	< 0.05
Class 4 & Class 3	23 (44%)	121 (61%)		

*t value of unpaired t test

Table 26 contd: The risk factors associated with infection by *T.gondii* among pregnant women.

Risk factor	Sero positives n=53	Sero negatives n=198	Odds ratio (95% Confidence Intrerval)	P value
Behavioural pattern				
Consumption of salad only				
Yes	37 (70%)	154 (78%)	0.66 (0.34 – 1.30)	> 0.05
No	16 (30%)	44 (22%)		
Drinking untreated water				
Yes	30 (57%)	127 (64%)	0.73 (0.39 – 1.35)	> 0.05
No	23 (43%)	71 (36%)		
Consumption of salad and drinking untreated water				
Yes	26 (49%)	121 (61%)	0.61 (0.33 – 1.13)	> 0.05
No	27 (51%)	77 (39%)		
Owning cat				
Yes	7 (13%)	28 (14%)	0.92 (0.37 – 2.24)	> 0.05
No	46 (87%)	170 (86%)		
Consumption of raw meat	0	0	-	-
Outdoor gardening				
Yes	10 (19%)	22 (11%)	1.86 (0.82 – 4.2)	> 0.05
No	43 (81%)	176 (89%)		
Babies characteristics				
Birth weight				
Normal	37 (70%)	150 (76%)	1.35(0.69-2.6)	>0.05
Low	16(30%)	48 (24%)		
Sex of the baby				
Male	18 (34%)	101 (51%)	0.49(0.26-0.93)	<0.05
Female	35 (66%)	97 (49%)		
Apgar score				
Normal	52 (98%)	194 (98%)	0.93 (0.1 – 8.5)	> 0.05
Low	1 (2%)	4 (2%)		

There were significantly more seropositives among mothers belonging to lower socio-economic status, when compared to mothers belonging to middle class ($p < 0.05$).

There was no statistically significant difference between the mean age of the seropositive and seronegative mothers, as estimated by unpaired t test ($t=1.298$ and $p > 0.05$).

The sero-prevalence of IgG anti-toxoplasma antibodies among multigravida was higher than that seen in primigravida, but it was not statistically significant ($p > 0.05$).

The babies of multigravida also showed a higher prevalence of IgG anti-toxoplasma antibodies, but it was not statistically significant ($p > 0.05$).

There was no significant association of previous history of abortion in the group which had IgG anti-toxoplasma antibodies, when compared with the group which was negative ($p > 0.05$).

The prevalence in gestational age as term or preterm was not significantly different in mothers who were seropositive and seronegative ($p > 0.05$).

The mode of delivery (normal vs. caesarean), the level of education and the occupation were not significantly different between seropositives and seronegatives ($p > 0.05$). None of the pregnant women gave history of consumption of raw meat.

The differences in the characteristics of IgG positive and IgG negative babies, born to IgG positive mothers is presented in table 27.

Table 27: Differences in the characteristics of IgG positive and IgG negative babies born to IgG positive mothers.

Risk factor	Seropositive babies n=40	Seronegative babies n=13	Odds ratio (95% Confidence Interval)	P value
Mother's gravida status				
Primi	12 (30%)	8 (62%)	0.26 (0.07 – 0.98)	> 0.05
Multi	28 (70%)	5 (38%)		
Birth weight				
Normal	25 (63%)	12 (92%)	7.2 (0.8 - 61)	> 0.05
Low	15 (37%)	1 (8%)		
Sex of the baby				
Male	13 (33%)	5 (38%)	0.77 (0.21 – 2.8)	> 0.05
Female	27 (67%)	8 (62%)		

There was no statistically significant difference, with respect to gravida status of mothers, birth weight of the baby, and sex of the baby, among the IgG positive or IgG negative babies, born to IgG positive mothers.

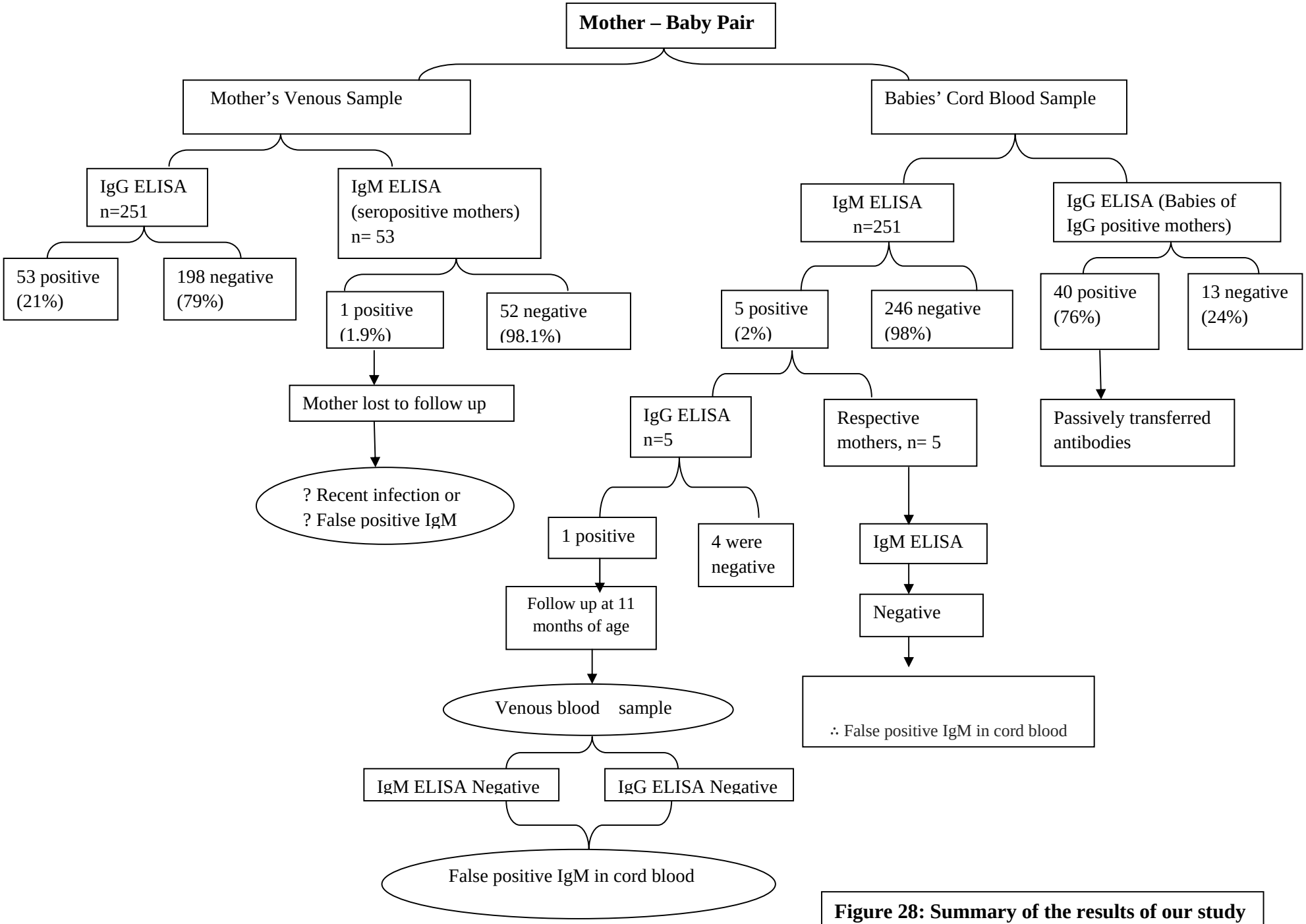


Figure 28: Summary of the results of our study

DISCUSSION

The study presented here estimates the prevalence of IgG antibodies to *T.gondii,* among pregnant women admitted for delivery from Kolar region. It also explores the possibility of vertical transmission of toxoplasma infection.

We found a seroprevalence of 21.1 % for anti-toxoplasma IgG antibodies in the population studied. We could detect a positive reaction for IgM in the cord blood samples of 5 (2%) babies, among 251 samples. Though, we could detect a positive reaction by the μ capture ELISA test, in the cord blood samples of these 5 babies, born to the above mothers, these could not be confirmed as true IgM antibodies suggestive of vertical transmission, as mothers of these babies did not have any detectable IgG class of antibodies to *T.gondii,* or on follow up of the newborn, there was no IgM antibody or IgG antibody to *T.gondii* detected.

Further, the titres of IgG anti-toxoplasma antibodies in the mothers or in the cord blood of newborns tested in our study were not suggestive of recent infection in any.

In this study, the seroprevalence of IgG anti-toxoplasma antibodies was found to be 21.1 %. This prevalence is comparable to 22 % of an average prevalence among women of reproductive age reported by a pan India study, involving serum samples from different parts of the country.[4]

The prevalence of anti-toxoplasma antibodies varies considerably among women of reproductive age from different parts of the world, which ranges from 10.3% in Japan[47] to 80.3 % in Democratic Republic of Congo.[3]

Though, the average prevalence in India is 22 %, the prevalence varies to some extent with geographical location: it could be as low as 4%, reported from Kerala[57] or as high as 77 % , reported from Kumaon region of Himalayas.[61]

In a study done in Bombay, the seroprevalence of IgG anti-toxoplasma antibodies among healthy adult voluntary blood donors was 30.9%.[59]

Among 499 women of child bearing age from Gujarat, the prevalence was 8.8 %. The lower seroprevalence of anti-toxoplasma antibodies is attributed to the dry, arid climate which is unfavorable for *T.gondii* oocysts survival.[4] In Karnataka, there are very few studies conducted on toxoplasmosis. The prevalence of IgG anti-toxoplasma antibodies among women of child bearing age, from coastal region of Karnataka, is reported to be 37 %.[4] This contrasts with our finding. The higher seroprevalence of anti-toxoplasma antibodies, in coastal region of Karnataka is attributed to the higher age in women sampled, climatic conditions (higher humidity are thought to favour the sustenance and development of toxoplasma oocysts), and significant number of households owning cats,[4] whereas in Kolar region, the climate is arid and less humid.

The prevalence of anti-toxoplasma antibodies among healthy voluntary blood donors in Bangalore was 20 %. This is in accordance to our finding of seroprevalence of 21 %.[78]

These observations show that within a state, there could be variations in prevalence of anti-toxoplasma antibodies. It is a reflection of many factors which influence the transmission of *T.gondii* from feline species to human beings.

Our study further supports the earlier studies, and thus the prevalence of anti-toxoplasma antibodies in India is around 21 % in general. This tells us that 79 % of the women in Kolar region, do not possess anti-toxoplasma antibodies, and are at a risk of acquiring primary infection during pregnancy, and may consequently transmit it to the newborns.

In our study, there were 53 anti-toxoplasma IgG positive mothers detected. The distribution of IgG antibody titres, among the positive mothers conformed to a normal distribution pattern. The arithmetic mean for the anti-toxoplasma IgG titres of 53 IgG positive mothers was 167. The sum of the average of 53 mother's IgG titres and 3 standard deviations was 425 IU/ml. When a distribution is such, the titres above this measure, i.e. average plus 3 standard deviations in serological tests are thought to suggest recent infection.

In our study, none of the pregnant women, had titres above the arithmetic mean plus 3 standard deviations (425 IU/ml). Thus, IgG titres suggestive of recent infection were not found in any of the pregnant women studied by us.

However, to compare our results, there are no studies reporting the anti-toxoplasma IgG titres among pregnant mothers. There are 2 studies, involving voluntary blood donors from Bombay[59] and Karnataka.[78] In both the studies, the mean titres reported were 376.8 IU/ml and 120.7 IU/ml respectively, which are not comparable with our results from pregnant women.

We have followed an algorithm to test the anti-toxoplasma antibodies in pregnant women. As per which, only those with positive IgG titres (more than 35 IU/ml) were tested for IgM. We have refrained from testing the IgM in remaining mothers. However, we have also tested IgM in mother's serum, whose babies' serum showed positive anti-toxoplasma IgM.

We detected IgM positive reaction in a mother, among the IgG positive mothers. This positivity accounts for 1.9% (1/53) prevalence. This prevalence of IgM is similar to the prevalence reported by other studies, all over India: a study in Kerala[57] (1%), pan India[4] study (1.43%), and a study in Delhi[62] (1.8%) among pregnant women. This pregnant woman, who gave a positive test for anti-toxoplasma

IgM, had an IgG titre of 53 IU/ml. According to CDC guidelines, the presence of both IgG and IgM anti-toxoplasma antibodies in a pregnant woman could be interpreted as a recent infection or a false positive IgM result.[93]

However, we could not confirm whether this positivity was due to real presence of anti-toxoplasma IgM or a false positive reaction, as the mother was lost to follow up, and we could not confirm the veracity of positive IgM reaction by doing a repeat test. The baby of the above mother was negative for both IgM and IgG anti-toxoplasma antibodies, implying no serological evidence of congenital toxoplasmosis.

Cord blood samples from 5(2 %) babies gave a positive IgM result in the μ capture ELISA test employed by us. In 4 babies, who gave a positive IgM ELISA test, their sera did not contain IgG antibodies against *T.gondii*; their respective mothers did not have either IgG or IgM anti-toxoplasma antibodies in their serum samples. Hence anti-toxoplasma IgM positive reaction in these babies' cord blood samples could not be due to vertical transmission and the presence of real IgM antibodies. This IgM reaction detected is due to false positivity of the test.

Previous studies have also reported such false positive reactions, while testing for IgM anti-toxoplasma antibodies by using commercial ELISA kits.[83]

We recommend, that the mother's IgG and IgM status against *T.gondii*, need to be tested parallely with the babies samples. If the mother's samples are negative for both these antibodies, the IgM detected in the cord blood should always be considered as false positive.

Even, if the mother's sample had IgG and / IgM, still the specificity of IgM detection in cord blood needs to be established, by following up of the baby and doing a repeat estimation later in life. In our study, one of the babies cord blood sample had both IgG and IgM class of antibodies to *T.gondii*. The respective mother was positive

only for IgG. The baby, on follow up at 11 months of age, did not have either IgG or IgM antibodies to *T.gondii*. The IgG antibodies that are passively transferred from mother to child through the placenta, have disappeared over a period of time, postnatally.

The diagnosis of congenital toxoplasmosis is excluded, when there is absence of IgG anti-toxoplasma antibodies at less than or equal to 12 months of age, when the infant is not on treatment.[94] This indicates that the initial IgM positivity in the cord blood sample was due to a false positive reaction. There are chances of both false positivity and false negativity with the testing of IgM.[44]

Thus, we could not find any evidence of vertical transmission of toxoplasmosis in the population studied. In contrast to our study, a study done in Trinidad[56], the seroprevalence of IgM anti-toxoplasma antibodies in newborns was 0.4 %. In another study done in Iraq[55], the seroprevalence of IgM anti-toxoplasma antibodies in infants was 0.33 %. However, these studies have not confirmed the specificity of IgM detection.

The titres of anti-toxoplasma IgG antibodies were also determined in the cord blood samples of babies of mothers who were positive for IgG antibodies. The anti-toxoplasma IgG antibodies could be detected in 40 (76%) cord blood samples only. The values seen in newborns at birth reflect the mother's IgG status, as these antibodies were passively transferred from the mother. To support this, the distribution of titres of anti-toxoplasma IgG antibodies among the babies conformed to a normal distribution pattern, as their mothers, and there was no significant difference between the mean titres of IgG positive mothers and IgG positive babies. There was a positive correlation between the mothers and babies IgG titres.

None of the cord blood samples had an IgG titre equal to or more than the mean plus three standard deviations of that of the respective mother's IgG. This lack of significantly higher titres, in the newborn further supports that there was no vertical transmission of toxoplasma infection, from mother to the child in the population studied in Kolar region.

We could not detect IgG anti-toxoplasma antibodies in cord blood samples of 13 (24%) babies, even though their mother's possessed anti-toxoplasma IgG antibodies. This seems to be due to an association with titres in the mothers, as there was almost consistent (95%) transfer of antibodies from the mother to the baby, when the mother had an IgG titre of 100 IU/ml or more. If the mother's titre was less than 100 IU/ml, only 31% (5/16) of mothers transmitted antibodies passively to their babies.

All the mothers of the IgG negative babies belonged to either lower middle class or lower class of socioeconomic status. Thus, other factors such as nutrition may influence the titres of antibodies, and subsequent transmission to the newborns.

Thus, with the prevalence of 21 % in Kolar region, the vertical transmission may not be that common. In countries where the prevalence is very high, with the tune of 70-90%, in France and Austria,[8] vertical transmission of toxoplasmosis and subsequent congenital toxoplasmosis is deemed important, and it is mandatory by law, that every pregnant woman should be tested for the toxoplasma serology. Our data does not indicate any such recommendation for Kolar region. However, we did not test the cord blood samples for IgA and IgE class of antibodies to *T.gondii,* which are said to increase the sensitivity and specificity of detection of vertical transmission.

There are no studies from India, to the best of our knowledge, which have undertaken studies on cord blood samples of newborns to explore the possibility of vertical transmission of toxoplasma infection.

We could find an association with lower socioeconomic status and seropositivity among pregnant women in our study; this may be due to conditions in lower socioeconomic strata owing to the environmental factors such as contact with soil. Similar findings have been reported in previous studies.[95]

Multigravida (55 %) outnumbered primigravida (45 %) in our study; this could be due to convenient sampling of our study. Since our hospital, is a tertiary referral centre, majority of the pregnant women are referred to our centre.

Exposure for a longer number of years in the environment predisposes to a higher prevalence of antibodies in the older population, due to active natural immunity to the agent, which is called cohort effect. As the age advances, the seroprevalence of anti-toxoplasma antibodies in the general population also increases. This effect has also been seen in earlier studies.[4, 59] But, in our study there was no significant difference between the mean age of primigravida and multigravida, this may explain the lack of cohort effect.

We could not find any association with multigravida, previous history of abortion, gestational age, mode of delivery (normal vs. caesarean), level of education, occupation, and owning cats with the seropositivity. There was no significant difference among woman who consumed salad and drank untreated water.

In the present study, none of the pregnant women gave history of consumption of raw meat, which is a known risk factor, in studies done on pregnant women, in Japan[47] and Democratic Republic of Congo.[3] The habit of sufficiently cooking meat might have interfered with the transmission of toxoplasmosis in Kolar region.

The study presented here shows that the prevalence of anti-toxoplasma IgG antibodies among pregnant women admitted for delivery was 21.1% in Kolar region, Karnataka. Though, this compares well with the average prevalence in India among the women of child bearing age. It differs from that reported from coastal Karnataka where the prevalence is higher probably due to difference in environmental factors compared to that of Kolar region. We observed a significantly higher prevalence among pregnant women belonging to lower socioeconomic strata; this could be related to the environmental exposure.

None of the cord blood samples from the babies of pregnant women tested by us could be confirmed to contain IgM class of antibodies specific for *T. gondii*, and false positives were encountered in 2 % of the samples tested, which emphasizes a need to be careful when interpreting IgM positivity in the cord blood samples tested in the μ capture ELISA test.

Though we could not detect any vertical transmission of toxoplasmosis in this study, we feel that routine screening of pregnant women and follow up of those who have IgM antibodies and testing a larger number of cord blood samples may provide a clear cut picture of vertical transmission of toxoplasmosis in Kolar region. It has been reported that, in addition to IgM detection, testing for the presence of IgA and IgE anti-toxoplasma antibodies in the venous blood sample of the newborn, after 10^{th} day of life may increase the sensitivity and specificity of detection of vertical transmission. Detection of such different class of antibodies to *T.gondii* from newborns may also facilitate in the quest to detect vertical transmission in Kolar region.

SUMMARY

We estimated the IgG antibodies to *T.gondii*, among pregnant women admitted for delivery from Kolar region to know the prevalence and the titre. We also tested IgM antibodies to *T.gondii* in cord blood samples of newborn babies, to know the possibility of vertical transmission of *T.gondii* infection from mother to the child.

Serum samples of 251 pregnant women, who were admitted for delivery at R.L.Jalappa Hospital, Kolar were tested for IgG class of antibodies to *T.gondii* using IgG ELISA NOVATEC IMMUNDIAGNOSTICA quantitative kit. We detected IgG anti-toxoplasma antibodies in 53 mothers, accounting for a seroprevalence of 21.1%. The mean antibody titre was 167±86 IU/ml. None of the mothers had a titre above the mean plus 3 standard deviations, suggestive of recent infection.

The seropositivity was significantly higher in women belonging to lower socioeconomic strata. Thus, toxoplasmosis is endemic in Kolar region, and about 79 % pregnant mothers lack anti-toxoplasma IgG antibodies. The anti-toxoplasma IgG antibodies could be detected in 40 (76 %) cord blood samples of the babies of serologically positive mothers.

Cord blood samples of 251 newborn babies were tested for IgM class of antibodies to *T.gondii* using IgM μ capture ELISA NOVATEC IMMUNDIAGNOSTICA kit. IgM positivity could be detected in 5 (2 %) of the cord blood samples. There were no detectable IgG antibodies to *T.gondii* in the serum samples of 4 of these IgM positive babies. Though the mother of the remaining 1 IgM positive baby had IgG antibodies to *T.gondii* in her blood sample, the newborn on follow up at 11 months of age, lacked both IgG and IgM class of antibodies to

T.gondii. Thus, there was no evidence to confirm that IgM positivity in the cord blood sample was due to vertical transmission of toxoplasma infection in the population studied. Therefore the validity of finding IgM positivity in the cord blood samples of the 5 babies as above could not be established, suggesting that these were false positive reactions.

CONCLUSION

In the study presented in dissertation, the prevalence of anti-toxoplasma IgG antibodies among pregnant women admitted for delivery was 21.1% in Kolar region, Karnataka. Thus, about 79% pregnant mothers, do not possess anti-toxoplasma antibodies, and are at the risk of acquiring primary infection during pregnancy.

The distribution of anti-toxoplasma IgG titres among the 53 mothers conformed to normal distribution pattern. The mean anti-toxoplasma IgG titres of IgG positive mothers were 167 ± 86. Only 2 (4%) mothers possessed a titre range of 301 IU/ml – 350 IU/ml. None of the mothers possessed a titre of mean plus 3 standard deviations (167+258), which amounts to 425 IU/ml and would suggest recent infection. There was a significant association between seropositivity and lower socioeconomic status.

A IgM positive reaction in 5 (2%) of the 251 cord blood samples tested were found to be false positives, as mothers of 4 of these babies did not contain either IgG/IgM antibodies to *T.gondii* in their serum samples, and on follow up of the newborn at 11 months of age, the child did not have either IgG or IgM antibodies to *T.gondii*. Thus, we could not detect any vertical transmission of toxoplasma infection in this study.

The anti-toxoplasma IgG antibodies could be detected in 40 (76 %) cord blood samples of the babies of seropositive mothers. The distribution of anti–toxoplasma IgG titres among the positive babies conformed to normal distribution pattern. The mean anti-toxoplasma IgG titres of IgG positive babies were 193 ± 88. There was no significant difference between the mean titres of IgG positive mothers and their babies. There was a positive correlation (r = 0.547) between the IgG titres in the seropositive mothers and the cord blood samples of their babies.

When the mother had an IgG titre of 100 IU/ml or more, there was almost consistent (95%) transfer of antibodies from the mother to the baby. If the mother's titre was less than 100 IU/ml, then only, 31% (5/16) of mothers transmitted antibodies passively to their babies. Thus, there seems to be an association between the titre in the mother and passive transfer of antibodies.

Our study could not detect any vertical transmission of toxoplasma infection. We recommend that routine screening of pregnant women, follow up of those who have IgM antibodies, and testing a larger number of cord blood samples not only for IgM, but also for IgA and IgE antibodies to *T.gondii* may provide a clearer picture of vertical transmission of toxoplasma infection in Kolar region.

BIBLIOGRAPHY

1. Muhie Y, Keskes S. Toxoplasmosis: emerging and reemerging zoonoses. Afr J App Microbiol Res. 2014;3:1-11.
2. Jones JL, Parise ME, Fiore AE. Neglected parasitic infections in the United States: Toxoplasmosis. Am J Trop Med Hyg. 2014;90:794-799.
3. Yobi D, Piarroux R, Olliver C L, Franck J, Situakibanza H, Muhindo H, et al. Toxoplasmosis among pregnant women : High seroprevalence and risk factors in Kinshasa, Democratic Republic of Congo. Asian Pac J Trop Biomed. 2014;4:69-74.
4. Singh S, Munawwar A, Rao S, Mehta S, Hazarika NB. Serologic prevalence of *Toxoplasma gondii* in Indian women of child bearing age and effects of social and environmental factors. PLOS Negl Trop Dis. 2014;8:e2737.
5. Dubey JP. The history of *Toxoplasma gondii* – the first 100 Years. J Eukaryot Microbiol. 2008;55:467-475.
6. Chaterjee KD. Genus Toxoplasma. Parasitology in relation to clinical medicine, 13th edn. New Delhi: Satish. K. Jain, 2009:129-133.
7. Weiss LM, Dubey JP. Toxoplasmosis: a history of clinical observations. Int J Parasitol. 2009;39:895-901.
8. Singh S. Mother to child transmission and diagnosis of *Toxoplasma gondii* infection during pregnancy. Indian J Med Microbiol. 2003;21:69-76.
9. Charles Nicolle image. Courtesy: http://toxo100.org/html/style/nicolle.gif
10. *Ctenodactylus gundii*, image. Courtesy: Muller JS, Go. https://www. google.co.in /imgres?imgurl=https%3A%2F%2Fs-media-cache-ak0.pinimg.com%2Foriginals%2F67%2F93%2F05%2F679305a9d5e96b092b6109c9fde54a82.jpg&imgrefurl=https%3A%2F%2Fwww.pinterest.com%2Fpin%2F462604192946579763%2F&docid=sqR3rBMxkodFvM&tbnid=rfcOWzZQD8qbNM%3A&w

=560&h=560&bih=490&biw=827&ved=0ahUKEwizoOqBkYnQAhXHro8KHeP9C58QMwg_KBcwFw&iact=mrc&uact=8

11. Tachyzoite image.Courtesy: https://en.wikipedia.org/wiki/Toxoplasma_gondii
12. Alfonso Splendore image.Courtesy: http://toxo100.org/html/index-en.html
13. Singh S. Toxoplasmosis in India. Indian J Ophthalmology. 1953;1:71-88.
14. Dubey JP. History of the discovery of the life cycle of *Toxoplasma gondii*. Int J Parasitol. 2009;39:877-882.
15. Dubey JP. Toxoplasmosis. In: Cox FEG, Wakelin D, Gillespie SH, Despommier DD, eds. Topley and Wilson's Microbiology and Microbial infections, Parasitology. 10th edn. London: Edward Arnold,2005: 421-442.
16. Taxonomy of *T.gondii*. Courtesy: Keas BE. https://msu.edu/course/zol/316/ tgontax. htm
17. Dubey JP. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cyst. Clin Microbiol Rev. 1998;11:267-299.
18. Beaver PC, Jung RC, Cupp EW. *Toxoplasma gondii*. Clinical Parasitology, 9th edn. Unitrd States of America: Lea & Febiger,1984:162-166.
19. Forzan MJ, Frasca S. Systemic toxoplasmosis in a five –month old Beaver, (*Castor caanadensis*). J Zoo Wild life Med. 2004;35:113-115.
20. Paris L. In: Magill AJ, Ryan ET, Hill DR, Solomon T, eds. Hunter's Tropical Medicine and Emerging Infectious Diseases, 9th edn. China: Elsevier Inc, 2013:765-775.
21. Gangneux FR, Darde ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012;25:264-296.

22. Al-Kappany YM, Rajendran C, Abu-Elwafa SA, Hilali M, Dubey JP. Genetic diversity of *Toxoplasma gondii* isolates in Egyptian feral cats reveals new genotypes. J Parasitol. 2010;96:1112-4.

23. Can H, Doskaya M, Ajzenberg D, Ozdemir HG, Caner A, Gulce Iz S, et al. Genetic characterization of Toxoplasma gondii isolates and toxoplasmosis seroprevalence in stray cats of Izmir, Turkey. PLOS ONE. 2014;9:e104930.

24. Biradar SS, Saravanan BC, Tewari AK, Sreekumar C, Sankar M, Sudhakar NR. Genetic characterization of *Toxoplasma gondii* isolates from chickens in India by GRA6 gene sequence analysis. Acta Parasitol. 2014;59:666-674.

25. Life cycle of *T.gondii* Courtesy: http://www.cdc.gov/parasites/images/toxoplasmosis/toxoplasma_lifecycle.gif

26. Halonen SK, Weiss LM. Toxoplasmosis. Handb Clin Neurol. 2013;114:125-145.

27. Gatkowska J, Wieczorek M. Behavioral changes in mice caused by *Toxoplasma gondii* invasion of brain. Parasitol Res. 2012;111:53-58.

28. Life cycle. Courtesy: http://www.cdc.gov/parasites/toxoplasmosis/biology.html

29. Noorbaksh S, Khosravi N, Zarabi V, Farhadi M, Tabatabaei A. Congenital Infection with *Toxoplasma gondii*: A Case Control Study in Tehran, Iran. Open Access Scientific Reports. 2012;1:199. doi:10.4172/scientificreports.199

30. Munoz-Roldan M, Heimesaat MM, Liesenfeld O. Toxoplasmosis. In: Farrar J, Hotez PJ, Junghanss T, Kang G, Lalloo D, White NJ, eds. Manson's Tropical Diseases,23rd edn. China: Elseviers, 2014:652-663.

31. Schwartzman JD, Maguire JH. Toxoplasmosis. In: Guerrant RL, Walker DH, Weller PF, eds. Tropical Infectious Diseases Principles, Pathogens, and Practice, Vol 2, 2nd edn. United States of America: Elsevier, 2006:1141-1151.

32. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004;363:1965-76.

33. Malarvizhi A, Viswanathan T, Lavanya V, Arul Sheeba Malar S, Moorthy K. Seroprevalence of *Toxoplasma gondii* in pregnant women. J Public Health Epidemiol 2012;4:170-177.

34. Soares JAS, Carvalho SFG, Caldeira AP. Profile of pregnant women and children treated at a reference center for congenital toxoplasmosis in the Northern state of Minas Gerais, Brazil. Rev Soc Bras Med Trop. 2012;45:55-59.

35. Sarkar MD, Anuradha B, Sharma N, Roy RN. Seropositivity of toxoplasmosis in antenatal women with bad obstetric history in a tertiary- care hospital of Andhra Pradesh, India. J Health Popul Nutr. 2012;30:87-92.

36. Li X-L, Wei H-X, Zhang H, Peng H-J, Lindsay DS. A Meta analysis on risks of adverse pregnancy outcomes in *Toxoplasma gondii* infection. PLOS ONE 2014;9:e97775.

37. Tanatawy NE, Taman A, Shalaby H. Toxoplasmosis and female infertility: Is there a co – relation? Am J Epidemiol 2014;2:29-32.

38. Abu-Madi MA, Behnke JM, Dabritz HA. *Toxoplasma gondii* seropositivity and co-infection with TORCH pathogens in high risk patients from Qatar. Am J Trop Med Hyg 2010;82:626-633.

39. Congenital toxoplasmosis scar image. Courtesy: https://aapos.org/terms/conditions/106

40. Feldman DM, Keller R. Toxoplasmosis, Parvovirus, and Cytomegalovirus in pregnancy. Clin Lab Med. 2016;36:407-419.

41. Palanisamy M, Madhavan B, Balasundaram MB, Andavar R, Venkatapathy N. Outbreak of ocular toxoplasmosis in Coimbatore, India. Indian J Ophthalmology. 2006;54:129-31.

42. John DT, Petri WA. *Toxoplasma gondii*. Markell and Voges Medical Parasitology, 9th edn. United States: Elsevier, 2006:140-372

43. Huekelbach J, Meyer-Cirkel V, Moura RCS, Gomide M, Queiroz JAN, Sawaljew P, et al. Waterborne toxoplasmosis, Northeastern Brazil. Emerg Infect Dis. 2007;13:287-289.

44. Montoya JG, Boothyrod JC, Kovacs JA. *Toxoplasma gondii*. Mandell, Douglas, and Bennett's Principles and Practices of Infectious Diseases. Vol 2, 7th edn. United States: Elsevier Inc, 2010: 3495-3525.

45. Singh S. Congenital toxoplasmosis: Clinical features, outcomes, treatment, and prevention. Trop Parasitol. 2016;6:113-22.

46. Meenken C, Rothova A, Kijlstra A, Ooosting J. Seasonal variation in congenital toxoplasmosis. Br J Ophthalmol. 1991;75:639.

47. Sakikawa M, Noda S, Hanaoka M, Nakayama H, Hojo S, Kakinoki S, et al. Anti-toxoplasma antibody prevalence, primary infection rate, and risk factors in a study of toxoplasmosis in 4,466 pregnant women in Japan. Clin Vaccine Immunol 2012;19:365-67.

48. Cong W, Dong X-Y, Meng Q-F, Zhou Na, Wang X-Y, Huang S-Y, et al. *Toxoplasma gondii* infection in pregnant women: A sero prevalence and case-control study in Eastern China. Bio Med Research International. 2015;2015.doi.org /10.1155 /2015/170278.

49. Quadros R M, Rocha G C, Romagna G, Oliveira J P, Ribeiro D M, Marques S M T. *Toxoplasma gondii* seropositivity and risk factors in pregnant women followed up by the Family Health Strategy. Rev Soc Bras Med Trop. 2015;48:338-342.

50. Andiappan H, Nissapatorn V, Sawangjareon N, Chemoh W, Lau YL, Kumar T, et al. Toxoplasma infection in pregnant women: a current status in Songklanagarind hospital, southern Thailand. BioMed Central. 2014;7:239.

51. Khameneh ZR, Hanifian H, Rostamzadeh A. Seroprevalence of toxoplasmosis in pregnant women in Urmia, Iran. Int J Enteric Pathog. 2016;4:e33350.

52. Ayi I, Sowah AO-K, Blay EA, Suzuki T, Ohta N, Ayeh –Kumi PF. Toxoplasma gondii infections among pregnant women, children and HIV–seropositive persons in Accra Ghana. Tropical Medicine and Health. 2016;44:17. doi: 10.1186/s41182-016-0018-5

53. Mattos CCB, Spegiorin LCJF, Meira CS, Silva TC, Ferreira ALC, Nakashima F, et al. Anti-*Toxoplasma gondii* antibodies in pregnant women and their newborn infants in the region of Sao Jose do Rio Preto, Sao Paulo, Brazil. Sao Paulo Med J. 2011;129:261-6.

54. Gontijo da Silva M, Vinaud MC, de Castro AM. Prevalence of toxoplasmosis in pregnant women and vertical transmission of *Toxoplasma gondii* in patients from basic units of health from Gurupi, Tocantins, Brazil, from 2012 to 2014. PLOS ONE. 2015; 10:e0141700. doi:10.1371/journal.pone.0141700

55. Al-Hari s FM, Saheb HS, Abdul-Sada KM. Investigation of toxoplasmosis in cord blood of newborns at Al-Najaf Province, Iraq by searching for IgG and IgM antibodies. Int J CurrMicrobiol App Sci. 2015;4: 314-321.

56. Adesiyun AA, Gooding R, Ganta K, Seepersadsingh N, Ramsewak S. Congenital toxoplasmosis in two health institutions in Trinidad. West Indian Med J. 2007;56:166-170.

57. Deepthy BJ, Singh S, Muthuswami R, Ravindran PC. Prevalence of *Toxoplasma gondii* specific IgG and IgM antibodies among young women of reproductive age group – a study conducted in Kerala, India. Int J Curr Res 2014;6:4568-4588.

58. Khurana S, Bagga R, Aggarwal A, Lyngdoh V, Shivapriya, Diddi K, et al. Serological screening for antenatal toxoplasma infection in India. Indian J Med Microbiol 2010;28:143-146.

59. Meisheri YV, Mehta S, Patel U. A prospective study of seroprevalence of toxoplasmosis in general population, and in HIV/AIDS patients in Bombay, India. J Postgrad Med. 1997;43:93-97.

60. Borkakoty B, Biswas D, Jakharia A, Mahanta J. Seroprevalence of Toxoplasma gondii among pregnant women in Northeast India. J Assoc Physicians India.2016;64:24-28.

61. Singh S, Nautiyal BL. Seroprevalence of toxoplasmosis in Kumaon region of India. Indian J Med Res. 1991;93:247-249.

62. Sharma S, Duggal N, Agarwal S, Mahajan RK, Anuradha, Hans C. Seroprevalence of Toxoplasma, Rubella, and CMV infections in antenatal women in a tertiary care hospital in North India. J Commun Dis. 2015;47:23-26.

63. Mittal V, Bhatia R, Singh VK, Sehgal S. Prevalence of toxoplasmosis in Indian women of child bearing age. Indian J Pathol Microbiol. 1995;38:143-145.

64. Sucilathangam G, Anna T, Velvizhi G. Seroprevalence of *Toxoplasma gondii* in pregnant women with bad obstetric history. Indian Journal of Research. 2013;2:240-242.

65. Prasoona KR, Srinadh B, Sunita T, Sujatha M, Deepika MLN, Vijayalakshmi B, et al. Seroprevalence and influence of Torch infections in high risk pregnant women : A

large study from South India. J Obstet Gynaecol India. 2015;65:301 .doi:10.1007/s13224-014-0615-3

66. Borkakoty BJ, Borthakur AK, Gohain M. Prevalence of *Toxoplasma gondii*infection amongst pregnant women in Assam, India. Indian J Med Microbiol 2007;25:431-432.
67. Chintapalli S, Padmaja IJ. Seroprevalence of toxoplasmosis in antenatal women with bad obstetric history. Trop Parasitol. 2013;3:62-66.
68. Sood N, Soni S, Vegad M, Gupta P. Seroprevalence of *Toxoplasma gondii* in women with bad obstetric history in Ahmedabad. Gujarat Medical Journal 2009;64:35-37.
69. Kishore J, Misra R, Paisal A, Pradeep Y. Adverse reproductive outcome induced by Parvovirus B19 and TORCH infections in women with high-risk pregnancy. J Infect Dev Ctries. 2011;5:868-873.
70. Singh S, Lodha R, Passi GR, Bhan MK. Cholestatic jaundice due to congenital *Toxoplasma gondii* infection. Indian J Pediatr. 1998;65:154-7.
71. Surendrababu NRS, Kuruvilla KA, Jana AK, Cherian R. Globe calcification in congenital toxoplasmosis. Indian J Pediatr. 2006;73:527-528.
72. Gupta A, Raja A, Mahadevan A, Shankar SK. Toxoplasma granuloma of brainstem: A rare case. Neurol India. 2008;56:189-191.
73. Mohanty S, Shah I, Bhatnagar S. Neonatal hepatitis with toxoplasmosis. J clin Neonatol. 2012;1:96-97.
74. Lune AA, Pujari SN, Lune SA. Ocular toxoplasmosis: A case report with review of literature. Med J DY Patil Univ. 2014;7:818-21.
75. Goud TG, Ramesh K. Opportunistic infections among HIV patients attending tertiary care hospital, Karnataka, India. Int J Curr Microbiol App Sci. 2014;3:824-829.

76. Sucilathangam G, Palaniappan N, Sreekumar C, Anna T. Serological survey of toxoplasmosis in a district in Tamil Nadu: hospital based study. Indian J Med Res. 2013;137:560-563.

77. Anuradha B, Preethi C. Seroprevalence of toxoplasma IgG antibodies in HIV positive patients in and around Khammam, Telangana state. Journal of Clinical and Diagnostic Research. 2014;8:1-2. doi: 10.7860/JCDR/2014/9211.4880

78. Sundar P, Mahadevan A, Jayshree RS, Subbakrishna DK, Shankar SK. Toxoplasma seroprevalence in healthy voluntary blood donord from urban Karnataka. Indian J Med Res . 2007;126:50-55.

79. Tekkesin N. Diagnosis of toxoplasmosis in pregnancy: a review. HOAJ Biology. 2012. doi: 10.7243/2050-0874-1-9

80. Tlamcani Z, Lemkhenete Z, Lmimouni BE. Toxoplasmosis: the value of molecular methods in diagnosis compared to conventional methods. J micobiol Infect Dis. 2013;3:93-99. doi: 10.5799/ahinjs.02.2013.02.0089

81. Koneman EW, Winn WC, Allen S, Janda W, Procop G, Schreckenberger PC, et al. *Toxoplasma gondii*. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th edn. United States of America: Lippincott Williams & Wilkins,2006:1306-1311.

82. Sudan V, Jaiswal AK, Shanker D. Recent trends in the diagnosis of toxoplasmosis. Clin Rev Opinions. 2013;5:11-17.

83. Liesenfeld O, Press C, Montoya JG, Gill R, Isaac-Renton JL, Hedman K, et al. False-positive results in Immunoglobulin M (IgM) toxoplasma antibody tests and importance of confirmatory testing: the Platelia Toxo IgM test. J clin Microbiol. 1997;35:174-178.

84. Montoya JG, Remington JS. Management of Toxoplasma gondii infection during pregnancy. Clin Infect Dis. 2008;47:554-556. doi: 10.1086/590149.

85. Ramachandran R, Radhan P, Anand R, Subramanian I, Santhosham R, Sai V. CNS toxoplasmosis in an immunocompetent individual. Radiol Case Rep. 2015;9:e00031. doi: 10.2484/rcr.v9i1.908.

86. Goodman and Gilman. In: Brunton LL, Chabner BA, Knollman BC, eds. The pharmacological basis of therapeutics, 12th edn. China: Mc Graw-Hill Companies, Inc, 2011:1420-1421

87. Neville AJ, Zach SJ, Wang X, Larson JJ, Judge AK, Davis LA. Clinically available medicines demonstrating anti-toxoplasma activity. Antimicrob Agents Chemother. 2015; 59:7161-9. doi: 10.1128/AAC.02009-15.

88. Patil HV, Patil VC, Rajmane V, Raje V. Successful treatment of cerebral toxoplasmosis with cotrimoxazole. Indian J Sex Transm Dis. 2011;32:44-6. doi: 10.4103/0253-7184.81255.

89. Madi D, Achappa B, Rao S, Ramapuram JT, Mahalingam S. Successful treatment of cerebral toxoplasmosis with clindamycin: case report. Oman Med J. 2012;27:411-412. doi: 10.5001/omj.2012.100.

90. Verma R, Khanna P. Development of *Toxoplasma gondii* vaccine. Human Vaccin Immunother. 2013;9:291-3.

91. Ghai OP. Newborn Infants. In: Paul VK, Bagga A, Sinha A, eds. Ghai Essential Pediatrics, 8th edn. New Delhi: Sathish. K. Jain, 2013:124-183.

92. Vasudevan J, Mishra AK, Singh Z. An update on B.G.Prasad's socioeconomic scale: May 2016. Int J Res Med Sci. 2016;4:4183-4186.

93. CDC website. http://www.cdc.gov/dpdx/toxoplasmosis/dx.html

94. Pomares C, Montoya JG. Laboratory diagnosis of congenital toxoplasmosis. J Clin Microbiol. 2016;54:2448-2454. doi: 10.1128/JCM.00487-16.

95. Yashodhara P, Ramalakshmi BA, Lakshmi V, Krishna TP. Socioeconomic status and prevalence of toxoplasmosis during pregnancy. Indian J Med Microbiol. 2004;22:241-243.

ANNEXURE

Patient Information Sheet

Purpose of the study

For the diagnosis of toxoplasmosis in pregnant women and their newborns. Toxoplasmosis is a disease transmitted from cats to man. It is caused by the parasite, called*Toxoplasma gondii*. When a pregnant woman is infected with this parasite, it may result in a child born with jaundice, big head and involvement of eyes.The purpose of this study would be to find out how many pregnant women get infected during pregnancyand how many newborns are born with this infection.

Procedure

Three ml peripheral venous blood from pregnant women and three ml cord blood from newborn babies will be collected aseptically and will be used to detect toxoplasma antibodies. IgG and IgM antibodieswill be detected in mothers and newborns samples respectively.

Risks and Benefits

Participation in the study is purely voluntary. There is no life threatening risk involved except for mild discomfort and rarely venous thrombophlebitis may occur while drawing blood. You will not be given any compensation in the study.

Confidentiality

All information that you provide will be considered confidential and no mention of your name or any other identifying information will appear on the samples or in any publication in connection with this study. If your child has any clinical features suggestive of toxoplasma infection results will be used to treat the child.

CONSENT FORM

Sl.no:

Name:

Age:

Hospital I.P No:

I have been explained in my own language in an understandable way, about the investigating procedure that is collection of blood sample from me and cord blood sample from my baby for the purpose of study. I am fully aware of the risks involved in the procedure. I am fully aware that I reservethe right to accept or reject the proposal of study. I hereby give my willful consent for the procedure, and will not hold the doctor or paramedical staff for any untoward incident.

Date:

Signature

WORKING PROFORMA

STUDY TITLE: SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN PREGNANT WOMEN ADMITTED FOR DELIVERY AND NEWBORNS AT KOLAR.

Sl.no: **Date:**

SOCIO- DEMOGRAPHIC DATA:

Name: Hospital I.P NO:

Age:

Address: House no: Village, Taluk:

District:

Education: Occupation:

Per capita income: Socioeconomic status:

BEHAVIOURAL DATA: (Y/N)

Owning cats:

Outdoor Farm work:

Outdoor Gardening:

Eating habits: Consumption of salad:

Drinking untreated water:

Consumption of raw meat:

CLINICAL HISTORY:

Obstetric history:

Gravida:

Para:

Antenatal history:

Antenatal scans:

Previous history of still births & miscarriages:

Past medical history:

Diagnosis:

Outcome of delivery:

Results: 1) Mother

2) Newborn

Clinical Microbiologist

KEY TO MASTER CHART

Abo	Abortion history	Nu	Nurse
Age	Age in years	Occ	Occupation
B.Pharm	Bachelor of Pharmacy	OG	outdoor gardening
B.IgG	Babies IgG test result	P	Primigravida
B. IgM	Babies IgM test result	PCI	Per capita income
CAT	Owning of cats		
Cl	Clerk	PD	Post dated
CRM	Consumption of raw meat	Ph	Pharmacist
DOC	Date of Collection	Po	Positive
DUW	drinking untreated water	PT	Pre term
Edn	Education status	PUC	pre-university college
En	Engineer	SAL	consumption of salad
F	Female child	SES	Socio economic status
Fa	farmer		as per BG Prasad
Ges	Gestational stage		classification
Gra	Gravida status	std	standard
Hw	House wife	T	Term
ID	Identification detail	Ta	tailor
IT	IT employee	Te	teacher
IgG titre	IgG titre in IU/ml	UE	Uneducated
Kgs	kilograms	V	vaginal delivery
L	Lower segment - caesarean section	Y	Yes
m	Male child		

M	Multigravida	
M. IgG	maternal IgG test result	**Note**: the alphabets and numbers used in
M. IgM	maternal IgM test result	ID columns are used only for
ML	Manual labourer	identification.
MOD	Mode of delivery	
N	No	
ND	Not done	
Ne	Negative	
NTU	Novatec units	

MASTER CHART - MOTHERS

MOTHER'S I.D	DOC	Age	Gra	Ges	Abo	MOD	Edn	Occ	PCI	SES	CAT	DUW	SAL	CRM	OG	M. IgG	IgG Titre	M. IgM	NTU
1/Su/113522	10/3/2015	22	P	T	N	V	10 std	Hw	1388	4	N	N	Y	N	N	Ne	8.95	ND	ND
2/La/125415	3/23/2015	18	P	PD	N	V	1 PUC	Hw	520	5	N	N	Y	N	N	Ne	6.24	ND	ND
3/Sh/125377	3/23/2015	25	P	PD	N	V	degree	Hw	595	5	N	Y	Y	N	Y	Ne	8.09	ND	ND
4/Si/125416	3/23/2015	22	M	T	Y	V	10 std	Hw	1,333	4	N	Y	Y	N	N	Po	306	Ne	0.75
5/Mu/125407	3/23/2015	30	P	T	N	V	Nurse	Nu	2,777	3	N	N	Y	N	N	Ne	22.03	ND	ND
6/Ra/126330	3/24/2015	33	M	T	N	V	degree	Te	2,083	3	N	N	N	N	N	Ne	7.87	ND	ND
7/Mu/119890	3/27/2015	25	M	T	N	V	5 std	Fa	694	5	N	N	Y	N	Y	Po	306	Ne	0.75
8/Su/119233	3/28/2015	20	P	PT	N	V	10 std	Hw	666	5	N	Y	Y	N	Y	Ne	2.21	ND	ND
9/La/128536	3/31/2015	20	P	PD	N	V	2 PUC	Hw	1,250	4	N	Y	Y	N	N	Ne	9.45	ND	ND
10/An/128483	3/31/2015	21	P	T	N	V	2 PUC	Hw	2,777	3	N	N	Y	N	N	Po	202	Ne	1.85
11/Ja/129404	2/4/2015	26	P	PD	N	V	UE	Hw	555	5	N	Y	Y	N	N	Ne	4.82	ND	ND
12/Mo/129266	2/4/2015	19	P	T	N	V	10 std	Hw	520	5	N	N	Y	N	Y	Ne	17.76	ND	ND
13/Sh/114889	3/4/2015	21	P	PT	N	V	7 std	Hw	500	5	N	Y	Y	N	N	Ne	8.29	ND	ND
14/As/129896	3/4/2015	18	P	PT	N	V	2 PUC	Hw	714	5	N	N	N	N	N	Ne	20.67	ND	ND
15/So/129697	3/4/2015	20	M	PD	Y	V	2 PUC	Hw	1,250	4	N	N	Y	N	Y	Ne	8.24	ND	ND
16/Mo/130363	4/4/2015	20	M	PT	Y	L	10 std	Hw	520	5	N	N	Y	N	N	Ne	12.86	ND	ND
17/Ch/130325	4/4/2015	20	P	PD	N	V	4 std	Fa	833	5	Y	Y	Y	N	N	Ne	34.3	ND	ND
18/Na/132207	9/4/2015	21	P	T	N	L	10 std	Hw	1,190	4	N	Y	Y	N	N	Po	274	Ne	2.56
19/Ge/132662	10/4/2015	20	P	T	N	V	3 std	Hw	416	5	N	Y	N	N	Y	Po	160	Ne	2.28
20/Ch/128083	10/4/2015	19	P	T	N	L	1 PUC	Hw	833	5	N	Y	Y	N	N	Po	47.75	Ne	0.9
21/La/1020925	10/4/2015	23	P	T	N	V	Nurse	Hw	555	5	N	Y	Y	N	Y	Ne	7.59	ND	ND
22/La/133375	11/4/2015	31	M	T	N	V	10 std	Fa	833	5	N	N	Y	N	Y	Po	300	Ne	0.57
23/Sa/133789	4/13/2015	24	M	T	N	V	10 std	Hw	1,041	4	N	N	Y	N	Y	Ne	8.48	ND	ND
24/Su/132996	4/13/2015	22	P	T	N	L	1 PUC	Hw	1,666	4	N	Y	Y	N	N	Po	174	Ne	0.42
25/So/137967	4/23/2015	19	P	T	N	V	8 std	Hw	833	5	N	N	Y	N	N	Ne	9.68	ND	ND
26/So/137547	4/23/2015	19	P	T	N	V	2 PUC	Hw	1,666	4	N	Y	Y	N	N	Ne	6.56	ND	ND
27/Ch/138024	4/24/2015	35	M	T	N	V	UE	ML	694	5	N	Y	Y	N	N	Po	95	Ne	1.28
28/Ar/137974	4/24/2015	28	P	PD	N	V	2 PUC	Hw	1,666	4	N	Y	N	N	N	Ne	8.27	ND	ND
29/Ar/138341	4/25/2015	19	P	T	N	V	10 std	Hw	1,666	4	N	N	N	N	N	Ne	4.19	ND	ND
30/Sw/42772	4/25/2015	22	P	T	N	V	degree	Hw	595	5	N	Y	Y	N	N	Ne	17.02	ND	ND
31/Ba/138726	4/25/2015	24	M	PD	N	V	10 std	Hw	625	5	N	N	N	N	N	Ne	7.69	ND	ND
32/Um/138676	4/25/2015	22	M	PT	N	V	2 PUC	Hw	333	5	N	Y	N	N	N	Ne	5.15	ND	ND
33/Ch/121743	4/25/2015	25	M	T	Y	V	10 std	Hw	1,666	4	N	Y	Y	N	Y	Ne	2.14	ND	ND
34/Sa/138675	4/26/2015	22	M	T	Y	V	5 std	Fa	1,000	4	N	Y	Y	N	N	Ne	4.54	ND	ND
35/Ro/138809	4/26/2015	18	P	PD	N	V	10 std	Hw	2,083	3	N	Y	N	N	N	Ne	4.16	ND	ND
36/Re/138667	4/26/2015	23	P	T	N	V	8 std	Hw	694	5	N	Y	Y	N	Y	Po	95.6	Ne	1.05
37/As/1021178	4/26/2015	24	P	PD	N	V	6 std	Hw	833	5	N	Y	Y	N	N	Ne	4.68	ND	ND
38/Re/138720	4/26/2015	30	M	T	N	V	2 PUC	Fa	1,190	4	Y	Y	Y	N	Y	Ne	11.78	ND	ND
39/Kh/137968	4/27/2015	30	M	T	Y	V	10 std	Hw	666	5	N	Y	Y	N	N	Ne	5.65	ND	ND
40/ Ra/137381	4/27/2015	21	P	T	N	V	7 std	Hw	952	4	N	Y	Y	N	N	Ne	4.92	ND	ND
41/Ra/122335	4/29/2015	20	M	T	Y	V	2 PUC	Hw	833	5	Y	Y	Y	N	N	Ne	3.76	ND	ND
42/La/139915	4/29/2015	21	P	T	N	V	10 std	Hw	833	5	N	Y	Y	N	N	Po	292	Ne	5.27
43/Am/141999	5/5/2015	25	M	T	N	L	6 std	Hw	740	5	N	Y	Y	N	N	Ne	4.4	ND	ND
44/ Ka/142446	5/5/2015	21	M	PD	N	V	8 std	Hw	1,666	4	N	Y	Y	N	Y	Ne	12.68	ND	ND
45/Ro/142888	6/5/2015	23	P	PT	N	V	2 PUC	Hw	833	5	N	Y	Y	N	Y	Ne	6.5	ND	ND
46/ Va/142535	6/5/2015	19	P	T	N	V	5 std	Hw	952	4	N	Y	Y	N	N	Ne	14.49	ND	ND
47/Ar/140996	6/5/2015	22	M	T	N	V	8 std	Hw	1,111	4	N	Y	Y	N	N	Ne	8.72	ND	ND
48/Ko/141090	8/5/2015	30	M	T	N	V	4 std	Hw	1,041	4	N	Y	Y	N	N	Ne	32.33	ND	ND
49/Ra/1021287	8/5/2015	30	M	T	Y	V	Nurse	Nu	1,190	4	N	Y	Y	N	N	Ne	2.27	ND	ND
50/Ma/142692	9/5/2015	25	M	T	N	V	7 std	Fa	925	5	N	Y	Y	N	N	Ne	5.38	ND	ND
51/Ay/142851	9/5/2015	24	P	PD	N	V	5 std	Hw	1,388	4	N	Y	Y	N	N	Ne	23.94	ND	ND
52/Na/144222	10/5/2015	28	M	T	N	V	4 std	Hw	952	4	N	Y	Y	N	Y	Ne	12.1	ND	ND
53/Mo/144050	9/5/2015	26	M	T	N	V	10 std	Hw	555	5	N	Y	N	N	N	Po	230	Ne	0.88
54/Sh/144188	10/5/2015	21	P	T	N	V	8 std	Hw	520	5	N	N	Y	N	N	Ne	5.01	ND	ND

MASTER CHART - MOTHERS

55/Ch/144296	11/5/2015	24	P	T	N	V	2 PUC	Ta	666	5	N	N	Y	N	N	Ne	4.2	ND	ND
56/De/77972	5/14/2015	22	P	PT	N	V	degree	Hw	740	5	N	Y	Y	N	N	Ne	3.11	ND	ND
57/So/145537	5/14/2015	22	P	T	N	V	10 std	Hw	595	5	N	Y	Y	N	Y	Ne	5.39	ND	ND
58/Pa/146336	5/16/2015	24	P	T	N	V	10 std	Hw	833	5	N	N	Y	N	Y	Ne	10.21	ND	ND
59/Ro/147843	5/20/2015	28	M	PD	N	V	2 PUC	Hw	1,333	4	N	N	Y	N	N	Ne	4.41	ND	ND
60/Re/132725	5/16/2015	24	M	T	N	V	6 std	Hw	833	5	N	N	Y	N	Y	Ne	32.04	ND	ND
61/Pu/146331	5/16/2015	28	P	T	N	V	1 PUC	Ta	925	5	N	N	Y	N	N	Ne	3.41	ND	ND
62/Fa/146740	5/17/2015	21	P	T	N	V	10 std	Hw	555	5	N	Y	Y	N	N	Ne	6.27	ND	ND
63/Bh/146738	5/17/2015	20	P	T	N	V	8 std	Hw	500	5	N	N	Y	N	N	Po	270	Ne	3.22
64/Ma/146777	5/17/2015	19	P	T	N	V	5 std	Hw	520	5	N	N	Y	N	N	Ne	3.12	ND	ND
65/An/146722	5/18/2015	19	P	PT	N	V	2 PUC	Hw	1,000	4	N	N	Y	N	N	Ne	4.41	ND	ND
66/Ra/148663	5/22/2015	29	M	T	Y	V	7 std	Hw	694	5	N	N	N	N	N	Po	256	Ne	0.75
67/No/150021	5/25/2015	22	M	T	N	V	5 std	Hw	952	4	N	N	Y	N	Y	Po	230	Ne	1.85
68/Sh/149578	5/25/2015	25	M	T	Y	V	2 PUC	Hw	833	5	Y	N	Y	N	Y	Ne	10.78	ND	ND
69/Gu/150065	5/26/2015	20	P	T	N	V	7 std	Hw	694	5	N	N	Y	N	Y	Ne	19.1	ND	ND
70/Ma/147882	5/28/2015	23	M	T	N	V	10 std	Hw	1,111	4	N	Y	Y	N	N	Ne	13.07	ND	ND
71/ Sh/151003	5/29/2015	20	P	T	N	V	7 std	Hw	714	5	N	Y	N	N	Y	Ne	4.34	ND	ND
72/Sa/149058	5/29/2015	20	P	T	N	V	10 std	Hw	833	5	N	Y	Y	N	N	Ne	6.14	ND	ND
73/Ra/152107	5/31/2015	23	P	T	N	V	9 std	Hw	1,666	4	N	N	Y	N	N	Ne	21.7	ND	ND
74/Sw/120390	1/6/2015	26	P	PD	N	V	1 PUC	Hw	1,388	4	N	N	Y	N	N	Ne	28.33	ND	ND
75/La/151997	1/6/2015	21	P	PD	N	V	6 std	Hw	1,666	4	N	Y	Y	N	Y	Po	202	Ne	0.88
76/An/157876	6/15/2015	22	M	T	Y	V	2 PUC	Ta	1,000	4	N	N	Y	N	N	Ne	7.1	ND	ND
77/Pa/150397	1/6/2015	22	M	T	N	V	10 std	Hw	625	5	N	N	Y	N	Y	Po	252	Ne	0.88
78/ Ma/152078	1/6/2015	23	M	T	N	V	degree	Hw	2,083	3	N	N	Y	N	N	Ne	2.12	ND	ND
79/ AS/152594	2/6/2015	20	P	T	N	V	8 std	Hw	1,333	4	N	Y	Y	N	N	Po	256	Ne	6.7
80/Jy/152587	2/6/2015	22	M	T	N	V	2 PUC	Hw	833	5	N	Y	Y	N	Y	Ne	17.41	ND	ND
81/An/160846	6/22/2015	24	M	PT	N	V	2 PUC	Hw	625	5	N	Y	Y	N	N	Ne	2.37	ND	ND
82/Su/150658	6/23/2015	19	P	PT	N	V	8 std	Hw	694	5	N	N	Y	N	N	Ne	4.65	ND	ND
83/Sa/165288	3/7/2015	22	M	T	N	V	UE	Hw	833	5	N	N	N	N	N	Po	87	Ne	1.28
84/Ko/1021905	7/7/2015	23	M	T	N	V	10 std	Hw	1,333	4	N	N	Y	N	N	Po	122	Ne	0.88
85/Sa/166524	7/7/2015	21	P	T	N	V	5 std	Hw	1,250	4	N	N	Y	N	N	Ne	23.03	ND	ND
86/Ar/104013	7/7/2015	25	M	T	N	V	5 std	Hw	520	5	N	N	Y	N	N	Ne	3.04	ND	ND
87/Pa/167183	8/7/2015	20	P	T	N	V	10 std	Hw	694	5	N	N	Y	N	N	Ne	27.9	ND	ND
88/Ge/167192	8/7/2015	24	P	T	N	V	10 std	Hw	1,333	4	N	N	Y	N	N	Ne	23.7	ND	ND
89/Ma/171355	18/7/2015	22	M	T	N	V	5 std	Hw	595	5	N	Y	Y	N	N	Ne	6.48	ND	ND
90/SU/ 160990	23/7/2015	30	P	T	N	V	6 std	Hw	694	5	N	Y	Y	N	N	Po	68.6	Ne	0.57
91/Sh/173955	25/7/2015	25	P	T	N	V	10 std	Hw	1,666	4	N	Y	Y	N	N	Po	35.6	Ne	1.71
92/Am/109238	18/8/2015	26	M	T	N	V	10 std	Hw	694	5	Y	N	N	N	N	Po	35.1	Ne	0.75
93/Pa/185315	19/8/2015	20	P	T	N	V	5 std	Hw	1,000	4	N	N	Y	N	N	Po	52.3	Ne	0.57
94/Sh/185347	20/8/2015	22	P	T	N	V	5 std	ML	1,041	4	Y	N	Y	N	N	Ne	28.1	ND	ND
95/Bh/185348	20/8/2015	24	P	T	N	V	10 std	Hw	1,666	4	N	Y	Y	N	N	Po	36.7	Ne	6.42
96/Ge/159788	22/8/2015	20	P	T	N	V	3 std	Fa	1,041	4	Y	N	N	N	N	Ne	6.5	ND	ND
97/Su/186693	8/24/2015	25	M	PT	Y	V	10 std	Hw	714	5	N	N	N	N	N	Po	210	Ne	1.71
98/Ka/184136	8/25/2015	23	M	T	Y	V	2 PUC	Hw	1,388	4	N	Y	Y	N	N	Ne	24.8	ND	ND
99/Ka/187169	8/25/2015	30	M	PT	N	V	3 std	Hw	555	5	N	N	N	N	N	Po	108.6	Ne	2.56
100/Sr/187199	8/26/2015	26	M	T	Y	V	10 std	Hw	1,666	4	N	Y	Y	N	N	Po	198.9	Ne	0.57
101/Sh/119081	8/27/2015	22	P	T	N	V	3 std	Hw	1,000	4	Y	Y	Y	N	N	Po	192.8	Ne	0.88
102/Sa/188778	8/29/2015	24	M	T	N	V	5 std	Hw	714	5	N	Y	N	N	N	Po	198	Ne	2.85
103/Sh/184335	1/9/2015	26	P	PT	N	V	7 std	Hw	1,111	4	Y	Y	Y	N	N	Ne	12.4	ND	ND
104/As/190174	1/9/2015	19	P	T	N	V	5 std	Hw	833	5	N	N	N	N	N	Ne	33.2	ND	ND
105/Ar/189115	3/9/2015	25	M	T	Y	V	3 std	Hw	833	5	N	Y	Y	N	Y	Po	52.7	Po	26.24
106/Sa/190673	3/9/2015	22	P	T	N	V	8 std	Hw	1,041	4	N	Y	Y	N	Y	Ne	32.9	ND	ND
107/An/191862	5/9/2015	18	P	T	N	V	5 std	Hw	833	5	N	Y	Y	N	N	Ne	25.7	ND	ND
108/Je/190990	6/9/2015	23	M	T	N	V	5 std	Hw	1,333	4	Y	Y	Y	N	N	Po	138.2	Ne	2.3
109/Su/125495	6/9/2015	26	M	T	Y	V	6 std	ML	694	5	N	N	N	N	N	Po	38.2	Ne	1.44
110/Sa/215632	1/11/2015	24	P	PD	N	V	2 PUC	Hw	1,666	4	N	Y	Y	N	Y	Po	40	Ne	3.5
111/Ru/221265	15/11/2015	24	P	PT	N	L	6 std	Hw	833	5	N	Y	N	N	N	Ne	10	ND	ND
112/Vi/210612	11/17/2015	32	M	T	N	V	UE	Hw	520	5	N	Y	Y	N	N	Ne	21	ND	ND
113/Go/221099	11/17/2015	26	M	T	N	V	5 std	Ta	1,111	4	N	Y	Y	N	N	Ne	27.7	ND	ND

MASTER CHART - MOTHERS

114/Mu/222369	11/18/2015	22	M	T	N	V	8 std	Hw	1,000	4	N	Y	Y	N	N	Ne	26.8	ND	ND
115/Ma/222542	11/19/2015	25	M	T	N	V	10 std	Hw	1,388	4	N	N	Y	N	Y	Ne	19	ND	ND
116/Pu/222535	11/19/2015	23	P	T	N	V	10 std	Hw	833	5	N	N	Y	N	N	Po	90.9	Ne	3.14
117/Re/ 208952	11/21/2015	19	P	T	N	V	8 Std	Hw	1,111	4	N	N	N	N	N	Po	138	Ne	0.85
118/Ar/223622	11/21/2015	21	P	PD	N	V	5 std	Hw	694	5	N	N	N	N	N	Ne	7.3	ND	ND
119/So/224039	11/23/2015	25	P	T	N	V	5 std	Hw	1,041	4	Y	Y	Y	N	N	Ne	15.2	ND	ND
120/Su/222932	11/23/2015	24	P	T	N	V	5 std	Hw	1,000	4	N	N	N	N	N	Ne	15.1	ND	ND
121/As/224600	11/24/2015	24	M	T	Y	V	4 std	ML	833	5	N	N	Y	N	N	Ne	19.9	ND	ND
122/ Ma/224741	11/25/2015	25	M	T	N	V	10 std	Hw	925	5	N	Y	Y	N	N	Po	36.9	Ne	5.97
123/Va/234662	12/18/2015	23	P	T	N	V	2 PUC	Te	1,000	4	N	N	N	N	N	Ne	10.4	ND	ND
124/As/235137	12/19/2015	23	P	T	N	V	6 std	Hw	833	5	N	Y	Y	N	N	Ne	23.8	ND	ND
125/Ma/235530	12/20/2015	21	M	T	N	V	3 std	Hw	833	5	N	N	Y	N	N	Ne	18.2	ND	ND
126/Sh/236916	12/24/2015	21	M	T	Y	V	5 std	Hw	833	5	N	Y	N	N	N	Ne	12.2	ND	ND
127/ Su/ 236747	12/23/2015	23	M	T	N	L	10 std	Hw	1,111	4	N	N	N	N	Y	Ne	14.8	ND	ND
128/Ch/237662	12/26/2015	23	P	T	N	V	8 std	Hw	1,333	4	N	Y	Y	N	N	Ne	10.6	ND	ND
129/Gi/238121	12/28/2015	30	P	T	N	V	Degree	Te	1,388	4	N	Y	Y	N	N	Ne	32.1	ND	ND
130/Sh/ 238124	12/28/2015	28	M	T	Y	V	7 std	Hw	714	5	N	Y	Y	N	N	Ne	19.8	ND	ND
131/Ai/ 238994	12/30/2015	24	P	T	N	V	6 std	Hw	833	5	N	N	N	N	N	Ne	14.2	ND	ND
132/Va/239310	12/31/2015	21	P	T	N	V	8 std	Hw	833	5	N	Y	Y	N	N	Ne	11.9	ND	ND
133/Su/145872	12/31/2015	28	M	T	N	V	2 PUC	Hw	1,333	4	N	Y	Y	N	N	Ne	26.5	ND	ND
134/Sa/240018	2/1/2016	21	P	T	N	V	5 std	Hw	833	5	N	N	Y	N	N	Po	89.7	Ne	8.23
135/Ma/240087	2/1/2016	20	M	T	Y	V	6 std	ML	520	5	N	N	N	N	N	Ne	3.2	ND	ND
136/Sh/230324	2/1/2016	23	P	T	N	V	10 std	Hw	952	4	N	Y	Y	N	N	Ne	8.1	ND	ND
137/Ar/240528	4/1/2016	25	P	T	N	V	8 std	Hw	952	4	N	Y	Y	N	N	Ne	26.7	ND	ND
138/Ve/242255	9/1/2016	20	M	T	N	V	5 std	Hw	1,000	4	N	Y	Y	N	N	Ne	28.1	ND	ND
139/Sa/243164	11/1/2016	26	M	T	Y	L	4 std	Hw	1,111	4	Y	N	Y	N	N	Ne	11.6	ND	ND
140/Da/ 243083	11/1/2016	18	P	PT	N	V	8 std	Hw	1,250	4	N	Y	Y	N	N	Ne	5.7	ND	ND
141/Ne/191355	12/1/2016	23	M	T	N	L	10 std	Ta	1,333	4	N	N	N	N	N	Ne	1.7	ND	ND
142/Dh/243931	1/13/2016	22	P	T	N	V	5 std	Hw	1,333	4	N	N	N	N	N	Ne	0.6	ND	ND
143/An/243941	1/13/2016	27	M	T	Y	V	3 std	Hw	694	5	N	Y	Y	N	N	Po	252	Ne	0.86
144/As/245004	1/16/2016	21	P	T	N	V	6 std	Hw	1,666	4	N	N	Y	N	N	Ne	2.3	ND	ND
145/Sa/245358	1/16/2016	26	M	T	N	V	2 PUC	Hw	833	5	N	Y	Y	N	N	Ne	1.7	ND	ND
146/Ra/231937	1/17/2016	19	P	T	N	V	4 std	Hw	833	5	N	N	N	N	N	Ne	4.3	ND	ND
147/Ch/177177	1/19/2016	23	M	T	Y	V	8 std	Hw	952	4	N	N	N	N	N	Ne	1.1	ND	ND
148/Ku/261711	3/15/2016	23	P	T	N	V	B.Pharm	Ph	2,083	3	N	N	N	N	N	Ne	4.2	ND	ND
149/Ru/268988	3/22/2016	28	M	T	N	V	4 std	Hw	1,111	4	N	Y	Y	N	N	Ne	2.6	ND	ND
150/Sh/264250	3/26/2016	20	P	T	N	V	5 std	Ta	1,250	4	N	N	N	N	N	Ne	1.6	ND	ND
151/Sa/269855	3/26/2016	26	M	T	N	V	10 std	Hw	952	4	N	Y	Y	N	N	Ne	1.8	ND	ND
152/Sh/273696	5/4/2016	26	P	T	N	V	10 std	Hw	1,000	4	N	Y	Y	N	N	Ne	2.6	ND	ND
153/AY/288249	3/29/2016	20	P	PT	N	V	10 std	Hw	833	5	N	Y	Y	N	N	Ne	3.7	ND	ND
154/Sh/288136	12/5/2016	25	M	T	N	L	2 PUC	Hw	1,666	4	Y	Y	Y	N	N	Po	256	Ne	1
155/Na/288247	12/5/2016	25	M	T	N	L	Degree	Cl	1,388	4	N	N	N	N	N	Po	176	Ne	2.2
156/An/280206	12/5/2016	23	M	T	N	L	10 std	Hw	952	4	Y	N	N	N	N	Po	248	Ne	1.2
157/Ve/284074	12/5/2016	29	M	T	N	L	3 std	ML	1,041	4	N	Y	Y	N	N	Ne	6.1	ND	ND
158/Pa/289099	5/15/2016	27	M	T	Y	V	10 std	Hw	1,333	4	Y	Y	Y	N	N	Ne	1	ND	ND
159/Me/289152	5/16/2016	26	P	T	N	V	2 PUC	Hw	1,388	4	Y	N	N	N	N	Ne	1.4	ND	ND
160/Sh/289519	5/16/2016	34	M	T	Y	V	7 std	Hw	625	5	N	Y	Y	N	N	Po	224	Ne	1.5
161/Sh/289608	5/16/2016	27	M	T	N	L	10 std	Hw	1,333	4	Y	N	N	N	N	Ne	1.3	ND	ND
162/Sh/162423	5/24/2016	27	M	T	Y	V	5 std	Hw	1,000	4	Y	Y	Y	N	N	Ne	1.9	ND	ND
163/La/293208	5/25/2016	24	M	T	N	V	10 std	Hw	1,388	4	Y	N	N	N	N	Ne	1.5	ND	ND
164/Ma/293741	5/26/2016	24	M	T	N	L	UE	ML	714	5	Y	Y	N	N	Y	Po	154	Ne	0.9
165/Sa/289956	5/27/2016	24	M	T	N	L	5 std	Hw	1,111	4	N	Y	Y	N	N	Ne	1.7	ND	ND
166/Ja/293782	5/27/2016	26	P	T	N	V	UE	Hw	833	5	Y	Y	Y	N	N	Ne	3.2	ND	ND
167/Ha/294137	5/27/2016	32	M	T	N	L	UE	Hw	714	5	N	Y	Y	N	N	Ne	5.9	ND	ND
168/Bh/294296	5/28/2016	22	P	T	N	V	5 std	Hw	1,041	4	N	Y	Y	N	N	Ne	1.4	ND	ND
169/Va/295195	5/31/2016	24	P	T	N	L	6 std	Hw	1,111	4	N	N	N	N	N	Ne	1.9	ND	ND
170/Jy/296494	1/6/2016	20	P	T	N	V	10 std	Hw	1,000	4	N	Y	Y	N	N	Ne	1.7	ND	ND
171/Jy/295994	1/6/2016	22	M	T	N	L	6 std	Hw	1,250	4	N	Y	Y	N	N	Ne	4.2	ND	ND
172/So/289449	2/6/2016	22	M	T	N	V	2 PUC	Hw	1,333	4	N	N	N	N	N	Ne	3.8	ND	ND

MASTER CHART - MOTHERS

173/Am/296850	3/6/2016	21	P	T	N	L	10 std	Hw	1,666	4	N	N	N	N	N	Ne	2.8	ND	ND
174/Mo/296868	3/6/2016	21	P	T	N	L	10 std	Hw	833	5	N	N	N	N	N	Po	167	Ne	2
175/Su/297454	3/6/2016	21	P	T	N	L	4 std	Hw	1,000	4	N	N	N	N	N	Ne	2.1	ND	ND
176/So/288560	4/6/2016	26	M	T	N	V	2 PUC	Ta	1,388	4	Y	N	N	N	N	Ne	2.1	ND	ND
177/Pa/286920	4/6/2016	24	M	T	N	V	5 std	Hw	833	5	N	Y	Y	N	N	Ne	2.6	ND	ND
178/La/297966	5/6/2016	21	M	T	Y	V	degree	Te	1,666	4	N	N	N	N	N	Ne	4.8	ND	ND
179/La/296391	6/6/2016	24	M	T	N	L	10 std	Hw	1,333	4	Y	N	N	N	N	Ne	3.4	ND	ND
180/Ra/297610	7/6/2016	18	P	T	N	L	10 std	Hw	714	5	Y	Y	Y	N	N	Ne	2.2	ND	ND
181/Va/298557	8/6/2016	27	P	T	N	V	5 std	ML	833	5	N	Y	Y	N	N	Ne	1.6	ND	ND
182/Ve/298666	8/6/2016	23	P	T	N	L	UE	ML	595	5	N	Y	Y	N	N	Ne	1.3	ND	ND
183/Pa/293821	11/6/2016	22	P	T	N	V	2 PUC	Hw	1,111	4	N	Y	Y	N	N	Ne	1.2	ND	ND
184/Ni/301897	6/15/2016	20	P	T	N	L	2 PUC	Hw	1,333	4	N	Y	Y	N	N	Ne	1.3	ND	ND
185/Sa/301920	6/16/2016	22	M	T	Y	L	10 std	Hw	833	5	N	Y	Y	N	N	Ne	5.9	ND	ND
186/An/301929	6/16/2016	24	P	T	N	V	2 PUC	Hw	1,666	4	N	Y	Y	N	N	Ne	3	ND	ND
187/Ma/302648	6/17/2016	22	M	PT	N	L	5 std	Hw	833	5	N	Y	Y	N	N	Ne	2.8	ND	ND
188/An/302631	6/17/2016	28	M	T	N	L	6 std	Hw	1,000	4	N	Y	Y	N	N	Ne	5.2	ND	ND
189/Na/219183	6/20/2016	25	M	T	Y	V	UE	Hw	833	5	N	Y	Y	N	N	Ne	1.2	ND	ND
190/An/303000	6/21/2016	24	M	T	N	V	10 std	Ta	1,333	4	N	Y	Y	N	N	Ne	2.3	ND	ND
191/Su/292408	6/21/2016	25	M	T	N	L	10 std	Hw	1,666	4	Y	N	Y	N	N	Po	266	Ne	2.8
192/Bh/304091	6/22/2016	21	M	T	N	L	2 PUC	Hw	1,111	4	N	Y	Y	N	N	Ne	5.3	ND	ND
193/Gu/303673	6/22/2016	20	P	T	N	L	2 std	ML	1,000	4	Y	Y	Y	N	N	Ne	1.9	ND	ND
194/La/303831	6/21/2016	24	M	T	N	L	5 std	Hw	1,041	4	N	Y	Y	N	N	Ne	6.4	ND	ND
195/Su/299186	6/22/2016	24	M	T	N	L	10 std	Hw	1,333	4	Y	Y	Y	N	N	Ne	5.4	ND	ND
196/Jy/ 305514	6/25/2016	30	M	T	N	V	2 PUC	Hw	833	5	N	Y	Y	N	N	Ne	5.5	ND	ND
197/Va/305225	6/24/2016	25	M	T	N	L	10 std	Ta	1,111	4	Y	Y	Y	N	N	Ne	22.7	ND	ND
198/Sw/284608	6/25/2016	25	M	T	N	L	Degree	IT	1,388	4	N	N	N	N	N	Po	204	Ne	1.4
199/Ma/307200	6/30/2016	28	M	PT	N	L	9 std	Hw	714	5	N	Y	Y	N	N	Ne	3.6	ND	ND
200/Ch/241090	4/7/2016	21	M	T	N	V	5 std	Hw	833	5	N	Y	Y	N	N	Ne	3.2	ND	ND
201/Am/298317	5/7/2016	23	P	T	N	V	8 std	Hw	1,250	4	N	Y	Y	N	N	Ne	3.5	ND	ND
202/Ch/298763	6/7/2016	21	P	T	N	L	2 PUC	Hw	1,666	4	Y	N	N	N	N	Ne	1.4	ND	ND
203/Ma/309602	6/7/2016	28	P	T	N	L	M.Sc	Te	2,083	3	N	N	N	N	N	Ne	2.6	ND	ND
204/In/309648	6/7/2016	22	P	T	N	L	2 PUC	Hw	1,333	4	N	N	N	N	N	Ne	5.1	ND	ND
205/Ka/309638	6/7/2016	18	P	T	N	L	7 std	Hw	833	5	N	Y	Y	N	N	Ne	6.7	ND	ND
206/Su/309731	6/7/2016	22	M	T	Y	L	UE	ML	520	5	N	Y	Y	N	N	Po	266	Ne	2.9
207/La/309961	7/7/2016	22	M	T	N	V	5 std	ML	833	5	N	Y	Y	N	N	Po	141	Ne	2.2
208/Aa/309474	12/7/2016	22	M	T	N	V	2 PUC	Hw	1,111	4	N	Y	Y	N	N	Ne	6.2	ND	ND
209/Ma/306380	12/7/2016	23	P	T	N	V	B.E	En	2,777	3	N	Y	Y	N	N	Ne	5.8	ND	ND
210/Sh/249945	7/14/2016	23	M	T	N	L	5 std	Hw	1,000	4	N	Y	Y	N	N	Ne	9.3	ND	ND
211/Am/313096	7/15/2016	23	P	T	N	V	4 std	Hw	1,666	4	N	Y	Y	N	N	Ne	2.2	ND	ND
212/Ru/314657	7/19/2016	22	P	T	N	L	10 std	ML	1,041	4	Y	Y	Y	N	N	Ne	13	ND	ND
213/Su/314524	7/19/2016	26	M	T	Y	L	2 PUC	Fa	2,083	3	N	Y	Y	N	N	Ne	2.2	ND	ND
214/Ka/312953	7/20/2016	24	M	T	N	L	10 std	Hw	1,333	4	N	Y	Y	N	N	Ne	2.4	ND	ND
215/Jy/311607	7/20/2016	25	M	T	N	L	10 std	Hw	833	5	N	Y	Y	N	N	Ne	0.7	ND	ND
216/Sh/315092	7/20/2016	28	M	PT	N	V	Degree	Te	2,083	3	N	Y	Y	N	N	Ne	0.2	ND	ND
217/Na/239960	7/20/2016	28	M	T	N	L	B. com	Hw	1,000	4	N	Y	Y	N	N	Ne	0.1	ND	ND
218/Va/249947	7/21/2016	22	M	T	N	V	5 std	Hw	1,111	4	Y	N	N	N	N	Ne	1.8	ND	ND
219/Sa/3903	7/22/2016	23	M	T	N	V	M.A	Hw	1,666	4	N	N	N	N	N	Ne	1.8	ND	ND
220/Cl/253294	7/15/2016	26	M	T	N	L	2 PUC	Hw	1,388	4	N	Y	Y	N	N	Ne	33	ND	ND
221/Su/311558	7/22/2016	25	M	T	Y	L	10 std	ML	833	5	N	Y	Y	N	N	Ne	0.1	ND	ND
222/Su/295826	7/23/2016	24	P	T	N	L	Diploma	Hw	1,111	4	N	Y	Y	N	N	Ne	0.2	ND	ND
223/Ma/316604	7/23/2016	29	M	T	N	V	10 std	Hw	1,333	4	Y	Y	Y	N	N	Ne	3.7	ND	ND
224/Ka/156402	7/23/2016	24	M	T	N	V	5 std	ML	833	5	N	Y	Y	N	N	Ne	1.7	ND	ND
225/Ma/313466	7/23/2016	25	M	T	N	L	Degree	Te	1,388	4	N	Y	Y	N	N	Ne	2.5	ND	ND
226/Su/289648	7/26/2016	33	M	T	Y	V	5 std	Hw	1,333	4	N	Y	Y	N	N	Ne	5.5	ND	ND
227/Am/318474	7/29/2016	25	M	T	Y	L	Degree	Te	2,083	3	Y	Y	Y	N	N	Ne	3.3	ND	ND
228/Ro/317164	7/25/2016	20	P	T	N	L	2 PUC	Hw	1,111	4	N	Y	Y	N	N	Ne	16.6	ND	ND
229/Me/318690	7/28/2016	24	M	T	N	L	8 std	Hw	1,000	4	N	Y	Y	N	N	Ne	3.4	ND	ND
230/Am/317060	7/24/2016	26	M	PT	N	L	8 std	Hw	1,333	4	N	Y	Y	N	N	Ne	1.9	ND	ND
231/La/315389	7/29/2016	21	M	T	Y	L	2 PUC	Hw	1,388	4	N	N	N	N	N	Ne	1.9	ND	ND

MASTER CHART - MOTHERS

232/Mu/318475	7/27/2016	23	M	T	N	L	UE	ML	833	5	N	Y	Y	N	N	Ne	1.7	ND	ND
232/Al/319179	7/30/2016	20	M	T	Y	V	8 std	Hw	1,666	4	N	N	N	N	N	Ne	13.5	ND	ND
234/Pr/321927	6/8/2016	25	M	T	Y	L	9 std	Hw	714	5	N	N	N	N	N	Po	118	Ne	1.7
235/Pa/323238	9/8/2016	20	P	T	N	L	Degree	Hw	833	5	N	Y	Y	N	N	Ne	1.9	ND	ND
236/Ra/323439	9/8/2016	32	M	PT	N	L	B. Ed	Te	1,388	4	N	Y	Y	N	N	Ne	12	ND	ND
237/Ya/323582	9/8/2016	23	M	T	N	L	UE	Hw	1,000	4	N	Y	Y	N	N	Po	180	Ne	1
238/Ar/326841	8/17/2016	29	M	T	N	L	8 std	Hw	1,388	4	N	Y	Y	N	N	Ne	0.3	ND	ND
239/Ku/155555	11/8/2016	22	M	T	N	L	5 std	Hw	952	4	N	N	N	N	N	Ne	11.2	ND	ND
240/Bh/286706	12/8/2016	27	P	T	N	L	3 std	Hw	833	5	N	Y	Y	N	N	Ne	5	ND	ND
241/Ga/317493	8/16/2016	25	M	T	Y	L	10 std	Hw	833	5	N	Y	Y	N	N	Ne	4	ND	ND
242/Ra/327016	8/17/2016	24	M	T	Y	L	UE	ML	694	5	N	N	N	N	N	Ne	13	ND	ND
243/Ka/322257	8/17/2016	27	M	T	N	L	2 PUC	Hw	1,333	4	N	Y	Y	N	N	Ne	9	ND	ND
244/Ra/329343	8/23/2016	22	M	T	N	L	10 std	Hw	1,388	4	N	Y	Y	N	N	Ne	20	ND	ND
245/So/329094	8/24/2016	32	M	T	N	L	8 std	Hw	833	5	N	Y	Y	N	N	Po	232	Ne	1.34
246/Pa/279052	8/25/2016	28	M	T	N	L	4 std	Hw	1,333	4	N	Y	Y	N	N	Po	41	Ne	0.8
247/Ta/329878	8/25/2016	22	M	T	N	L	10 std	Hw	2,777	3	Y	Y	Y	N	Y	Ne	2.3	ND	ND
248/Pa/251290	8/31/2016	24	M	T	N	L	10 std	Hw	833	5	N	Y	Y	N	N	Ne	3.5	ND	ND
249/Na/313342	8/29/2016	27	M	T	N	L	2 PUC	Hw	952	4	N	N	N	N	N	Ne	3.1	ND	ND
250/Ya/336058	7/9/2016	30	M	T	N	L	2 PUC	Hw	1,111	4	N	Y	Y	N	N	Ne	2.3	ND	ND
251/Ya/306543	9/15/2016	22	M	T	N	L	2 PUC	Ta	1,666	4	Y	Y	Y	N	N	Ne	4.8	ND	ND

MASTER CHART - BABIES

Sl.No	BABIES I.D	Baby's Sex	Baby's Weight (Kgs)	APGAR SCORE	B. IgM	NTU	B. IgG	IgG Titre
1	1/ B/O Su/113522	f	3.26	1' -7/10, 5'-9/10	Ne	2.36	ND	ND
2	2/B/O La/125415	m	3.1	1' -7/10, 5'-9/10	Ne	1.89	ND	ND
3	3/ B/O Sh/125377	f	2.8	1' -7/10, 5'-9/10	Ne	2.21	ND	ND
4	4/ B/O Si/125416	f	2.2	1' -7/10, 5'-9/10	Ne	1.99	Po	232
5	5/ B/O Mu/125407	m	2.94	1' -7/10, 5'-9/10	Ne	2	ND	ND
6	6/ B/O Ra/126330	f	2.82	1' -7/10, 5'-9/10	Ne	2.36	ND	ND
7	7/B/O Mu/119890	m	3.29	1' -7/10, 5'-9/10	Ne	1.69	Po	220
8	8/B/O su/119233	m	2.2	1' -7/10, 5'-9/10	Ne	2.99	ND	ND
9	9/ B/O La/128536	f	3.2	1' -7/10, 5'-9/10	Ne	1.97	ND	ND
10	10/ B/O An/128483	m	3.1	1' -7/10, 5'-9/10	Ne	1.86	Po	220
11	11/ B/O Ja/129404	m	3.32	1' -3/10, 5'-5/10	Ne	2.15	ND	ND
12	12/ B/O Mo/129266	m	3.66	1' -7/10, 5'-9/10	Ne	1.96	ND	ND
13	13/B/O Sh/114889	f	1.74	1' -3/10, 5'-5/10	Ne	2.56	ND	ND
14	14/ B/O As/129896	f	1.62	1' -3/10, 5'-7/10	Ne	2.46	ND	ND
15	15/B/O So/129697	f	3.36	1' -7/10, 5'-9/10	Ne	2.37	ND	ND
16	16/ B/O Mo/130363	m	1.58	1' -7/10, 5'-9/10	Ne	1.59	ND	ND
17	17/ B/O Ch/130325	f	2.62	1' -7/10, 5'-9/10	Ne	1.58	ND	ND
18	18/ B/O Na/132207	m	2.8	1' -7/10, 5'-9/10	Ne	1.94	Ne	9.3
19	19/B/O Ge/132662	f	2.86	1' -7/10, 5'-9/10	Ne	1.78	Po	123
20	20/ B/O Ch/128083	m	3.25	1' -7/10, 5'-9/10	Ne	2.15	Ne	3.8
21	21/B/O La /1020295	f	2.36	1' -7/10, 5'-9/10	Ne	1.7	ND	ND
22	22/ B/O La/133375	m	2.75	1' -7/10, 5'-9/10	Ne	1.76	Po	97
23	23/ B/O Sa/133789	f	2.7	1' -7/10, 5'-9/10	Ne	1.95	ND	ND
24	24/ B/O Su/132996	f	2.8	1' -7/10, 5'-9/10	Ne	3.04	Po	52
25	25/ B/O So/137967	f	2.2	1' -7/10, 5'-9/10	Ne	2.29	ND	ND
26	26/ B/O So/137547	m	2.24	1' -7/10, 5'-9/10	Ne	2.03	ND	ND
27	27/ B/O Ch/138024	m	2.7	1' -7/10, 5'-9/10	Ne	2.14	Po	64
28	28/ B/O Ar/137974	m	3.09	1' -7/10, 5'-9/10	Ne	2.28	ND	ND
29	29/ B/O Ar/138341	f	3.14	1' -7/10, 5'-9/10	Ne	3.25	ND	ND
30	30/ B/O Sw/42772	m	2.79	1' -7/10, 5'-9/10	Ne	2.95	ND	ND
31	31/ B/O Ba/138726	m	3.22	1' -7/10, 5'-9/10	Ne	2.6	ND	ND
32	32/ B/O Um/138676	m	1.98	1' -7/10, 5'-9/10	Ne	1.71	ND	ND
33	33/ B/O Ch/121743	m	3.12	1' -7/10, 5'-9/10	Ne	2.69	ND	ND
34	34/ B/O Sa/138675	f	2.27	1' -7/10, 5'-9/10	Ne	3.01	ND	ND
35	35/ B/O Ro/138809	f	3.24	1' -7/10, 5'-9/10	Ne	1.87	ND	ND
36	36/ B/O Re/138667	f	2.77	1' -7/10, 5'-9/10	Ne	2.99	Ne	15
37	37/ B/O As/1021178	f	3.22	1' -7/10, 5'-9/10	Ne	3.12	ND	ND
38	38/ B/O Re/138720	f	2.23	1' -7/10, 5'-9/10	Ne	2.23	ND	ND
39	39/ B/O Kh/137968	m	3.7	1' -8/10, 5'-9/10	Ne	1.67	ND	ND
40	40/B/O Ra/137381	f	3.2	1' -7/10, 5'-9/10	Ne	2.47	ND	ND
41	41/ B/O Ra/122335	m	2.27	1' -7/10, 5'-9/10	Ne	4.41	ND	ND
42	42/ B/O La/139915	f	3.15	1' -7/10, 5'-9/10	Ne	6.65	Po	314

MASTER CHART - BABIES

43	43/ B/O Am/141999	m	2.62	1' -7/10, 5'-9/10	Ne	6.09	ND	ND
44	44/ B/O Ka/142446	f	3.16	1' -7/10, 5'-9/10	Ne	5.35	ND	ND
45	45/ B/O Ro/142888	m	1.8	1' -7/10, 5'-9/10	Ne	4.76	ND	ND
46	46/ B/O Va/142535	f	2.27	1' -7/10, 5'-9/10	Ne	2.71	ND	ND
47	47/ B/O Ar/140996	f	3.16	1' -7/10, 5'-9/10	Po	12.94	ND	ND
48	48/ B/O Ko/141090	f	2.66	1' -7/10, 5'-9/10	Ne	4.62	ND	ND
49	49/ B/O Ra/1021287	m	3.3	1' -7/10, 5'-9/10	Ne	5.16	ND	ND
50	50/ B/O Ma/142692	m	2.7	1' -7/10, 5'-9/10	Ne	4.61	ND	ND
51	51/ B/O Ay/142851	f	3.02	1' -7/10, 5'-9/10	Ne	3.48	ND	ND
52	52/ B/O Na/144222	m	2.61	1' -7/10, 5'-9/10	Ne	7.29	ND	ND
53	53/ B/O Mo/144050	f	2.75	1' -7/10, 5'-9/10	Ne	2.07	Po	244
54	54/ B/O Sh/144188	m	2.74	1' -7/10, 5'-9/10	Ne	3.85	ND	ND
55	55/ B/O Ch/144296	m	2.7	1' -7/10, 5'-9/10	Ne	6.31	ND	ND
56	56/ B/O De/77972	m	2.06	1' -7/10, 5'-9/10	Po	12.37	ND	ND
57	57/ B/O So/145537	f	3.09	1' -7/10, 5'-9/10	Ne	2.63	ND	ND
58	58/ B/O Pa/146336	f	2.64	1' -7/10, 5'-9/10	Po	13.7	ND	ND
59	59/ B/O Ro/147843	f	3.3	1' -7/10, 5'-9/10	Ne	6.33	ND	ND
60	60/ B/O Re/132725	f	2.9	1' -7/10, 5'-9/10	Ne	3.6	ND	ND
61	61/ B/OPu/146331	f	3.11	1' -7/10, 5'-9/10	Ne	2.98	ND	ND
62	62/ B/O Fa/146740	f	2.78	1' -7/10, 5'-9/10	Ne	2.29	ND	ND
63	63/ B/O Bh/146738	f	2.4	1' -7/10, 5'-9/10	Po	11.71	Po	356
64	64/ B/O Ma/146777	m	2.65	1' -7/10, 5'-9/10	Ne	3.44	ND	ND
65	65/ B/O An/146722	m	1	1' -3/10, 5'-5/10	Po	11.72	ND	ND
66	66/ B/O Ra/148663	m	2.33	1' -7/10, 5'-9/10	Ne	3.57	Po	128
67	67/ B/O No/150021	f	3.35	1' -7/10, 5'-9/10	Ne	8.39	Ne	12.4
68	68/ B/O Sh/149578	m	2.1	1' -7/10, 5'-9/10	Ne	4.68	ND	ND
69	69/ B/O Gu/150065	f	2.2	1' -7/10, 5'-9/10	Ne	4.47	ND	ND
70	70/ B/O Ma/147882	m	2.82	1' -7/10, 5'-9/10	Ne	2.09	ND	ND
71	71/ B/0 Sh/151003	f	3.19	1' -7/10, 5'-9/10	Ne	4.49	ND	ND
72	72/ B/O Sa/149058	m	2.62	1' -7/10, 5'-9/10	Ne	4.46	ND	ND
73	73/ B/O Ra/152107	f	2.9	1' -7/10, 5'-9/10	Ne	3.83	ND	ND
74	74/ B/O Sw/120390	f	2.3	1' -7/10, 5'-9/10	Ne	5.2	ND	ND
75	75/ B/O La/151997	f	3	1' -7/10, 5'-9/10	Ne	5.96	Po	65
76	76/ B/O An/157876	f	3.19	1' -7/10, 5'-9/10	Ne	4.94	ND	ND
77	77/ B/O Pa/150397	f	2.73	1' -7/10, 5'-9/10	Ne	3.4	Po	91
78	78/ B/O Ma/152078	m	3.41	1' -7/10, 5'-9/10	Ne	3.78	ND	ND
79	79/ B/O AS/152594	f	2.25	1' -7/10, 5'-9/10	Ne	7.33	Po	105
80	80/ B/O Jy/152587	m	3.12	1' -7/10, 5'-9/10	Ne	4.06	ND	ND
81	81/ B/O An/160846	m	1.32	1' 8/10, 5'-9/10	Ne	4.68	ND	ND
82	82/ B/O Su/150658	m	1.96	1' -7/10, 5'-9/10	Ne	4.09	ND	ND
83	83/ B/O Sa/165288	m	2.5	1' -7/10, 5'-9/10	Ne	2.62	Po	124
84	84/ B/O Ko/1021905	f	2.91	1' -7/10, 5'-9/10	Ne	3.32	Po	216
85	85/ B/O Sa/166524	f	2.55	1' -7/10, 5'-9/10	Ne	2.98	ND	ND
86	86/ B/O Ar/104013	f	2.5	1' -7/10, 5'-9/10	Ne	2.67	ND	ND
87	87/B/O Pa/167183	m	2.68	1' -7/10, 5'-9/10	Ne	5.79	ND	ND
88	88/B/O Ge/167192	m	3.1	1' -7/10, 5'-9/10	Ne	2.37	ND	ND
89	89/B/O Ma/171355	m	2.83	1' -7/10, 5'-9/10	Ne	2.78	ND	ND

MASTER CHART - BABIES

90	90/B/O SU/160990	f	3.19	1' -7/10, 5'-9/10	Ne	2.9	Ne	22
91	91/B/O Sh/173955	m	3	1' -7/10, 5'-9/10	Ne	3.93	Ne	8.3
92	92/B/O Am/109238	F	3.1	1' -7/10, 5'-9/10	Ne	0.87	Ne	1.5
93	93/B/O Pa/185315	F	2.9	1' -7/10, 5'-9/10	Ne	0.87	Ne	9.7
94	94/B/O Sh/185347	m	2.96	1' -7/10, 5'-9/10	Ne	1.15	ND	ND
95	95/B/O Bh/185348	f	2.87	1' -7/10, 5'-9/10	Ne	1.05	Ne	2.5
96	96/B/O Ge/159788	f	2.4	1' -7/10, 5'-9/10	Ne	0.97	ND	ND
97	97/B/O Su/186693	m	920 Gm	1' -3/10, 5'-5/10	Ne	0.84	Po	128
98	98/B/O Ka/184136	F	2.8	1' -7/10, 5'-9/10	Ne	0.81	ND	ND
99	99/B/O Ka/187169	F	1.57	1' -7/10, 5'-9/10	Ne	0.75	Po	180
100	100/B/O Sr/187199	f	3.6	1' -7/10, 5'-9/10	Ne	1.08	Po	232
101	101 /B/OSh/119081	m	2.36	1' -7/10, 5'-9/10	Ne	0.88	Po	150
102	102/ B/o Sa/188778	f	2.39	1' -7/10, 5'-9/10	Ne	3.22	Po	199
103	103/B/O Sh/184335	f	2.08	1' -7/10, 5'-9/10	Ne	0.87	ND	ND
104	104/B/O As/190174	f	2.66	1' -7/10, 5'-9/10	Ne	1.15	ND	ND
105	105/B/O Ar/189115	f	3.06	1' -7/10, 5'-9/10	Ne	1.28	Ne	1.8
106	106/B/O Sa/190673	m	3	1' -7/10, 5'-9/10	Ne	1.05	ND	ND
107	107/ B/O An/191862	m	2.5	1' -7/10, 5'-9/10	Ne	1.15	ND	ND
108	108/B/O Je /190990	F	2.09	1' -7/10, 5'-9/10	Ne	1.28	Po	250
109	109/B/O Su/125495	m	3.16	1' -7/10, 5'-9/10	Ne	0.75	Ne	4
110	110/B/O Sa/215632	m	2.36	1' -7/10, 5'-9/10	Ne	0.75	Ne	3.7
111	111/B/o Ru/221265	f	1.48	1' -7/10, 5'-9/10	Ne	0.88	ND	ND
112	112/B/O Vi/210612	m	3.4	1' -7/10, 5'-9/10	Ne	0.88	ND	ND
113	113/B/O Go/221099	m	2.86	1' -7/10, 5'-9/10	Ne	1.08	ND	ND
114	114/B/O Mu/222369	m	2.53	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
115	115/ B/O Ma/222542	m	2.34	1' -7/10, 5'-9/10	Ne	0.88	ND	ND
116	116/B/O Pu/222535	m	3.5	1' -7/10, 5'-9/10	Ne	0.88	Po	212
117	117/B/O Re/208952	f	2.3	1' -7/10, 5'-9/10	Ne	0.75	Po	270
118	118/B/o Ar/223622	m	3.67	1' -7/10, 5'-9/10	Ne	0.75	ND	ND
119	119/B/O So/224039	m	2.7	1' -7/10, 5'-9/10	Ne	1.08	ND	ND
120	120/B/O Su/222932	F	2.62	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
121	121/B/O As/224600	f	3.32	1' -7/10, 5'-9/10	Ne	0.42	ND	ND
122	122/B/o Ma/224741	f	3.2	1' -7/10, 5'-9/10	Ne	0.75	Ne	1.6
123	123/B/O Va/234662	F	2.12	1' -7/10, 5'-9/10	Ne	1.08	ND	ND
124	124/B/o As/235137	m	2.53	1' -7/10, 5'-9/10	Ne	0.87	ND	ND
125	125/B/o Ma/235530	m	2.52	1' -7/10, 5'-9/10	Ne	0.75	ND	ND
126	126/B/O Sh/236916	m	2.98	1' -7/10, 5'-9/10	Ne	1.08	ND	ND
127	127/B/O Su/ 236747	F	2.92	1' -7/10, 5'-9/10	Ne	1.85	ND	ND
128	128/B/O Ch/237662	f	2.6	1' -7/10, 5'-9/10	Ne	1.08	ND	ND
129	129/B/O Gi/238121	f	2.98	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
130	130/ B/O Sh / 238124	f	3.18	1' -7/10, 5'-9/10	Ne	0.87	ND	ND
131	131/B/O Ai/ 238994	m	2.68	1' -7/10, 5'-9/10	Ne	1.85	ND	ND
132	132/B/O Va/239310	M	3.52	1' -7/10, 5'-9/10	Ne	0.75	ND	ND
133	133/B/o Su/145872	f	3.07	1' -7/10, 5'-9/10	Ne	1.15	ND	ND
134	134/B/O Sa/240018	m	2.46	1' -7/10, 5'-9/10	Ne	1.05	Po	224
135	135/B/O Ma/240087	m	2.8	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
136	136/B/O Sh/230324	m	2.85	1' -7/10, 5'-9/10	Ne	0.75	ND	ND

MASTER CHART - BABIES

137	137/B/O Ar/240528	M	3.15	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
138	138/ B/O Ve/242255	m	2.93	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
139	139/B/O Sa/243164	f	2.53	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
140	140/B/O Da/ 243083	m	2.01	1' -7/10, 5'-9/10	Ne	0.88	ND	ND
141	141/B/O Ne/191355	f	2.68	1' -7/10, 5'-9/10	Ne	0.75	ND	ND
142	142/B/O Dh/243931	f	2.22	1' -7/10, 5'-9/10	Ne	0.42	ND	ND
143	143/B/O An/243941	f	1.82	1' -7/10, 5'-9/10	Ne	0.57	Po	318
144	144/B/o As/245004	m	2.08	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
145	145/B//o Sa/245358	m	2.8	1' -7/10, 5'-9/10	Ne	0.88	ND	ND
146	146/B/o Ra/231937	f	2.65	1' -7/10, 5'-9/10	Ne	0.75	ND	ND
147	147/ B/O Ch /177177	f	2.79	1' -7/10, 5'-9/10	Ne	0.42	ND	ND
148	148/B/O Ku/261711	m	3.05	1' -7/10, 5'-9/10	Ne	0.88	ND	ND
149	149/B/O Ru/268988	f	2.7	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
150	150/B/o sh/264250	f	2.2	1' -7/10, 5'-9/10	Ne	0.88	ND	ND
151	151/B/o Sa/269855	f	2.6	1' -7/10, 5'-9/10	Ne	0.57	ND	ND
152	152/B/O Sh/273696	m	3.44	1' -7/10, 5'-9/10	Ne	0.75	ND	ND
153	153/B/O AY/288249	m	2.3	1' -7/10, 5'-9/10	Ne	1.21	ND	ND
154	154/B/O Sh/288136	F	2.96	1' -7/10, 5'-9/10	Ne	1.2	Po	170
155	155/B/O Na/288247	f	2.62	1' -7/10, 5'-9/10	Ne	3.57	Po	69
156	156/B/O An/280206	f	2.69	1' -7/10, 5'-9/10	Ne	1.48	Po	322
157	157/B/o Ve/284074	m	2.96	1' -7/10, 5'-9/10	Ne	0.77	ND	ND
158	158/B/O Pa/289099	f	2.38	1' -7/10, 5'-9/10	Ne	1.52	ND	ND
159	159/B/O Me/289152	m	3.58	1' -7/10, 5'-9/10	Ne	0.96	ND	ND
160	160/B/O Sh/289519	f	3.2	1' -7/10, 5'-9/10	Ne	0.98	Po	208
161	161/B/O Sh/289608	m	3.2	1' -7/10, 5'-9/10	Ne	0.84	ND	ND
162	162/B/o Sh/162423	F	3.06	1' -7/10, 5'-9/10	Ne	1.59	ND	ND
163	163/B/O La/293208	f	2.7	1' -7/10, 5'-9/10	Ne	1.39	ND	ND
164	164/B/O Ma/293741	F	2.48	1' -7/10, 5'-9/10	Ne	1.76	Po	154
165	165/B/O Sa/289956	M	3	1' -7/10, 5'-9/10	Ne	0.85	ND	ND
166	166/B/O Ja/293782	F	2.69	1' -7/10, 5'-9/10	Ne	1.22	ND	ND
167	167/B/O Ha/294137	M	3.1	1' -7/10, 5'-9/10	Ne	1	ND	ND
168	168/B/O Bh/294296	m	3.3	1' -7/10, 5'-9/10	Ne	0.97	ND	ND
169	169/B/O Va/295195	f	2.7	1' -7/10, 5'-9/10	Ne	2.47	ND	ND
170	170/B/o Jy/296494	f	2.71	1' -7/10, 5'-9/10	Ne	0.89	ND	ND
171	171/B/o Jy/295994	f	2.85	1' -7/10, 5'-9/10	Ne	1.47	ND	ND
172	172/B/O So/289449	f	2.94	1' -7/10, 5'-9/10	Ne	1.47	ND	ND
173	173/B/O Am/296850	m	3	1' -7/10, 5'-9/10	Ne	0.85	ND	ND
174	174/B/O Mo/296868	f	2.63	1' -7/10, 5'-9/10	Ne	1.04	Po	140
175	175/B/O Su/297454	f	2.63	1' -7/10, 5'-9/10	Ne	1.02	ND	ND
176	176/B/O So/288560	m	3.13	1' -7/10, 5'-9/10	Ne	1.42	ND	ND
177	177/B/O Pa/286920	m	2.75	1' -7/10, 5'-9/10	Ne	1.05	ND	ND
178	178/B/o La/297966	m	2.82	1' -7/10, 5'-9/10	Ne	2.27	ND	ND
179	179/B/O La/296391	M	3.22	1' -7/10, 5'-9/10	Ne	1.85	ND	ND
180	180/B/oRa/297610	f	2.67	1' -7/10, 5'-9/10	Ne	7.88	ND	ND
181	181/B/O Va/298557	f	3.22	1' -7/10, 5'-9/10	Ne	1.04	ND	ND
182	182/B/oVe/298666	m	2.4	1' -7/10, 5'-9/10	Ne	1.04	ND	ND
183	183/B/oPa/293821	f	2.6	1' -7/10, 5'-9/10	Ne	0.07	ND	ND

MASTER CHART - BABIES

184	184/B/o Ni/301897	m	2.54	1' -7/10, 5'-9/10	Ne	1.18	ND	ND
185	185/B/O Sa/301920	f	2.65	1' -7/10, 5'-9/10	Ne	1.26	ND	ND
186	186/B/O An/301929	f	2.92	1' -7/10, 5'-9/10	Ne	1.01	ND	ND
187	187/B/o Ma/302648	m	3.5	1' -7/10, 5'-9/10	Ne	1	ND	ND
188	188/B/o An/302631	m	3.5	1' -7/10, 5'-9/10	Ne	1.6	ND	ND
189	189/B/o Na/219183	f	3.1	1' -7/10, 5'-9/10	Ne	1.18	ND	ND
190	190/B/o An/303000	m	3.17	1' -7/10, 5'-9/10	Ne	1.26	ND	ND
191	191/B/O Su/292408	m	2.34	1' -7/10, 5'-9/10	Ne	0.6	Po	202
192	192/B/O Bh/304091	m	2.69	1' -7/10, 5'-9/10	Ne	1.59	ND	ND
193	193/B/O Gu/303673	f	2.86	1' -7/10, 5'-9/10	Ne	1.72	ND	ND
194	194/B/O La/303831	m	2.32	1' -7/10, 5'-9/10	Ne	1.33	ND	ND
195	195/B/o Su/299186	m	2.5	1' -7/10, 5'-9/10	Ne	1.01	ND	ND
196	196/B/O Jy/ 305514	m	2.86	1' -7/10, 5'-9/10	Ne	1	ND	ND
197	197/B/O Va/305225	f	2.38	1' -7/10, 5'-9/10	Ne	1.71	ND	ND
198	198/B/O Sw/284608	f	2.76	1' -7/10, 5'-9/10	Ne	1.66	Po	196
199	199/B/O Ma/307200	m	2.32	1' -7/10, 5'-9/10	Ne	2.43	ND	ND
200	200/B/O Ch/241090	m	2.18	1' -7/10, 5'-9/10	Ne	1.65	ND	ND
201	201/B/O Am/298317	m	2.83	1' -7/10, 5'-9/10	Ne	0.64	ND	ND
202	202/B/O Ch/298763	m	2.65	1' -7/10, 5'-9/10	Ne	0.71	ND	ND
203	203/B/O Ma/309602	f	2.8	1' -7/10, 5'-9/10	Ne	0.89	ND	ND
204	204/B/O In/309648	f	1.9	1' -7/10, 5'-9/10	Ne	1.01	ND	ND
205	205/B/O Ka/309638	m	2.82	1' -7/10, 5'-9/10	Ne	0.77	ND	ND
206	206/B/O Su/309731	m	2.65	1' -7/10, 5'-9/10	Ne	0.72	Po	342
207	207/B/O La/309961	f	2.8	1' -7/10, 5'-9/10	Ne	0.71	Po	91
208	208/B/O Aa/309474	f	3.24	1' -7/10, 5'-9/10	Ne	1.26	ND	ND
209	209/B/O Ma/306380	m	3.15	1' -7/10, 5'-9/10	Ne	1.43	ND	ND
210	210/B/O Sh/249945	f	2.95	1' -7/10, 5'-9/10	Ne	1.12	ND	ND
211	211/B/O Am/313096	m	2.6	1' -7/10, 5'-9/10	Ne	1.26	ND	ND
212	212/B/ORu/314657	M	2.18	1' -7/10, 5'-9/10	Ne	1.41	ND	ND
213	213/B/O Su/314524	f	2.98	1' -7/10, 5'-9/10	Ne	1.12	ND	ND
214	214/B/O Ka/312953	f	2.46	1' -7/10, 5'-9/10	Ne	0.9	ND	ND
215	215/B/O Jy/311607	m	2.56	1' -7/10, 5'-9/10	Ne	0.83	ND	ND
216	216/B/O Sh/315092	f	1.98	1' -7/10, 5'-9/10	Ne	1.28	ND	ND
217	217/B/O Na/239960	m	2.7	1' -7/10, 5'-9/10	Ne	1.47	ND	ND
218	218/B/O Va/249947	f	3.24	1' -7/10, 5'-9/10	Ne	0.65	ND	ND
219	219/B/O Sa/3903	m	2.18	1' -7/10, 5'-9/10	Ne	1.09	ND	ND
220	220/B/O Cl/253294	F	2.58	1' -7/10, 5'-9/10	Ne	0.89	ND	ND
221	221/B/O Su/311558	f	2.78	1' -7/10, 5'-9/10	Ne	1.18	ND	ND
222	222/B/O Su/295826	f	2.6	1' -7/10, 5'-9/10	Ne	0.69	ND	ND
223	223/B/O Ma/316604	f	2.6	1' -7/10, 5'-9/10	Ne	1.09	ND	ND
224	224/B/O Ka/156402	f	3.6	1' -7/10, 5'-9/10	Ne	1.53	ND	ND
225	225/B/O Ma/313466	f	2.31	1' -7/10, 5'-9/10	Ne	1.26	ND	ND
226	226/B/O Su/289648	m	3.5	1' -7/10, 5'-9/10	Ne	1.18	ND	ND
227	227/B/O Am/318474	m	2.6	1' -7/10, 5'-9/10	Ne	0.13	ND	ND
228	228/B/O Ro/317164	f	2.5	1' -7/10, 5'-9/10	Ne	1.24	ND	ND
229	229/B/O Me/318690	m	2.76	1' -7/10, 5'-9/10	Ne	1.94	ND	ND
230	230/B/O Am/317060	f	2.2	1' -7/10, 5'-9/10	Ne	0.96	ND	ND

MASTER CHART - BABIES

231	231/B/O La/315389	F	2.4	1' -7/10, 5'-9/10	Ne	0.24	ND	ND
232	232/B/O Mu/318475	f	2.8	1' -7/10, 5'-9/10	Ne	1.79	ND	ND
233	232/B/O Al/319179	m	2.5	1' -7/10, 5'-9/10	Ne	1.47	ND	ND
234	234/B/O Pr/321927	f	2.8	1' -7/10, 5'-9/10	Ne	3.09	Po	340
235	235/B/O Pa/323238	m	3.08	1' -7/10, 5'-9/10	Ne	3.33	ND	ND
236	236/B/O Ra/323439	M	2.28	1' -7/10, 5'-9/10	Ne	1.6	ND	ND
237	237/B/O Ya/323582	f	2.43	1' -7/10, 5'-9/10	Ne	1.84	Po	314
238	238/B/O Ar/326841	m	2.87	1' -7/10, 5'-9/10	Ne	1.69	ND	ND
239	239/B/O Ku/155555	f	2.8	1' -7/10, 5'-9/10	Ne	1.3	ND	ND
240	240/B/o Bh/286706	m	2.23	1' -7/10, 5'-9/10	Ne	1.7	ND	ND
241	241/B/O Ga/317493	M	2.92	1' -7/10, 5'-9/10	Ne	1.06	ND	ND
242	242/B/O Ra/327016	f	2.5	1' -7/10, 5'-9/10	Ne	1.9	ND	ND
243	243/B/O Ka/322257	m	2.62	1' -7/10, 5'-9/10	Ne	2.6	ND	ND
244	244/B/O Ra/329343	f	4.11	1' -7/10, 5'-9/10	Ne	1.4	ND	ND
245	245/B/O So/329094	m	2.86	1' -7/10, 5'-9/10	Ne	1.7	Po	290
246	246/B/O Pa/279052	f	2.68	1' -7/10, 5'-9/10	Ne	1.6	Po	80
247	247/B/O Ta/329878	m	2.9	1' -7/10, 5'-9/10	Ne	1.05	ND	ND
248	248/B/O Pa/251290	f	3.03	1' -7/10, 5'-9/10	Ne	1.3	ND	ND
249	249/B/O Na/313342	f	3.3	1' -7/10, 5'-9/10	Ne	0.91	ND	ND
250	250/B/O Ya/336058	m	1.99	1' -7/10, 5'-9/10	Ne	1.1	ND	ND
251	251/B/O Ya/306543	f	2.35	1' -7/10, 5'-9/10	Ne	1.6	ND	ND