# Detection of Cryptosporidium Oocysts in Raw Meat in Misan City/ Iraq

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**Abstract--- Background:** Intestinal parasites are widespread in developing countries. Cryptosporidium has appeared to be one of the most common parasites. The study aimed to detect the contamination of raw meat with Cryptosporidium oocyst in Misan city and elevate most type of contaminated meat. Materials and Methods: This study was done from September 2017 to May 2018, 60 samples of meat (30 beef and 30 sheep) 90 from meat product samples. **Results:** The study showed that contamination percentage of (60) raw meat ;30 from cattle and 30 from sheep; reached 6.7 % and 3.33% in cattle and sheep respectively with high prevalence contamination in meat of cattle. Sausages samples recorded higher contamination rate (10%) than hamburger and luncheon (6.7%) and (3.4%) respectively. **Conclusions:** The present study reported the detection of Cryptosporidium oocysts in meat and meat products and there is possibility of transmission of oocysts to human during consumption. The results of our study clarify that contamination of meat products may be occurred during preparation, since sausage and hamburger do not exposed to heat completely during preparation.

Keywords--- Cattle, Cryptosporidium, Iraq, Misan, Raw Meat, , Sheep.

#### I. INTRODUCTION

Meat supply the majority of the nutrients required for human health. Meat is known as a critical wellspring of high organic worth protein and micronutrients including for example nutrients A, B6, B12, D,E and iron (Hamasalim 2012). but also considered substantial sources of pathogenic contamination, *Cryptosporidium spp.* is an intestinal protozoan parasite resides in the intestine of humans and animals, there are really 20 perceived types of Cryptosporidium, which have been disconnected from a huge decent variety of hosts and is passed in the stool of a contaminated individual or creature, influences epithelial cells of the small digestive tract and now and then stomach, nerve bladder, liver, trachea, and lungs in different warm-blooded creatures including people (Hunter and Thompson 2005). He is considered to have the force for nourishment transmission, and surely, there has been a cryptosporidiosis-related erupt (Robertson and Fayer 2012). Similarly as it causes misery and demise in animals, cryptosporidiosis in sheep and calves is viewed as a hotspot that contaminates people (Mirzaei 2007). Tainted calves can oust a lot of oocysts in their stools and corrupt crisp nourishment, drink, and recreational water that ascents to pollution that influences people and different animals (Del Coco et al. 2008).

The major clinical sign in cryptosporidiosis is self-restricting gastroenteritis. In any case, in people who are immune compromised, it might cause ceaseless looseness of the bowels and episodes of the runs I n infant

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ruminants, which can bring about high horribleness and monetary effect (Mackenzie et al. 1994). There are numerous investigations performed already to gauge the nearness of parasite in human ,and animal feces in Iraq such as (Al-Alousi et al. 2003, Al Kaby 2005, Altamimi and Al-Zubaidi 2013,).

In our present investigation, we didn't discover any information about the pollution of Cryptosporidium oocyst in crude meat in Misan city, Iraq. Along these lines, this examination is viewed as the primary preliminary in our region planned to decide and look at the sullying paces of Cryptosporidium spp. oocysts in meat of sheep and steers.

## II. MATERIALS AND METHODS

#### Sample Preparation

This study was done from September 2017 to May 2018, 60 samples of meat (30 beef and 30 sheep) 90 from meat product samples (hamburger, sausage and luncheon, 30 from each sample) were collected from different butcheries, supermarket and retail stores in different areas of Misan city in Iraq. homogenized method was used for detection of cryptosporidium oocyst.

#### Detection of Cryptosporidium

15 g was cut from each one of meat samples. The samples were gathered by putting them in an ice container after cutting immediately. Samples were relocate to the lab to be examined for oocysts. Samples were suspended in 50 Ml of 0.85 % NaCl solution and vigorously shaked with glass beads for 10 min. The substance were tapped and washed with saline, at that point moved to rotator tube and turned at 3000 RPM for 5 minutes. The supernatant was disposed of and the residue transmitted to a spotless oil free slide for recognition of Cryptosporidium oocyst utilizing distinctive indicative procedures, including direct smear, iodine strategy, recoloring technique by changed Zeil-Nelseen recolor. (Sharma and Chattopadhyay 2015).

The statistical analysis was done by independence sample t test (Petrie and Watson 2003).

### III. **RESULTS**

Out of 30 of each, just 2 (6.7%) of beef and 1 (3.33%) of sheep were found as positive samples for Cryptosporidium oocysts. Table 1.

Cryptosporidium oocyst detected in meat products as three sausages samples out of 3 (10 %) recording high contamination while 2 (6.7%) out of 30 hamburger samples and one positive sample of luncheon (3.4%) were found as positive for Cryptosporidium oocyst using direct smear method. Table 2. Iodine staining method (Fig. 1), modified Zeil-Nelseen stain (Fig. 2).

#### IV. DISCUSSION

Cryptosporidium is an apicomplexan parasite of general government assistance and creature esteem, first portrayed in the mid-twentieth century; Cryptosporidium muris and Cryptosporidium parvum are the fundamental species delineated. Transmission happens by means of the fecal-oral course. It has low irresistible portion; that contamination can be started by a very little quantities of oocysts; hypothetically a solitary oocyst could prompt disease in an adept individual. Prolonged survival in moist environments and excessive chlorine tolerance

make Cryptosporidium ideally transmissible (Hunter and Thompson 2005).

Cryptosporidium is one of the essential food borne and waterborne illness in the world. Nourishment borne illnesses are probably the sincere issue in creating nations.

One of the most substantial routs of contamination with Cryptosporidium spp. is contact with creatures, for example, dairy cattle, calves and sheep which are basic supplies of this parasite (Okhuysen et al.1999). Oocyst transmission is fecal-oral, either legitimately or by implication through drinking or recreational water, (for example, pools) that has been debased with Cryptosporidium. Transmission can likewise occur through eating nourishment which can be sullied at any phase during appropriation and planning by nourishment handlers, wash water, readiness surfaces, gear. albeit anyway the essential nourishments entangled in transmission are crude products of the soil, propels identified with unpasteurized milk and crushed apples, crude meat and sauce has likewise been legally approved (Fayer et al. 2000).

Several investigations showed that there are significant infection rate with cryptosporidiosis of humans and several domestic animals throughout the world and Iraq (Al-Gelany 1998, Al-Alousi et al. 2003, Zorona et al. 2006) .Cryptosporidium oocyst transmitted by water have been attested in a number of waterborne outbreaks, The primary archived waterborne erupt of cryptosporidiosis happened in Texas (D'Antonio et al. 1985). It was identified with sewage spillage into well water, similarly Had (2014) announced it in filtrated savoring water Iraq nation.

Oocysts of Cryptosporidium parvum had specified in vegetables by Ali et al. (2018) in " green onion, parsley, radish, leek, and celery " at 5 of 80 (6.25%), 6 of 80 (7.50%), 5 of 80 (6.25%), 5 of 80 (6.25%), and 5 of 80 (6.25%), individually, while Hasan et al. (2018) distributed that the frequency of Cryptosporidium oocysts were 32 % and 46% in credulous ovine and caprine milk tests in blended (sheep and goats) raising administration creatures in Iraq.

Our present investigation can be viewed as one of only a handful hardly any examinations in Iraq and everywhere throughout the world on discovery of cryptosporidium oocysts in meat and meat results of hamburger and sheep. Our outcomes concurred halfway with consequences of study done in city of Kolkata, where Sharma and Chattopadhyay (2015) introduced that the complete pervasiveness of cryptosporidium spp. contamination as 6.0% in the meat tests of chicken and lambs .

Faeco-oral method of transmission might be basic between household creatures and man (Mackenzie et al. 1994).Uninterrupted contact with animals and their faeces play important function in contamination of raw meat, The higher incidence of Cryptosporidium oocysts in cattle meat than sheep may be as a result of the ability of cattle and calves to excrete Cryptosporidium parvum in their faeces, which may persevere on the creature cover up to defile inferred remains meat. (Smith 1993). Investigation of Alemayehu et al. (2013) had been recorded higher pervasiveness (27.8%) of Cryptosporidium in calves defecation than in sheep (22.2%) and kids (12.2%). Fayer and Xiao (2008) and Wang et al. (2010) detailed comparative outcomes and proposed that the explanation might be because of generally escalated calves the executives framework or packed lodging arrangement of these creatures when contrasted with children and sheep, and this can bring about moderately low invulnerability of calves.

The detection of cryptosporidium oocysts of present study in meat products; hamburger, sausage , lunchen; had

been reported previously in study done by Samaher (2010) who pointed the occurrence of cryptosporidium oocyst in (45 )examples of minced meat, wiener and lunchen with level of 21%, 12%, 4% individually. Meat and meat products might be a huge vehicle for these nourishment borne sicknesses, with the capacity to spread the infection to an enormous number of purchasers who eat polluted crude or half-cooked meat (Pepin et al. 1997).

The consequences of present investigation explain that tainting of meat items might be happened during planning, since sausage and hamburger do not exposed to heat completely during preparation.

Moriarty et al. (2005) noticed that heat temperature treatments of meat with 60°C for 45 sec, or on the other hand 75°C for 20 sec are adequate to render oocysts of cryptosporidium parvum connected to a hamburger surface and oocysts can stay reasonable during gentle warmth medicines, for example low temperature.

# V. CONCLUSIONS

The present investigation revealed the identification of cryptosporidium oocyst in meat and meat items and there probability to transmit oocyst to human during utilization. Data of our results recommend the need for further large scale studies to clarify the association of contamination with different risk factors for more reliable conclusions.

Table 1: Cryptosporidium Oocyst in Raw Meat Samples

Samples	Total	Positive	Percentage(%)
beef	30	2	6.7
sheep	30	1	3.33

Table 2: Cryptosporidium Oocyst in, Sausages, Hamburger and Hot Dogs Meat Samples in Misan City, Iraq

Meat sample	Total	Infected	Percentage(%)
sausages	30	3	10
hamburger	30	2	6.7
luncheon	30	1	3.4



Figure 1: Oocyst of Cryptosporidium by Iodine Stain with Magnification 400X



Figure 2: Oocyst of Cryptosporidium by Modified Ziehl-Neelsen Stain with Magnification 1000X

# REFERENCES

- [1] Al Kaby S (2005) Epidemiological study parasite spore of Cryptosporidium causes diarrhea in Al-Qadissyia . *Master province - College of Veterinary Medicine - University of Al-Qadisiya*;.
- [2] Al-Alousi TI, Al-Ani AJ, Al-Rawi SA (2003) Cryptosporidiosis in children and calves in Mosul, *Iraq Medical journal of Tikrit University* 119 pp.
- [3] Alemayehu R, Oda G, Fufa A, Rahmeto A, Desta B, Bekele M, Etana D, Kassahun A, Eystein S (2013) *Cryptosporidium* in calves, lambs and kids at Haramaya, Eastern Ethiopia. *Ethiopian Veterinary Journal* 17:81–94.
- [4] Al-Gelany BAA (1998) Study of epidemiology of cryptosporidiosis in Baghdad M. Sc. Thesis, College of Veterinary Medicine, *Baghdad University* 127 pp. (in Arabic).
- [5] Ali HS, Mageed SN, Jahed Khaniki GR, Shariatifar N, Yunesian M, Rezaeian M, Saleh KK (2018) . Contamination of Cryptosporidium spp. Oocysts in Raw Vegetables Produced in Koya City, Iraq. *Journal* of Food Quality and Hazards Control 5: 89-93.
- [6] Altamimi MKA, Al- Zubaidi M, Hasan AB (2013) Prevalence of Cryptosporidium andersoni in cattle in Babylon province, Iraq. *Al-Anbar Journal of Veterinary Sciences* 6(2): 37-42.
- [7] D'Antonio RG, Winn RE, Taylor JP, Gustafson TL, Current WL, Rhodes MM, Gary JGW, Zajac RA (1985) A waterborne outbreak of cryptosporidiosis in normal hosts. *Annals of Internal Medicine* 103: 886– 888.
- [8] Del Coco VF, Cordoba MA, Basualdo JA (2008) Cryptosporidium infection in calves from a rural area of Buenos Aires, Argentina. *Veterinary Parasitology* 158: 31-35.
- [9] Fayer R, Morgan U, Upton SJ (2000) Epidemiology of Cryptosporidium: transmission, detection and identification. *International Journal for Parasitology* 30:1305-1322.
- [10] Fayer R, Xiao L (2008) Cryptosporidium and Cryptosporidiosis. 2nd ed. CRC press, London, New York.
- [11] Had A (2014) Isolation and Identification of Cryptosporidium spp. by Reverse Osmosis System of Tap Water in Baghdad. *Journal of Baghdad for Science* 11(2):894-899.
- [12] Hamasalim HJ (2012) Quality assessment of the imported canned beef sold in Sulaimani markets. *KSU Journal of natural sciences* 15(4):1-6.
- [13] Hasan MH, AL-Taee AF, AL-Hubaety AK, ALAni A.J (2018) Detection of Cryptosporidium in ovine and caprine raw milk in relation to rearing management . *Assiut Veterinary Medical Journal* 64(156): 47-50.
- [14] Hunter PR, Thompson RC (2005) The zoonotic transmission of *Giardia* and *Cryptosporidium*. International Journal for Parasitology 35:1181–90.
- [15] Mackenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blaire KA, Peterson D E, Kazmierczak JJ, Addis DG, Fox KR, Roes JB, Davis JPA (1994) A Massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. *The New England Journal of Medicine* 331(3): 161-167.
- [16] Mirzaei M (2007) Prevalence of Cryptosporidium spp. infection in diarrheic and non-diarrheic humans in Iran. *Korean Journal of Parasitology* 45(2):133-7.

- [17] Moriarty EMG, Duffy JM, McEvoy S, Caccio JJ, Sheridan DM, Blair IS (2005) The effect of thermal treatments on the viability and infectivity of Cryptosporidium parvum on beef surfaces. *Journal of Applied Microbiology* 98:618–623.
- [18] Okhuysen PC, Chappell CL, Crabb JH, Sterling CR, DuPont HL (1999) Virulence of three distinct Cryptosporidium parvum isolates for healthy adults. *Journal of Infectious Disease* 180: 1275-1281.
- [19] Pepin M., Russo P, Pardon, P (1997) Public health hazards from small ruminants meat products in Europe. *Revue Scientifique et Technique (International Office of Epizootics)* 16(2): 415–425.
- [20] Petrie A, Watson P (2003) Statistics for Veterinary and Animal Science Black Well Science Co.. London . England.
- [21] Robertson LJ, Fayer R (2012) Cryptosporidium. In: Robertson LJ, Smith HV (eds), Foodborne protozoan parasites. *Nova Publishers, in press. ISBN*: 978-1-61470-008-1.
- [22] Samaher HSN (2010) Detection of Cryptosporidium parvum and Toxoplasma gondii in some Meat and Meat products. *College Of Basic Education Researches Journal 10* (4): 511-520.
- [23] Sharma KP, Chattopadhyay UK (2015). Isolation and Identification of Cryptosporidium spp. from Raw Meat Samples Sold in Open Markets of the City of Kolkata. *International Organization of Scientific Research-Journal of Agriculture and Veterinary Science* 8(2): 16-19.
- [24] Smith JL (1993) Cryptosporidium and Giardia as agents of foodborne disease. *Journal of Food Protection* 56: 451–461.
- [25] Wang Y, Feng Y, Cui B, Jian F, Ning C, Wang R, Zhang L, Xiao L (2010) Cervine genotype is the major Cryptosporidium genotype. *Parasitology Research* 106 (2):341-347.
- [26] Zorona M, Katic-Radivojevic S, Kulisic Z (2006) Cryptosporidium infection in lambs and kids in Serbia. *Acta Veterinaria* 56 : 49 -54.