Study the prevalence of *Neospora caninum* in serum and milk of sheep in Al- Fallujah city

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Abstract

This study was conducted to determine the prevalence of *Neospora caninum* of sheep in Al- Fallujah city. One hundred and twenty seven (78 females and 49 males) blood serum and 52 milk samples of different ages and sexes of sheep were examined by using indirect enzyme linked immunosorbent assay (ELISA) during the period from 10/ 12/2012-15/2/ 2013 in Al- Fallujah city. The total infection rate was 3.91%, which divided into 4.72% in sera and 1.92% in milk. Sheep above 4 years old was showed a high infection rate 11.11%, and no infection rate was recorded in the age group under 3 years old. The results of the study revealed that the infection rates were higher in females 6.41% than males 2.04% and in pregnant 11.53% than lactating 3.84%. This study was the first report about the seroprevalence of *N. caninum* of sheep by using ELISA in Iraq.

دراسة انتشار طفيلي *Neospora caninum* في مصل وحليب أغنام مدينة الفلوجة

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الخلاصة

هدفت هذه الدراسة إلى تحديد الانتشار المصلي لطفيلي *Neospora caninum* في أغنام مدينة الفلوجة للمدة 2012/12/10– 2013/2/15 من خلال فحص 127عينة مصل (78 إناث و49 ذكور) و52 عينة حليب باستعمال مقايسة الممدص المناعي المرتبط بالأنزيم (الاليزا) غير المباشرة أظهرت النتائج ان نسبة الإصابة الكلية 3.91% توزعت بواقع 4.72% في المصول و1.92% في الحليب. وكانت أعلى نسبة للإصابة في الحيوانات التي بلغت أعمارها أكثر من 4 سنوات (11.11%) ولم تسجل أية إصابة في الأعمار التي قلت أعمارها عن 3 سنوات، كما بينت النتائج أن نسبة الإصابة في الإناث أعلى ما هو عليه في الذكور وبلغت (6.41%)، (2.04%) على التوالي وفي الحوامل (11.53%) والمرضعات (3.84%).

Introduction

Neospora caninum is an Apicomplexan protozoan (1), can be infect a wide range of intermediate animals and has been recognized as an important causative agent of economic and reproductive losses in the livestock industry, especially in dairy cattle (2). It is known to cause abortion and premature birth (3). The ingestion of oocysts is the only demonstrated mode for postnatal (horizontal) transmission in herbivores (4) or by ingestion of infected tissues with tachyzoites or tissue cysts in dogs, and it can be transmitted transplacentally (vertically; congenitally) from an infected dam to her fetus during pregnancy (5); The transplacental transmission has been induced experimentally in cattle, dogs, sheep, goats, monkeys, cats, mice and occurs naturally in many hosts (6). Studies that address the epidemiology of neosporosis in small ruminants are very scarce; The only available reports in the Middle East investigated the prevalence of infection in horses, cattle, buffalos and wild canines (7). The seroprevalence of *N. caninum* in Jordan in sheep and goats were 4.3% and 5.7% respectively (8). It has been

recently shown to be a cause of abortion of sheep (9); According to the stage of gestation at infection time and can cause fetal mummification, embryo resorption, stillbirth, and the birth of weak or apparently healthy but congenitally infected offspring(10). Natural infections in sheep are rare (11) and reports being restricted to a few cases(12). In sheep, despite the availability of serological tests for neosporosis to identify infected dams, it is also important to determine those in a herd at risk of abortion; The enzyme-linked immunosorbent assay (ELISA) can be helpful in studies on reproductive failure related to neosporosis (13), and in Europe and Asia, studies have been performed to evaluate this test for individual infection and bulk milk samples(14) Due to the importance of disease and has no data about the seroprevalence of sheep neosporosis in Al-Fallujah city before this study was conducted.

Materials and Methods

- **Animal Samples:** One hundred and twenty seven (78 females and 49 males) blood and 52 milk samples of different ages and sexes of sheep were randomly collected during 10/12/ 2012-15/2/ 2013 in Al- Fallujah city.
- **Blood samples:** Blood samples (about 5 ml) were collected from the jugular vein after cleaning and disinfected the area by using ethyl alcohol (70%); without anticoagulant additives. The blood was allowed to clot and centrifugation (3000 rpm/ 10 minute). The serum was separated and transferred to 1.5 ml sized Eppendorff tubes (1.5 ml) and stored at $-20C^0$ until used (15).
- **Milk Samples:** Milk samples (about 4 ml) were collected at different reproductive periods from ewes in test tubes and transferred to Eppendorff tubes (1.5 ml) and stored at $-20C^0$ till used (16).
- **Enzyme Linked Immunosorbent Assay (ELISA)[*]:** Serum and milk samples were examined by ELISA according to kit procedure.

Results and Discussion

- **Infection rate:** The total infection rate of *N. caninum* was 3.91%, which divided into 4.72% in serum and 1.92% in milk without significance difference ($P \geq 0.05$). (Table, 1).

Table (1) Infection rate of *Neospora caninum* in sheep by ELISA

Samples	No. of Samples Examined	Positive	Percentage (%)
Serum	127	6	4.72
Milk	52	1	1.92
Total	179	7	3.91

*** P≥ 0.05**

In last years, ELISA has become one of the most commonly used test for the serological diagnosis of *N.caninum* that enables rapid analysis of samples and was extremely useful for large scale screening of herds (17). Also, it is a technique that enables rapid determination of antibody levels in body fluids like serum and milk (18). It was based on crude extracts or recombinant proteins were developed to detect *Neospora*-specific antibodies, no cross-reaction was seen between those produced by *Neospora* infection and those by *Toxoplasma*, *Cryptosporidium*, *Sarcocystis*, *Eimeria*, or *Babesia* (19). Natural infection of sheep by *Neospora caninum* is uncommon, with a few abortion cases (20, 21). However, several serological surveys conducted in different countries, including Brazil (22, 23), which have demonstrated prevalence of infection ranging from 6.4 to 29% (24, 25); in Parana and in Sao Paulo obtained frequencies of 9.5% and 9.2%, respectively (22,26) was observed a high seroprevalence 29.0% in sheep from the Amazonic region, But (12) was observed only three positive samples 0.45% of 660 analyzed in England. These findings were disagree with our results

[*] ***Neospora caninum*** **Indirect Multi- species ID.VET –Franc.**

3.91%; But they were closely to (27) who reported an infection rate 3.7% in lambs that aborted in Slovakia, in Jordan 4.3% (8). Recent serological surveys indicate a very low 0.6% in New Zealand to high 30.8% in Brazil prevalence in asymptomatic sheep (28). In the Czech Republic and in Slovakia, studies reported a prevalence of 12% and 3.8%, respectively (23, 27). In Uberlandia and Minas Gerais, reported that 8.81% and 8.1% of sheep had anti-*N. caninum* IgG antibodies, respectively. The different of frequencies of infection between regions could be due to different sampling methods; Indeed, there was a great variability of results regarding to the region studied (23). In a study of neosporosis in Minas Gerais using different serological methods suggested that variations among frequency rates may also be due to different environment contamination. In addition, the results can vary according to the extent of canine presence and animal age (29). (8) identified the presence of more than one dog in the herd as a risk factor for neosporosis. There was effect of gender, age and race in the infection rate (24,25). Milk ELISA testing offers an attractive alternative to serologic diagnosis of *N. caninum* infection because it can be conveniently and economically incorporated into existing animal recording programs that routinely collect (30) and milk samples are easier to collect than blood samples and sampling can be done by the animal owner or attendant. Several available ELISA kits have been previously validated for use on either whole or skim milk ELISA assays have also been described for use with milk samples (18). An indirect ELISA on individual milk samples demonstrated a 90% sensitivity and 90% specificity relative to serum (31) Previously, the only prevalence data available has been from individual dairy herds and the prevalence's ranged from 10 to 31% (32,33) or from the New Zealand dairy cattle population (6.75-8.5%) (34) In our study the overall prevalence in milk was 1.92%, this result was lower than that reported in United States 18.3%.(30), northeast and north Thailand (46%) and (24%) respectively, the central region of Thailand and Vietnam (6%) (35). Significant difference between these results in different areas could be not only the proportion of infected animals but also their antibody levels lactation stage and milk yield. The differences in farming management and climate may in part explain these significant differences in the prevalence. Climatic factors affect the abundance of viable parasitic stages in the environment for definitive and intermediate hosts and influence the overall prevalence. It is known that moist conditions are favorable for development of *N. caninum* oocysts, potentially making transmission to intermediate hosts more efficient (8).

- **Infection rate according to age:** The high infection rate of *N. caninum* was recorded in the age group above 4 years old 11.11% followed by the age group between 3-4 years 7.5%; While no infection rate was recorded in the age group under 3 years old. with significance difference (P≤0.05). (Table. 2).

Table (2) Infection rate of *N. caninum* in sheep according to age

Age (year)	No. of Sample Examined	Positive	Percentage (%)
<1	19	0	0
1-2	20	0	0
2-3	21	0	0
3-4	40	3	7.5
>4	27	3	11.11
Total	127	6	4.72

***(P≤0.05)**

(36) reported that *N. caninum* antibodies prevalence increases with age. (24, 37) were found no significant association between age and seroprevalence and the later suggesting an effective trans-placental transmission from infected dams to their calves . Our results were showed an association between serological status and sheep age, A

high positive seroprevalence of *N. caninum* was recorded in the age group above 4 years old 11.11%; that was agree with (8) who determined that the seroprevalence of *N. caninum* was significantly higher in sheep and goats older than 4 years of age than in younger animals due to horizontal transmission by ingestion of oocysts that shed by the final host. Our results showed that no infection rate was recorded in the age group under 3 years old that may be due to the fact that this age may have a good resistance against *N.caninum* due to maternal immunity.

- **Infection rate according to sex:** A high infection rate was recorded in the females 6.41% than males 2.04% without significance difference (P≥ 0.05) (Table. 3).

Table (3) Prevalence of *Neospora caninum* infection in sheep according to sex

Sex	No. of Sample Examined	Positive	Percentage (%)
Male	49	1	2.04
Female	78	5	6.41
Total	127	6	4.72

***(P≥ 0.05)**

Our results were showed that the high infection rate of *N. caninum* was recorded in the females than males, that agreement with (38) who found the infection rate was higher in females than males 43. 36% and 15% respectively. In New Zealand a seroprevalence of 0.63% in ram sera (9) that was less than the infection rate of our study 2.04% may be due to deferent samples collection, areas of the study and the tests that used in addition to the deferent climatic conditions that affect in the viability of the oocysts.

- **Infection rate according to reproductive stage:** A high infection rate of *N. caninum* 11.53% was recorded in pregnant ewes than lactation ewes 3.83% without significance difference (P≥ 0.05). (Table. 4)

Table (4) Infection rate of *N. caninum* in sheep according to reproductive stage

Reproductive stages	No. of Sample Examined	Positive	Percentage (%)
Pregnant	26	3	11.53
Lactation	52	2	3.84
Total	78	5	6.41

*** (P≥ 0.05).**

Serological surveys for *N. caninum* in sheep have reported variable findings, with seroprevalence of below 2% to over 10% regardless of any history of abortion. A studies from the United Kingdom found 0.45% prevalence in ewes that aborted (12), and Switzerland 10.3% in sheep exhibiting a persistent abortion problem (10). This seroprevalence was in line with the seroprevalence (0.45%) reported from, albeit aborted sheep in the UK (12). The level of infection was; however, lower than reported from South American sheep (9.2-9.5%) (22) and (24)We agree with the explanation of (30) they have shown an increases in serum antibody level as gestation progresses. Also (39) who suspected that increased rainfall may pose direct and indirect stresses to elevated heat production in response to cold temperatures, behavioral stress, impaired food quality, and diminished hygiene that could trigger *N. caninum*-associated abortion in latently infected cattle.

- **Infection rate according to area:** Table (5) showed that the infection rate of *N. caninum* was recorded only in the Al-Shehabi village 8.95% with significance difference (P≤0.05) and in the milk samples 3% without significance difference (P≥0.05) (Table. 6).

Table (5) Infection rate of *N. caninum* in sera according to areas of the study

Areas	No. of Sample Examined	Positive	Percentage (%)
Al-Fallujah Center	41	0	0
Al-Shehabi	67	6	8.95
Al-Saqlawia	19	0	0
Total	127	6	4.72

*** (P≤0.05).**

Table (6) Infection rate of Neospora caninum in milk according to areas

Areas	No. of Sample Examined	Positive	Percentage (%)
Al-Fallujah center	0	0	0
Al-Shehabi	33	1	3
Al-Saqlawia	19	0	0
Total	52	1	1.92

***(P≥0.05)**

The variation in the infection rates in deferent areas from the previous studies may be due to variation in the areas of the studies and different climatic and geographical conditions or characteristics of the tests that used (Sensitivity and Specificity) (40). The climatic factors affect the abundance of viable parasitic stages in the environment for definitive and intermediate hosts and influence the overall prevalence; It is known that moist conditions are favorable for development of *N. caninum* oocysts, potentially making transmission to intermediate hosts more efficient (8). Climatologically differences between geographical areas may also affect oocyst survival and subsequently the disease prevalence (41).

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