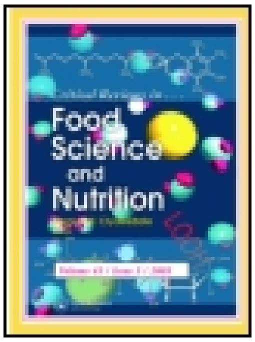
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Toxoplasma infection and milk consumption: Meta-analysis of assumptions and evidences

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Running title: meta-analyses of milkborne toxoplasmosis

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Abstract

Toxoplasmosis is the most widespread infection worldwide. It occurs within congenital contamination, organ transplant or immune system depression. Primary infection is mainly foodborne with the ingestion of raw or undercooked meat; unwashed fruit-vegetables, unhygienic water or contaminated milk. Gaps in current knowledge about the risk assessment of *Toxoplasma gondii* by milk consumption are noted. Contradictory data are observed within risk assessment of milk consumption and toxoplasmosis occurrence. While some papers reported positive correlations between drinking milk and infection transmission to human, other studies stated non significant influence of milk or milk products consumption. New debate about the detection of the parasite in the milk matrix from different hosts raised interrogations. To figure out the real contribution and the potential correlations of milkborne way in toxoplasmic infection, meta-analysis approach was investigated. Overall analysis showed heterogeneous responses and led to sate that statistically: dairy matrix (other than milk); Bovidae products; agricultural population and countries in Africa, Europe and Southeast Asia are not linked to milkborne toxoplasmosis. The most involved factors are Capridae products, immune-depressed population and North America, Middle East and Latin territories. The current work advanced which parameters could affect the public health and should be envisioned in further epidemiological analysis.

Key words

Toxoplasmosis, risk factor, dairy consumption, meta-analysis

INTRODUCTION

Toxoplasmosis is one of the most prevalent parasitic infections of man and animals caused by *Toxoplasma gondii*. The protozoan infects virtually all warm-blooded animals including human, livestock and marine mammals. The importance of toxoplasmosis mainly lies among pregnant population, transplant and immune compromised individuals. The infection leads to huge damages in public health sector and veterinary field as well.

Contamination occurs mainly within: congenital transmission (when a primary maternal infection is passed transplacentally to the fetus); organ transplant/blood transfusion and foodborne way by ingestion one of the three forms of the parasite. *Toxoplasma gondii* is one of three pathogens (together with Salmonella and Listeria) which account for >75% of all deaths due to foodborne disease in the USA (Dubey *et al*., 2011). The foodborne transmission can occur by the ingestion of: raw or undercooked meat; unwashed fruit-vegetables, unhygienic water, or contaminated milk. In a retrospective study performed by the Brazilian Ministry of Health, it was found that from January 2000 to March 2013, more than 8,000 outbreaks of foodborne diseases (FBD) occurred in Brazil. Of these total, *T. gondii* was responsible for five outbreaks and milk and its derivatives vehiculated the causative pathogens of 337 FBD outbreaks (da Silva *et al*., 2015). Gaps concerning the risk assessment of *Toxoplasma* by milk consumption are noted (Boughattas, 2015).

T. gondii has been detected in the milk of several hosts, including sheep (Fusco *et al*., 2007), goat (Bezzara *et al*., 2013), donkey (Mancianti *et al*., 2014), cat (Powell *et al*., 2001), camel (Ishag *et al*., 2006), dog (Chamberlain *et al*., 1953), buffalo (Dehkordi *et al*., 2014) and even human breast feed (Azeb *et al*., 1992). It is assumed that any type of milk is a potential source of infection if consumed raw (EFSA, 2007). Acute clinical toxoplasmosis has been reported in humans after drinking goat milk (Sacks *et al*., 1982). It has often been thought that the risk of acquiring an infection with *Toxoplasma* by drinking cow's milk, if any, is minimal. However some seroepidemiology surveys stated significantly association with raw cows' milk consumption and *T. gondii* infection (Alvarado-Esquivel *et al*., 2010, da Silva *et al*., 2014).

Moreover, the high seropositivity for *T. gondii* of camels in Sudan, Egypt, Iran and China, suggested also a potential public health issue for nomads/local residents who consume raw camelian milk (Toaleb *et al*., 2013; Hamidinejat *et al*., 2013; Wang *et al.*, 2013). Likewise, the recent interest in donkey milk and its parasitic contamination should raise more consideration (Martini *et al*., 2014).

Risk assessing studies associating *T. gondii* infections and milk consumption reported contradictory data. While some papers reported positive correlations between drinking milk and infection transmission to human in Poland (Paul, 1998), USA (Jones *et al*., 2009);) Mexico (Alvarado-Esquivel *et al*., 2010) and Turkey (Gencer *et al*., 2014), other studies stated non significant influence of milk or milk products consumption among pregnant women in Jordan (Nimri *et al*., 2004) and high risked populations in Kyrgyzstan (Minebaeva *et al*., 2013). In addition, Alvarado-Esquievel *et al*., 2013 showed in their recent article that *T. gondii* exposure was negatively associated with consumption of raw milk. Emphasizing this disagreement, a recent paper launched a debate about milk contamination: Dehkordi *et al*., 2013 reported the detection, by different approaches, of the toxoplasmic DNA in milk of different hosts naturally infected including low sensitive hosts to the parasite. Scientists commented the findings pointing out some gaps and rising thus interrogations (Dubey and Jones, 2014; Boughattas, 2015).

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Within the reported conflicting data, meta-analysis approach can facilitate to draw a relevant conclusion among the biased published facts. Indeed, meta-analysis is used to analysis previous findings from observational studies, helping thus concise results synthesis. This will lead to epidemiological indicators drawing required by health care professionals and policy makers. Additionally, in meta-analysis sample size is increased as studies are combined, resulting in a better and stronger statistical basis. Meta-analysis approach was previously investigated in the framework of congenital toxoplasmosis (Thiebaut *et al*., 2007) and toxoplasmic schizophrenia (Torrey *et al*., 2007). Consequently, as comprehensive epidemiological information is needed to assess the health significance of raw/unpasteurized milk consumption, this statistical methodology was investigated. The aim of the current study is to identify the “real” contribution of the dairy products in toxoplasmosis occurrence as well as the involvement of the different characteristics.

META-ANALYSIS

(1) Data collection and selection

Systematic search of scientific literature was conducted to identify quantitative contribution of the consumption of milk/dairy products in toxoplasmosis with checking if animal origin, geography and populations characteristics are involved. We used different online databases and the Institute of Scientific Information (ISI) Web of Science as they provide access to previously peer-reviewed published data. We employed a broad search using combinations of the following keywords: “toxoplasma”, “toxoplasmosis”, “milk”, “dairy”, “cheese”, “cream”, “risk factor”, “seroepidemiology”. From the listed references published until November 2014, we examined the title and abstract of each of these references to assess their potential of meeting the selection criteria for inclusion in our work. No language or geography restriction was used.

Papers were excluded if they were reviews, described experimental infection, investigated only the diagnosis of the parasite in milk, the sero-epidemiology study focused on animal flocks, the analysis focus on global population not the infected one, no serological investigations was done, no numeric values were reported and the analyzed milk was boiled or pasteurized or in powder. When the milk nature wasn’t mentioned, it was assumed by default to be a risk factor. In case of publication duplicate, the first published paper was taken in consideration. If an article was considered relevant, but data were missing or not available in the format needed for our meta-analysis, the corresponding authors were contacted by E-mail and asked for supplementary information before the final decision of exclusion or inclusion of the paper is made. Reference lists of identified papers were also screened to maximize sensitivity of the search.

Data abstracted from the contributing studies consisted of the: target sero-positive population, geographic area of the study, technique of diagnosis, the dairy matrix and animal origin of the matrix. Mandatory numeric values included population size, seropositive and control prevalence. When measures were reported in percentage, odd ratio or risk ratio, conversion to crude size was achieved when possible. Measurements from different: populations, matrix or experimental conditions within a single study were considered independent observations. When parameters are reported dependant (e.g mix of the matrix milk/egg), they were not considered for the analysis.

(2) Analysis

For each parameter, the effect size (a value obtained from a study summarizing differences between positive and control groups, comparable across studies) was calculated. Each parameter should be with a minimum of two observations to be included in the correlation analysis. So when unique: target population, country or dairy product are reported, correlation can't be investigated. To avoid data loss, subgroups within each parameter was privileged. The countries, from where studies were extracted, were grouped according to the geographic location within Africa (Sudan, Ethiopia), Middle-East (Saudi Arabia, Egypt, UAE, Turkey), Southeast Asia (Pakistan, Iran, Kyrgyzstan), Europe (United Kingdom, France, Norway, Germany), North America (British Columbia, USA) and Latin (Brazil, Mexico) groups. The matrix enclosed two subgroups milk and dairy (sour cream, cheese, ice cream, butter). The target populations were divided into congenital group (women, pregnant women and patients with CT), immune-depressed group (HIV, cancer, ocular infection or any other health impairing), agriculture group (workers and farm resident/owners) and immune-competent group (global population and blood donors). The animal origin of the dairy matrix was designed according to the host denomination bovidae (cow, buffalo), capridae (goat, sheep), etc. When no indication was reported, it was assumed that the host can be from any animal species.

The mean effect size for each group of studies was estimated by determining the weighted mean of the log odds ratios and its variance from the individual studies, using MetaWin v.2.1 software (Rosenberg *et al.*, 2000). According to it, as the number of observations gathered in on dataset is below 75, fixed model assumption was conducted with 4,999 bootstrapped

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iterations. Heterogeneity of observations should be observed (pQ(t) < 0.0005 when tested against a chi-square distribution) to conduct correlation analysis.

For the proportion meta-analysis, values are transformed from percentage to the log odds scale as it offers better statistical properties for meta-analysis.. Considering a study in which r out of n patients were observed to have an event, leads to a proportion of r/n. The associated effect size is log odds: $\ln(r/(n-r))$ with SE $\mathrm{sqrt}(1/r + 1/(n-r))$ (Hunter *et al.*, 2014).

Publication bias was investigated in the current work. It is defined by the selective publication of significant over non-significant data or positive over negative results. Studies are more likely published when reporting significant results emphasizing them more. Unlike other type of scientific papers, meta-analysis can provide an approach to identify if the significance of the effect is due or not to the applied factor. In the current meta-analysis, it was assessed by Spearman's rho rank correlation. A significant correlation (p(Rs) < 0.0005) would indicate publication bias.

RESULTS

The established process of selection resulted in 49 independent observations gathered from 39 peer-reviewed papers which formed the basis of our meta-analysis. One paper was in French: Baril *et al.*, 1996; two papers were in Portuguese: Cademartori *et al.*, 2008 and Garcia *et al.*, 1999 and the rest were in English. For the current analysis, some relevant papers weren't included as full texts of articles were unavailable: de Camargo *et al.*, 1995; Fu *et al.*, 2004 and Makki *et al.*, 2010.

Applying the selection criteria, other references were discarded: Boyer et al., 2005 as they reported the milk consumption factor associated with egg consumption (dependent variables); Gebremedhin *et al*., 2014 and Ogendi *et al*., 2013 as no serology test was investigated to animal owners (absence of seropositive population); Proctor & Banerjee, 1994 and Mohamed *et al*., 2014 as they analyzed the milk factor according to the global population not only the infected one without numeric differentiation ; Loges *et al*., 2012; Gencer *et al*., 2014; and Xavier *et al*., 2013 as they didn't report control values.

Even if the selection leads to papers number decrease, the results are more suitable and reliable for robust meta-analysis.

The homogeneity statistic Q, which is an estimate of the among-study variance, was large enough to be significant (df: 48/Q_t: 213.3673, $p(Q_t)$: 0.00000), consequently data were considered to be heterogeneous and eligible to be subjected to further single factor categorical analyses without any apparent publication bias when investigating Spearman Rank-Order Correlation: Rs: 0,149; *p:* 0,30829.

The overall effect size was in favor of correlation between milk consumption and toxoplasmosis occurrence (lnE+ 0.1997; 95%CI: 0.1274 to 0.2719), even if 16 observations extracted from 13 papers reported negative effect size :Baril *et al*., 1996; Elsheikha *et al*., 2009; Ertug *et al*., 2005; Esquivel *et al*., 2013; Ferreira *et al*., 2014; Flatt & Shetty 2009; Fouladvand *et al*., 2010; Heuklebach *et al*., 2007; Kapperud *et al*., 1996; Marques *et al*., 2008; Okwuzu *et al*., 2014; Sroka *et al*., 2010 and Tavares *et al*., 2012.

When pursuing the categorical analysis, dairy products consumption was not retained significantly involved in toxoplasmosis occurrence, as their confidence intervals were

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overlapping zero (lnE+: 0.0938; 95%CI: -0.0149 to 0.2026 / lnE+: 0.3036; 95%CI: 0.2006 to 0.4066 for dairy an milk matrix respectively). Indeed only two papers reported significant positive correlation: El Deeb *et al*., 2012 and Ahmed *et al*., 2014 (Figure 1).

Non significant association was also retained with bovidae origin, milk from mixed origins, agricultural population and countries in Africa, Europe and Southeast Asia, as their confidence intervals were overlapping zero (Table2).

Toxoplasmosis occurrence is retained strongly correlated with capridae milk consumption (lnE+: 0.4718) as well as milk from any origin, except bovine, (lnE+: 0.1807).

Immune-depressed population seemed to be the most sensitive target to toxplasmosis via milkborne way (lnE+: 0.4078). Significant correlation is also retained within Congenital Toxoplasmosis group and surprisingly Immune competent population (lnE+: 0.1733 and lnE+:0.2492 respectively).

Correlation within geographic location stated that North America territories are the most subjected to milkborne toxoplasmosis followed by Middle East region and Latin countries (LnE+: 1.9269/0.3999/0.1582 respectively) (Table 2)

Papers reporting their result, within the seropositive population, as a percentage of contamination were subjected to the proportion meta-analysis. It is retained that only 12 papers advanced significant positive association between milk consumption and toxoplasmosis occurrence (Ahmed *et al*., 2014; Endris *et al*., 2014, Ertug *et al*., 2005; Ferreira *et al*., 2014; Flatt & Shetty 2009; da Silva *et al*., 2014; Hailu *et al*., 2014; Higa *et al*., 2010; Khan *et al*., 2011; Marques *et al*., 2008; Sroka *et al*., 2010 and Tavares *et al*., 2012).

Graphic presentation showed that the positive association reported in Tavares *et al*., 2012 is however non significant as the SE bars overlaps with the zero axis (Figure 2). Marques *et al*., 2008; Khan *et al*., 2011 and Sroaka *et al*., 2010 reported the highest risk whereas Ferreira *et al*., 2014; Hailu *et al*., 2014 and Flatt & Shetty 2009 reported the lowest risk.

DISCUSSION

Overview

Ingestion of unpasteurized milk was considered a possible source of *T. gondii* infection in children of rural areas (Azeb *et al*., 1992; Sacks *et al*., 1982; Radon *et al*., 2004). In addition, toxoplasmic evidence was described in breast fed child whose mother acquired toxoplasmosis showing, thus, the importance of milk in the spread of *T. gondii* infection. The toxoplasmic transmission was attributed both to tachyzoites in the milk and to suckling trauma (Ishag *et al*., 2006) as well as to tissue cyst excretion. Indeed, during pre-lactation, relatively stable mammary cells could harbor *T. gondii* cysts. Those silent cysts could be secreted from the mammary gland cells by exocytosis, coated by host cell membranes, similar to milk fat globules secretion allowing milk contamination inside the gland. This contamination is worsened due to smaller concentration of proteolytic enzymes in the intestine of children and suckling animals, increasing the survival of *T. gondii* forms (Hiramoto *et al*., 2001). It was experimentally confirmed that tachyzoites survived in the goat milk for three to seven days at 4˚C (Spisak *et al*., 2010) and in homemade fresh cheese for a period of 10 days (Hiramoto *et al*., 2001) proving thus that raw goat milk can serve as a source of *Toxoplasma* infection.

Proponents of raw milk claim that unpasteurized milk and soft cheeses are more nutritive than pasteurized dairy, consequently there is great concern regarding whether it is safe to consume raw (unpasteurized) milk (http://www.cdc.gov/foodsafety/rawmilk/raw-milk-index.html). It was reported that many Americans consume products from food cooperatives (Dubey *et al*., 2014). In addition, a recent study identified drinking unpasteurized goat milk as a risk factor for recently-acquired toxoplasmosis in pregnant women in the USA (Jones *et al*., 2009). European reports advanced that raw milk consumption was a significant risk factor in Lausanne but not in Naples, Milan, Copenhagen, Oslo and Brussels (Cook *et al*., 2000). Austria recommend to its pregnant women no raw milk or raw milk products consumption unless boiling; Republic of Ireland stated that milk should be pasteurized; UK advised to avoid unpasteurized goat's milk or products that are made from it and Sweden warn pregnant women to avoid mould- ripened or washed rind cheese, even if it is made of pasteurized milk, giving examples such as brie, gorgonzola, chèvre, vacherol and taleggio. (AD HOC group: www.food.gov.uk/sites/default/files/multimedia/pdfs/committee/acmsfrtaxopasm).

Toxoplasmosis seems to play deeper public health role particularly in rural areas where milk pasteurization is not common regardless the animal origin of the dairy product.

Goats

With similar casein structure to human milk, goat milk is the most used substitute for children and people especially those with allergy to cow milk. It is easily digested because of smaller fat size and its distribution characteristics. It is also alkaline and better tolerated by those with lactose intolerance. It contains higher amount of cholesterol than buffalo or camel milk, higher calcium than cow milk but lower B6 and B12 amounts. However, it is not advised to feed infant exclusively raw goat's milk (Basnet *et al*., 2010).

According to Tenter *et al*., 2000 unpasteurized goat milk is an important source of human toxoplasmic infection. Within an experimental infection of goats by *T. gondii*, analyses indicate that the parasite can be excreted in goat's milk and can survive in fresh cheese made by cold-enzyme treatment (Dubey *et al*., 2014). Outbreaks of human toxoplasmosis, involving the ingestion of raw goat milk, have also been reported by Sacks *et al*., 1982 who recorded an outbreak in 10 members of a family composed of 24 individuals. Other studies attempted to analyze the risk factor of raw goat milk consumption among different populations including Averlino *et al*., 2003; Barbosa *et al*., 2009 and Moura *et al*., 2013 in Brazil all targeting pregnant women; Almushait *et al*., 2012 in Saudi Arabia targeting the same population; Jones *et al*., 2009 in USA questioning a global population; Alvarado-Esquivel *et al*., 2013, in Mexico focusing on agricultural workers and Gebremedhin *et al*., 2013 in Ethiopia investigating women population.

Jones *et al*., 2009 showed in their results that only 1.6% of the control patients indicated that they drank unpasteurized goat's milk in the past 12 months, so consumption of unpasteurized goat's milk must be relatively uncommon in the United States. There was no evidence that the risk resulting from consumption of unpasteurized goat's milk was associated with travel outside the United States. Investigating the detection of the parasite in naturally infected milk, researchers from Italy stated that toxoplasmic DNA was found in 10 milk samples from 77 goats with positive serology (Mancianti *et al*., 2013). In Brazil, the DNA of the parasite was detected in 6.05% of the milk samples by Bezzara *et al*., 2013 when da Silva *et al*., 2015 reported 2.06% rate from seronegative goats. Using molecular tools, the presence of *T. gondii*

DNA was confirmed in 32.56% of milk samples in Slovakia (Spisak *et al*., 2010) and in 1.07% samples in Iran (Tavassoli *et al*., 2013).

Sheep

The phylogenetic proximity between sheep and goats, as well as the lack of studies involving the role of ovine milk as a potential source of toxoplasmosis transmission, highlights the importance of studying this species. Researchers suspected the consumption of undercooked ovine meat to be a major risk factor for human infection (Boughattas *et al*., 2014). Indeed, a case of acute toxoplasmosis was reported in a breast feed infant, whose mother acquired toxoplasmosis by ingesting raw sheep meat (Bonametti *et al*., 1997).

Sheep milk contains high level of butterfat however; it is lower in saturated fat than cow or goat milk. It contains especially higher amounts of calcium than cow milk, as well as zinc and vitamins A, D, E. Sheep milk involvement in toxoplasmosis occurrence was investigated as potential risk factors by Gebremedhin *et al*., 2013 in Ethiopia targeting women population and by Barbosa *et al*., 2009 in Brazil targeting pregnant females. *T. gondii* DNA was detected by molecular tools in 4 milk samples out of the 117 examined in Campania region in Italy (Fusco *et al*., 2007). In Brazil, positive PCR were observed in 7 of 139 milk samples. The authors stated that these results need deeper investigations since sampling was done every 14 days not daily and that only a 200µl milk sample was processed in the analysis, a very small volume compared to the 500 ml produced by a ewe/dairy (Camossi *et al*., 2011). In Slovakia, detection of genomic *DNA* in milk samples was varying from 3.6% in seronegative hosts to 20% in seropostive animals and (Luptakova *et al*., 2013; 2014). Elsewhere in Iran, two different studies reported rates of 4.63% and 6.48% positive milk samples using molecular tools (Tavassoli *et al*., 2013; Dehkordi *et al*., 2013).

Cows

Cow milk, is the most consumed milk worldwide given its abundance and low cost production. Like any other milk, it is a source of vitamins, minerals and proteins needed for the organism development. However, cow milk is characterized by higher amount of cholesterol than buffalo or camel milk, higher fat and proteins than human milk and is linked to milk allergies and intolerances ranging from atopic dermatitis, diarrhea, and constipation.

In the past, it has often been thought that the risk of acquiring an infection with *T. gondii* by drinking cows' milk, if any, is of negligible importance because cattle are resistant to *T. gondii* infection. Lately, it was found that 14.1% of the seropositive pregnant women in Brazil had the habit of consuming untreated cow's or/and goat's milk (Moura *et al*., 2013). When risk factors analysis were conducted, unpasteurized/raw milk consumption was negatively associated with toxoplasmic infection among agricultural workers in Mexico (Alvarado-Esquivel *et al*., 2013) as well as in Brazil when targeting global immune-competent population (Olivera *et al*., 2003). However, more recent studies reported significant association between *T. gondii* infection and consumption of raw cow's milk: Heuklebach *et al*., 2007 and daSilva *et al*., 2014 in Brazil when analyzed pregnant women population; Esquivel *et al*., 2010 in Mexico when targeting patients with visual and hearing impairment, Cancer, HIV or undergoing hemodialysis; Elsheikha *et al*., 2009 in Egypt when focusing on blood donors' population and Fouladvand *et al*., 2010 in Iran when considering high school girls.

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So far, studies attempting to isolate the parasite from milk matrix in naturally infected cow are scarce. Dehkordi *et al.*, investigation in 2013 reported the detection of *T. gondii* in bovine milk by different approaches: in 4% of the cases by cell line cultivation, 4% by cat bioassay, 3% by Capture ELISA and 3.5% by PCR. By experimental infection, Hiramoto et al., 2001 studied the infectivity of *T. gondii* in bovine milk and homemade cheese.

Their data stated toxoplasmosis transmission by milk or fresh cheese, unpasteurized or inadequately processed. Indeed authors reported that milk and homemade cheese could keep up the infectivity of *T. gondii* cysts for periods up to 10 days for homemade cheese and up to 20 days for milk, under regular refrigeration conditions.

Buffaloes

Water buffalo (*Bubalus bubalis*) is one of the best adapted species of livestock to hot and humid tropical areas (http://www.fao.org/agriculture/dairy-gateway/milk-and-milk-products/milk-composition/en/#.VF24PIJlrhA). In several countries, water buffaloes are the major source of meat, milk and milk products. In general, buffaloes, like cattle, are considered resistant to toxoplasmic infection. However, recently it was reported that 87.79% of buffaloes had anti-*Toxoplasma gondii* antibodies in Turkey (Beyhan *et al.*, 2014). There is no valid report of isolation of viable *T. gondii* from buffalo meat. Lately, Dehkordi *et al.*, 2013 reported isolation of viable *T. gondii* from milk of 7 of 164 buffaloes in Iran, but the validity of these results has been questioned (Dubey *et al.*, 2014). The report, the first investigating buffaloe milk contamination, raised concerns regarding transmission of *T. gondii* by consuming raw products from buffaloes. Traditionally used to make mozzarella in Italy, buffalo milk is similar to cow's milk. However, it is characterized by lower cholesterol than cow or goat milk, higher proportion in calcium and richer source of nutrients such as vitamins and minerals. Risk factors analysis reported the consumption of raw buffalo dairy products in British Columbia (Proctor & Banerjee 1994) and Egypt (Elsheikha *et al.*, 2009) among global population and blood donors, respectively.

Camels

Camels play a very important role in the national income of different countries mainly in Africa and Middle East, as they are an important source of meat, milk and hide (Toaled *et al.*, 2013). It was shown that camels can produce an adequate amount of milk in drought areas where other domestic animals have very low production. The most important factor in camel milk is water content. The water content of camel milk fluctuates from 84%to 90%. With the increase in water content of the milk, there is a decrease in the fat content. Camel milk is three times richer in vitamin C than cow's milk and has 10 times more iron. It is usually used to: treat type 1 diabetes (contains insulin like molecules), strengthen cellular immune response (high level of lactoferrin with antimicrobial activity) and reduce allergic response in children.

Considered as an important food source, contamination of camel products (meat and milk) by *T. gondii* would be a foodborne concern. The parasite was previously detected in the camel meat from slaughtered animals in Saudi Arabia (Hilali *et al.*, 1995). The detection via cat bioassay showed that all the inoculated cats shed toxoplasmic oocysts after ingestion camel meat. The sero-reactivity of *Toxoplasma* in camels' herders is an alert to a closer look into its economic impact as well as its public health significance especially among nomads, local residents, Tibetans and pastoralists who consume raw camel milk.

An experimental infection study confirmed the excretion of *T. gondii* tachyzoites in the milk of female camels. Suckling calf-camel acquired toxoplasmosis from milk of their experimentally infected mother. Histopathological examination revealed the presence of *T. gondii* tachyzoites and cysts in the brain of suckling calf-camels and the mice that were inoculated with milk of infected she-camels (Ishag *et al*., 2006). Very few studies investigated the detection of natural infection of the parasite within camel milk. Dehkordi *et al* in 2013 analyzed 160 camel milk samples by different approaches. They reported a rate of contamination of 3.12% by both cell line cultivation and bioassay in cat, 1.87% by capture ELISA and 2.5% by PCR. Gebremedhin *et al*., 2014 focused on a seroepidemiological investigation in *Camelus droedarius*. The conducted survey within camel owners revealed that 32.3% of respondents may consume uncooked camel meat and 100% of them consume raw camel milk. Moreover, when discussing the origin of the dairy product investigated in risk factors analysis (Minbaeva *et al*., 2013), the corresponding author underlines that “Shubat” (dairy product) widely consumed in the desert and steppe regions near Kazakhstan, was made from fermented camel milk without any apparent pasteurization or heat treatment when it is made at home.

Donkey

The composition of donkey milk is the closest to woman’s milk (relatively poor in protein and fat but rich in lactose) and can be used as a valid substitute for babies, children and adults with IgE-mediated cow milk allergy (Vincenzetti *et al*., 2014). Moreover, its ability to modulate the aged immune system, including the intestinal mucosal immune response makes it suitable for elderly. Donkey milk is also recommended as an aid in the prevention of atherosclerosis and tumor therapy (Mancianti *et al*., 2014) or a treatment to conjunctivitis according to some ethnic believes. . Some studies suggested that donkey milk has a strong inhibitory activity against some bacteria due to the high contents of lysozyme and lactoferrin However, little is known about infection by *T. gondii* in donkeys. Recently, studies start to underline it involvement as a food sources. Its meat is consumed in some countries (Miao *et al*., 2013) and its milk is more and more “praised”.

So far, only three studies focused on *T. gondii* prevalence in the milk matrix of donkeys. Haridy *et al* in 2010 were able to detect the antibodies against toxoplasmosis in the milk of pregnant Egyptian donkey’s female by ELISA. They reported a contamination rate of 46.3%. Even if the donkey meat is not consumed by the population, the milk seems to be frequent food source especially in rural and poor zones without any safety control. In Europe, Mancianti *et al*., 2014 investigated the detection of the parasite in Italian donkeys using molecular tools. Three of the six tested milk samples were retained contaminated by n-PCR technique. Another Italian study focusing on the quality and safety of milk in donkeys reported 22.22% of toxoplasmic parasitemia (Martini *et al*., 2014). When the authors investigated the characteristics of the positive samples, they revealed lower percentages of casein and ash as well as larger average globule diameter and fewer globules/ml. It was supposed that changes in milk quality could be linked to the release of enzymes as a result of an antibody response; this can alter the composition of milk and the fat globule membrane.

According to the European legislation reported in Mancianti *et al*., 2014; raw milk from any species can be sold immediately after milking and directly by the producer to the consumer, or to a local milk seller which in turn is the supplier to final consumers, without any thermal treatment except refrigeration between 0 and 4°C. Given that, the recent increase of donkey milk demand and in the light of these preliminary observations, donkey milk may be considered as potential risky source of human infection.

In conclusion, this current work, as best as I know, is the first meta-analysis focusing on milk/dairy consumption and toxoplasmosis occurrence. By no way do I assert to have included every previous performed study. However, using the meta-analysis approach, it was possible to identify the variable involved in the milk-borne contamination. Milk consumption is linked to the infection more than dairy products consumption; capridae milk is the most risky matrix; bovideae milk is not associated to toxoplasmosis occurrence; agricultural population and countries in Africa and Southeast Asia seem resistant to the infection by milkborne contamination. The results of this meta-analysis should provide epidemiological indicators needed by health care professionals and policy makers and improve the understanding of environmental contamination and the risk factors to take in consideration in further investigations. Special awareness should be envisaged especially by the vulnerable consumers ones (cases of low immune system defenses, elderly, convalescence, infants with cow milk allergy) with the urge of heat treatment of the milk before consumption.

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Table 1. Summary of the studies included in the meta-analysis

Study	Country	Target	Matrix	Host
Abu-Zeid, 2002	UAE	IC	milk	any
Ahmed et al., 2014	Egypt	CT	milk/cheese	any
Almushait et al., 2012	Saudi Arabia	CT	milk	goat
A-Raouff & Elbasheir, 2014	Sudan	CT	milk	any
Averlino et al., 2003	Brazil	CT	milk	goat
Averlino et al., 2004	Brazil	CT	milk	goat
Barbosa et al., 2009	Brazil	CT	milk	goat/sheep
Baril et al., 1999	France	CT	cheese	mix
Cademartori et al., 2008	Brazil	CT	milk	any
Cook et al., 2000	Europe	CT	milk	any
da Silva et al., 2014	Brazil	CT	Milk/cheese	Bovine
El Deeb et al., 2012	Egypt	CT	dairy	any
El Ghandour et al., 2010	Egypt	CT	dairy	any
Elsheikha et al., 2009	Egypt	IC	milk/cheese	cow/buffalo
Endris et al., 2014	Ethiopia	CT	milk	Any
Ertug et al., 2005	Turkey	CT		Any
Esquivel et al., 2010	Mexico	ID	milk	Bovine
Esquivel et al., 2013	Mexico	Agr	milk	Bov+Cap

Fallah et al., 2008	Iran	CT	milk	Any
Ferreira et al., 2014	Brazil	ID	milk	Any
Flatt & Shetty 2009	UK	CT	Milk/ cheese	Any
Fouladvand et al., 2010	Iran	CT	milk	Bovine
Garcia et al., 1999	Brazil	ID	milk	Any
Gebremedhin et al., 2013	Ethiopia	CT	milk	goat/sheep
Hailu et al., 2014	Ethiopia	CT/ID	milk	Any
Heuklebach et al., 2007	Brazil	CT	Milk/ Cheese/ ice cream	Cow
Jones et al., 2009	USA	IC/CT	milk	Goat
Kapperud et al., 1996	Norway	CT	milk	Any
Khan et al., 2011	Pakistan	CT	milk	Any
Marques et al., 2008	Brazil	IC	cheese	Any
Minbaeva at al, 2013	Kyrgyzstan	mix	sour cream	Mix
Moura et al., 2013	Brazil	CT	milk	cow/goat
Okwuzu et al., 2014	Nigeria	ID	milk	Any
Olivera et al., 2003	Brazil	IC	Butter/ice cream	Cow
Radon et al., 2004	Germany	ID	milk	Any
Sacks et al., 1982	USA	Agr	milk	Goat

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Santos et al., 2009	Brazil	Agr	milk	Any
Sroka et al., 2010	Brazil	CT	Milk/cheese	Any
Tavares et al., 2012	Brazil	CT	Milk/cheese	Any

Table 2. Summary of categorical correlations by meta-analysis

	df (Nb studies-1)	lnE+	95% CI
Origin			
Any	27	0,1807	0,0846 to 0,2767
Mix	3	-0,1265	-0,6050 to 0,3520
Capridae	9	0,4718	0,2709 to 0,6726
Bovidae	6	0,1224	-0,0788 to 0,3236
Matrix			
Milk	32	0.3036	0.2006 to 0.4066
Dairy	15	0.0938	-0.0149 to 0.2026
Population			
CT	32	0.1733	0.0843 to 0.2623
IC	5	0.2492	0.0497 to 0.4488
ID	4	0.4078	0.0124 to 0.8032
Agricultural group	2	-0.1492	-1.4407 to 1.11423
Geography			
Africa	4	0.2725	-0.2327 to 0.7777
Europe	5	0,1684	-0,0253 to 0,3621
Middle East	7	0.3999	0.1285 to 0.6713
Latin	21	0.1582	0.0611 to 0.2552

Southeast Asia	3	-0.0376	-0.6577 to 0.5825
North America	3	1.9269	0.7840 to 3.0699

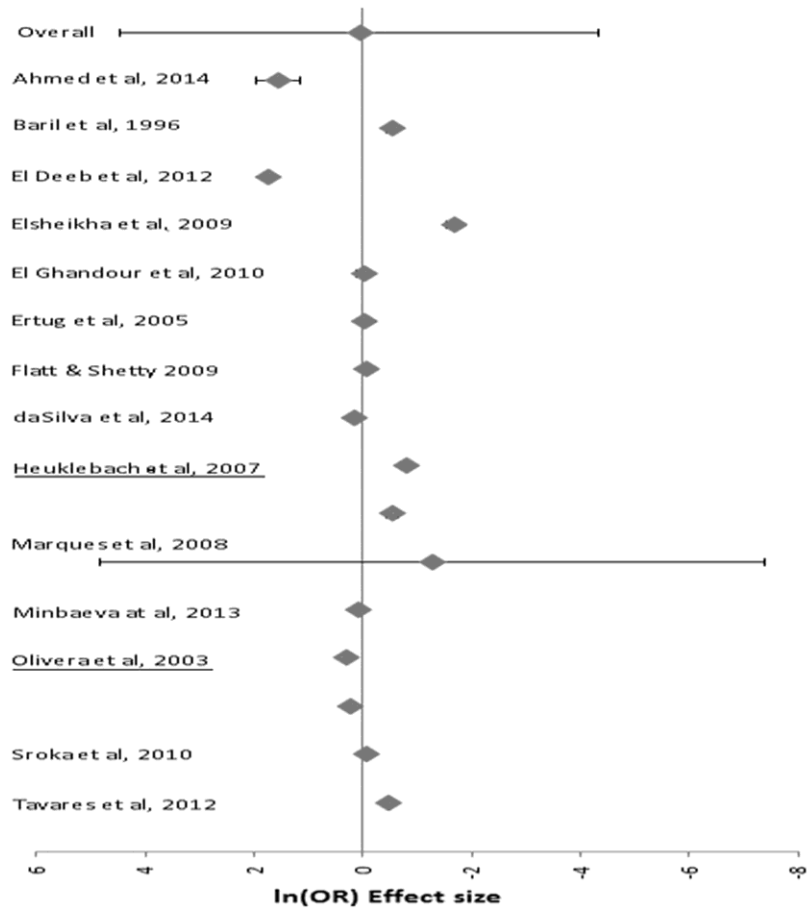

Figure1. Contribution of dairy consumption in toxoplasmosis occurrence. Axis "X" represents the effect size ln(OR); axis "Y" represents the investigated studies and bars represent standard errors of the effect size. Effect sizes above zero indicate positive response; those below zero indicate negative response. When overlapping with zero axis, the response is considered non significant. The underlined studies investigated the analysis of two different dairy matrixes.

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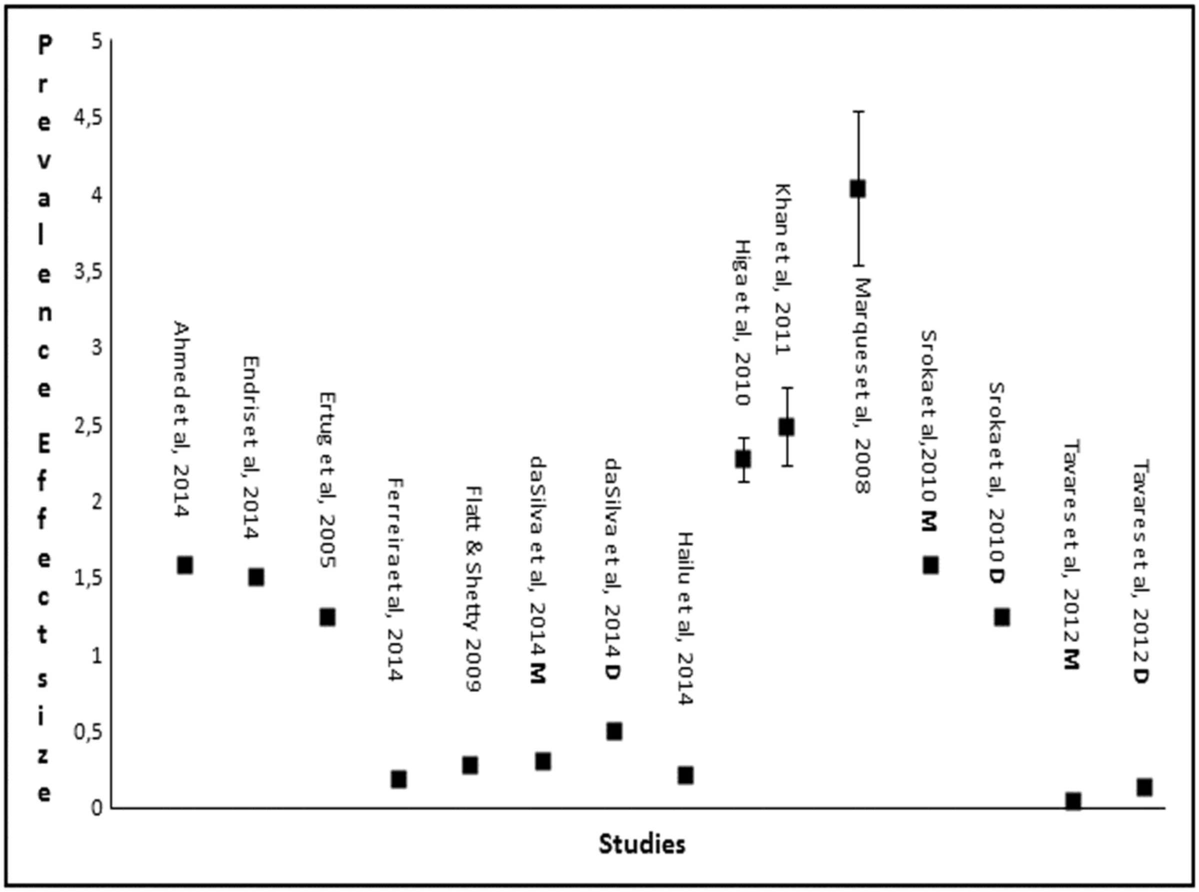

Figure2. Proportions meta-analysis correlation between milk consumption and *Toxoplasma* infection. Bars represent standard errors of the effect size. Effect sizes above zero indicate positive response; those below zero indicate negative one. When overlapping with zero axis, the response is considered non significant.