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Using Elisa and nested PCR for detection of the toxoplasmosis in milk and the influence of infection and some factors on milk composition in the Iraqi local and Shami goats

Entesar Hussain Madi

Dep. of Zoonotic disease/College of Veterinary Medicine/University of Baghdad/Iraq

Firas Rashad Al-Samarai

Dep. of Veterinary Public Health/ College of Veterinary Medicine/University of Baghdad/Iraq

Abstract--This study concluded detection of *Toxoplasma gondii* in milk, immunologically by using Elisa and nested PCR(nPCR) based on B1 gene, also to investigate the effect of toxoplasmosis, parity, breed and flock on some milk composition in the Iraqi local and Shami goats in the middle of Iraq. A total of 80 milk samples of the lactating goats were collected. Results of this study showed the prevalence of Toxoplasmosis was 21.25% and 28.75% by Elisa and nPCR respectively without significant differences. The sensitivity of Elisa was a low (30.43%) whereas the specificity was a high (82.45%). The degree of agreement estimated by Kappa coefficient revealed a slight agreement (0.14) between two methods. The results indicated that goats infected with toxoplasmosis have significantly ($P<0.05$) higher in protein and lactose percentage whereas the effect was not significant for each of fat, SNF and pH. On the other hand, the effect of parity was significant ($P<0.05$) on fat, protein, lactose, solid nonfat and non-effect on PH. As well as effect of breed and flock was significant ($P<0.05$) on protein, lactose and solid nonfat without significant effect on fat and PH. In conclusion the detection of toxoplasmosis is very important because of its effect of protein and lactose%.

Keywords---milk composition, *T.gondii*, nested PCR, Elisa, local goats, shami goats.

Introduction

Toxoplasmosis is a serious public health concern that continues to be exacerbated by the presence of the parasite in goat milk, consumer demands for pathogen-free goods have focused government authorities and the food industry's attention on the need of having safe, high-quality products, raw milk is sold and distributed commercially in a variety of ways in different nations (**Mancianti et al., 2013**). Despite the fact that raw goat's milk has been proved to be a vector for disease transmission, unpasteurized dairy products have gained appeal among customers who believe raw milk improves the immune system and provides other health advantages (**Basnet et al., 2010**). Toxoplasmosis in humans can be spread vertically through the placenta or horizontally by the intake of foods contaminated with bradyzoites and tachyzoites, analogous as unpasteurized milk or cheese, undercooked meat, or ignoble fruits and vegetables (**Tenter et al., 2000**). These cases make up the majority of *T. gondii*'s disease burden, which is among the highest of all foodborne parasitic diseases globally (**Torgerson et al., 2015**), third among all food-borne diseases in the United States, and second among food-borne parasitic infections in Europe (**Batz et al., 2012 and Bouwknecht et al., 2018**). A few studies reported the presence of tachyzoites in milk of different species as goat, sheep, cow, buffalo, and camel (**Dubey, 1998; Dehkordi et al., 2013 and Medani and Mohamed, 2016**). **Sharifdini et al. (2015)** explained that molecular approaches were shown to be superior than parasitological methods in general, in compared to parasitological approaches, the sensitivity of nPCR was higher than that of real-time PCR. Other PCR-based methods were also developed to identify the presence of parasite DNA in the samples. **Martinez et al. (2003)** introduced nPCR-ELISA, in which PCR products are hybridized to polystyrene beads, allowing for quick colorimetric examination (**Škorpíková, 2021**). The aim of the study was the effect of toxoplasmosis on some milk components due to the lack of studies in Iraq.

Materials and Methods

Samples collection

The research was conducted in Iraq at the Ruminant Research Station of the General Authority for Agricultural Research /Ministry of Agriculture /Abu Ghraib/ Baghdad. And in AL-Dibuni Research Station for Researches / Wasit. Milk samples were taken from 80 each female animal using plastic tubes with a capacity of 100 ml, and analyzed on the same day using a milk analyzer type **Julie Z7 Bulgaria/ Germany** and washing machine with sodium thiosulphate every 10 sample to find out the proportions of the components of protein, fat, SNF, lactose and pH was examined with a pH meter. Also, milk samples kept at -20 °C for Elisa and DNA extraction process.

Nested PCR

This technique was performed for direct detection of *Toxoplasma gondii* based on B1 gene from milk samples. This method was carried out according to method described by (**Halleyantoro et al., 2019**) as following steps: These primers were provided by (Scientific Researchers. Co. Ltd / Iraq) as following table:

Table (1) B1 gene PCR primer and B1 gene Nested primer

Primers	Sequence 5'-3'		PCR product size
B1 gene PCR primer	F	GGAAGTGCATCCGTTTCATGAG	160 bp
	R	GGCGACCAATCTGCGAATACACC	
B1 gene Nested primer	F	TGCATAGGTTGCAGTCACTG	131 bp
	R	TCTTTAAAGCGTTCGTGGTC	

Statistical analysis

A total of 80 milk samples were collected randomly and subjected to Elisa and nPCR to detect the infected animals. To evaluate the Elisa test, a comparison was done between the results to obtain sensitivity, specificity, and Kappa coefficient. The odds ratios were estimated to identify the risk of some factors. Two types of odds ratios were estimated; crude odds ratio and the adjusted odds ratio by using logistic regression. To evaluate the effect of some factors on milk content the data of 80 animals were analysed using the following model:

$$Y_{ijkl} = \mu + B_i + P_j + H_k + F_l + e_{ijkl}$$

Where Y_{ijkl} is the observed trait, μ is the overall means, B_i the effect of breed (Shami and local goats)

P_j the effect of i^{th} parity ($i = 1, 2, 3, 4$ and more), H_i the effect of health status (infected or healthy)

F_k the effect of flock. e_{ijkl} is the random error.

Results and Discussion

Serological test and molecular test in milk

Out of 80 goats milk examined by using indirect IgG ELISA and nPCR. In milk indirect IgG ELISA, positive samples were 17(21.25 %), while in nPCR, positive samples was 23(28.75%) with no significant difference (**Table 2**). The Kappa coefficient (0.140) indicated a slight agreement between ELISA and nPCR in milk with 95% CI (-0.0886 to 0.368) (**Table 3**). A bioassay utilizing cats and mice, cell line culture, capture ELISA, and PCR can all be used to demonstrate *Toxoplasma* excretion in milk (**Dehkordi et al., 2013**). **Saad et al. (2018)** found that from 30 the infected was 27 (90%) by Elisa and from 27 cases the infected animals was 1 (3.70%) by qPCR. Also **Sadek et al., (2015)** found LAT 18(38.3%), and PCR in 5 (22.7%), in smears of milk of goats in Egypt; nevertheless, **Dubey et al.(2020)** stated that there is a real danger of misdiagnosis because it is very difficult to

identify tachyzoites due to the fat droplets in milk. In Iraq **Ali, (2018)** found that the prevalence was 25%, 00.00% using Elisa and conventional PCR respectively.

As well as, in Mongolia by using nPCR **Iacobucci et al. (2019)** found zero in milk. In Poland **Jańczak et al., (2017)** reported that after being released in goat milk, *T. gondii* may thrive in fresh cheese made with cold enzyme treatment, according to the findings, to stop spread to humans and animals, raw milk should never be drunk, Fresh goat cheese made with cold enzymes and raw milk should also be avoided (**Dubey et al., 2020**).

Table (2): Comparison between infection rate using ELISA and nPCR for milk.

Type of test	Total No	Infected No	Infection %	McNemar test (Paired proportions)	P-value
nPCR	80	23	28.75	9.62	0.32
ELISA	80	17	21.25		

Means non-significant ($p > 0.05$)

Table (3): Some parameters for evaluation of Elisa compared with nPCR in milk.

	Milk-nPCR		
Milk-Elisa	-	+	
-	47	16	63 (78.7%)
+	10	7	17 (21.2%)
	57 (71.2%)	23 (28.7%)	80
Weighted Kappa ^a			0.140
Standard error			0.117
95% CI			-0.0886 to 0.368
Sensitivity			30.43%
Specificity			82.45%

Milk composition evaluation

Effect of some genetic and non-genetic factors on some milk composition.

Effect of health status

Results revealed that the effect of healthy status was significant ($p < 0.05$) on protein% and lactose%, with no significant effect on SNF%, fat% and PH (**Table 4**). There are insufficient studies on the effect of infection with *Toxoplasma* on the

milk composition of goats. Although there was a study showed that toxoplasmosis had not affected on milk production in sheep additionally, there were no variations in the milk composition of the seropositive and seronegative sheep groups when comparing fat, protein, and lactose levels (**Klauck et al., 2016**). In addition, a study in donkeys found that infection with *Toxoplasma* does not lead to significant differences in milk composition and milk production, except of casein and ash (**Martini et al., 2014**).

Table (4) Effect of health status on some milk composition in goats

Health status	No.	Fat%	Protein%	Lactose%	SNF	pH
Healthy	63	4.12±0.14 a	3.10±0.0 4a	4.60±0.04 a	8.37±0.12 a	6.72±0.04 a
Infected	17	4.00±0.23 a	3.24±0.1 1b	4.84±0.17 b	8.80±0.30 a	6.78±0.07 a

Means with a different letter in the same column are significantly different (P<0.05)

Effect of Parity

The effect of parity was significant ($p<0.05$) on fat%. The fat% was significantly higher in parity 4 and 2 than parity 1 and 3. It stayed also significant ($p<0.05$) on protein %, lactose % and SNF % in parity 1, but not on PH **Table (5)**. **Addass et al. (2013)** discovered a considerable rise in milk fat with increasing parity, which was consistent with **Ehoche et al. (1990)**. The negative link between milk fat and yield, according to the researchers, explains their findings. This means that when milk yield is prioritized, milk fat concentration decreases (**Al-Azawi et al., 2015 and Addass et al., 2013**).

Table (5) Effect of parity on milk composition.

Parity	No.	Fat%	Protein%	Lactose%	SNF	pH
1	23	3.83±0.14b	3.34±0.10 a	4.98±0.15 a	9.06±0.27a	6.65±0.06a
2	23	4.51±0.18a	3.08±0.06 b	4.59±0.09 b	8.37±0.15b	6.84±0.08a
3	18	3.47±0.22b	3.04±0.07 b	4.56±0.10 b	8.26±0.18b	6.79±0.09a
≥4	16	4.55±0.35a	2.99±0.07 b	4.37±0.09 b	7.69±0.16b	6.64±0.06a

Means with a different letter in the same column are significantly different (P<0.05) SNF=solid non-fat

Parity, breed, season, and lactation stage had no influence on pH content, according to (**Beyene and Seifu, 2000 and Addass et al., 2013**). Also, A decrease in percentage of protein, lactose, SNF is associated with the decreasing in the milk production (**Dhiab et al., 2012**). Lactose synthesis might result in a

rise in milk yield, hence Shamis with high lactose content had a high milk output (**Lin et al., 2016**). Parity influenced dairy sheep milk yield (141, 144, and 148 kg/d in the 1st, 2nd, and 3rd parturition, respectively), but not milk protein ratio (5.59 %, 5.68 %, and 5.99 % in the 1st, 2nd, and 3rd parturition, respectively) (5.13 % , 5.60 % , and 6.05 %through the 1st, 2nd, and 3rd parturition, respectively) (**Sevi et al ., 2000**). Difference could be due to the milk output from primiparous dairy goats had a lower yield (257.8 kg/lactation) than milk output from multiparous dairy goats (3021 kg/lactation), and parity affects milk fat and protein concentrations and milk yield of dairy goats as stated by **Carnicella et al. (2008)**. The lipid and protein concentrations of goat milk were equal over the first five parities (**Zeng et al., 2008**).

Effect of flock

Results revealed that the effect of herd was significant ($p < 0.05$) on protein%, lactose% and SNF% in flock (AL-Dibuni) but there is no significant on fat%, PH (**Table 6**). These differences could be attributed to the difference in the level of management and the flock size (**Mohsin et al., 2019**).

Table (6) Effect of flock on some milk composition in goats.

Flock	No.	Fat%	Protein%	Lactose%	SNF	pH
Abu Ghraib	55	4.08±0.15a	3.04±0.04b	4.51±0.06b	8.20±0.11b	6.74±0.05a
AL-Dibuni	25	4.12±0.17a	3.33±0.09a	4.97±0.14a	9.04±0.24a	6.73±0.06a

Means with a different letter in the same column are significantly different (P<0.05)

Effect of breed

Results revealed that the effect of breed was significant higher ($p < 0.05$) on protein%, lactose% and SNF% in shami but there is no significant on fat%, PH (**Table 7**). Milk composition might differ depending on the goat's age, body size and weight, udder size, and lactation phase, diet, breed, managing, ecological disorder, individually, season and location all contributed to the variety (**Park, 2007**). Breed, diet, and seasonal variables all contributed to the difference in total fat concentration in goat milk (**Lai et al., 2016**). The pH content was unaffected by breed, lactation stage, season, or parity (**Beyene and Seifu, 2000**).

According to **Mohsin et al. (2019)** milk chemical and mineral content varies significantly between breeds ($p < 0.05$), According to **Yakan et al. (2019)**, milk obtained from pastured goats was found to be healthier, and early lactation stage goats produced healthier milk than late lactation stage goats.

Table (7) Effect of breed on some milk composition in goats.

Breed	No.	Fat%	Protein%	Lactose%	SNF	pH
shami	49	3.96±0.13a	3.20±0.06a	4.76±0.08a	8.65±0.16a	6.73±0.04a
local	31	4.29±0.22a	3.00±0.05b	4.48±0.08b	8.16±0.14b	6.74±0.07a

Means with a different letter in the same column are significantly different (P<0.05)

Conclusion

The results of the current study confirmed that the Toxoplasmosis can be detected in milk using nPCR and confirmed that Elisa was not efficient for detection. Also, the results showed that the infection effect the milk composition. Hence further studies needs to investigate the effect of infection on milk yield of goats.

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