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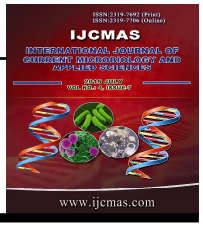

Original Research Article

Seroprevalence of *Neospora caninum* in Goats in Wasit Province – Iraq

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A B S T R A C T

This report proposes an improved adaptation for the laboratory application of a direct transesterification method in the simultaneous analysis of a large number of dry samples. This paper describes a microscale direct transesterification method that features an acid-catalyzed direct alcoholysis of seven microbial biomasses with variable lipid content. The aim of the study was to develop a quantitative method for fatty acid determination that offers a high conversion rate using a minimum number of samples and reactants. The microscale transesterification method was efficient and had a conversion rate of 98.06 ± 0.87% using approximately 3 mg of dried biomass in an acid-catalyzed transesterification process at 70 °C over 20 hours. This method was effective, efficient, and technically attractive for analytical and comparative purposes. It has direct applicability for the screening of strains for lipid-rich biomass production.

Keywords

Neospora caninum, Goat, ELISA, Iraq

Introduction

According to study of Dubey *et al.* (1988), *Neospora caninum* is intracellular a coccidian parasite belongs to phylum of Apicomplexa, also described as a new genus and species. It's includes two species: *Neospora caninum* and *Neospora hughesi* (Ortega-Mora *et al.*, 2003).

On first find out in dogs with encephalomyelitis in 1984 by (Bjerkas *et al.*, 1984). Domestic and wild canids such as dog and coyote are considered both definitive and intermediate hosts. Cattle, sheep, goats, horses and deer are intermediate hosts (Dubey, 2003; Dubey and Schares, 2011). There are two mode of transmission in intermediate hosts which can be infected by vertically (transplacental from the dam to her fetuses) or horizontally infection ingestion of contaminated feed or water with Oocyst; while in definitive hosts can be infection by ingestion of infected tissues. (McAllister *et al.*, 1998; De Marez *et al.*, 1999; Wouda *et al.*, 1999; Dubey, 1999; Anderson *et al.*, 2000; Bergeron *et al.*, 2000; Dubey *et al.*, 2006). Also horizontally in neonatal calf by ingestion of contaminated colostrums with tachyzoite (Uggla *et al.*, 1998).

Neosporosis are infection by *N.caninum* that can cause more clinical signs Characterized primarily by abortion (Larson *et al.*, 2004).

In addition to canids infections, *N. caninum* occurs normally in cattle (where it is an important cause of abortion), goats and deer (Thurmond and Hietala, 1996).

In sheep, neosporosis can cause abortion, neonatal mortality and clinical signs (Ueno, 2009). *N. hughesi*is considered as equine parasite (Dubey and Schares, 2011). In horses, neosporosis can cause abortion, protozoalmyelo encephalitis and neuromuscular disorder (Carrie *et al.,* 2007).

Although neosporosis is a major problem in cattle and dogs cosmopolitanally, but Dubey reported by serological, molecular tests showing that *N. caninum* can cause clinical infections in goats, Abortions, fetal deaths and stillbirths have been documented in goats & sheep due to *N. caninum* in 2003. Antibodies against *N. caninum* were reported in goats in France (Chartier *et al.,* 2000), Taiwan (Ooi *et al.,* 2000), Turkey (Sevgili *et al.,* 2003), Sri Lanka (Naguleswaran *et al.,* 2004), Argentina (Moore *et al.,* 2007), Brazil (Faria *et al.,* 2007), Southern Jordan (Al-Majali *et al.,* 2008), Northern Jordan (Abo-Shehada and Abu Halaweh, 2010), Poland (Czopowicz *et al.,* 2011) and Iran (Sadrebazzaz *et al.,* 2006; Haddadzadeh *et al.,* 2007; Hajikolaei *et al.,* 2007; Salehi *et al.,* 2010; Hosseini *et al.,* 2011; Asadpour *et al.,* 2012).

Goats are economically important in many countries, including Iraq, where this species is a valuable source of meat and milk for humans, particularly in the southern region, where 85% of the goats are concentrated, from total estimated number 1474845 (Fahad and Abbas, 2008).

Low animal productivity is often caused by a lack of information on disease control, and, in this regard, there is a severe lack of information about neosporosis in goats. Some studies have reported occurrences of seropositive goats in different Brazilian states, such as Bahia (Uzeda *et al.,* 2007), Sao Paulo (Modolo *et al.,* 2008), Rio Grande do Norte (Lima *et al.,* 2008) and Alagoas (Anderlini *et al.,* 2011).

However, in the state of Iraq, there are still no studies on neosporosis in goats.

The main aim of this study is to investigate the prevalence of specific IgG antibodies to *N. caninum* by using ELISA kit in sera of goat in Wasit province and the effect of age, sex and seasons on the prevalence of the parasite., also to study of some of blood parameter like RBCs count, PCV, Hb, MCV, MCHC, WBCs and Differential leukocyte count.

Materials and Methods

The study was performed in 5 regions of Wasit province, blood samples were collected randomly from 106 goats during 6 –months-period (Oct. 2014-March 2015).

Blood sampling plan

Blood samples were collected randomly from 106 goats obtained for the serological & hematological tests almost (10ml) were collected via jugular venipuncture under a septic condition (Bassert and McCurnin, 2013), which is transported to the laboratory at 4°C, (5ml) are transfer to Vacutainer® tubes without additives and centrifuged for 15 min at 1000 rpm at room temperature to separate the serum, which is stored at -20°C until analyzed. Other amount of blood (2ml) collected into 2.5 ml anticoagulant tube (EDTA k3) for hematological analysis (Belic *et al.,* 2010).

ELISA test: (IDvet, France) according to the manufacturer's instructions.

Testing procedure

Allow all the reagents to come to room temperature (21°C±5°C) before use Homogenize all reagents by inversion or Vortex.

Samples preparation

In order to avoid differences in incubation times between specimens, it is possible topreparea96-wellplate containing the test and control specimens, before transferring the mintoan ELISA microplate using a multi channel pipette.

Serum or plasma samples

90 µl of Dilution Buffer 2 to each micro well was added. 10 µl of the Negative Control to wells A1 and B1 was added and 10 µl of the Positive Control to walls C1 and D1.

10 µl of each sample to be tested to the remaining wells was added and incubated for 45 ± 4 min at 21°c (± 5°C). Empty the wells. Each well was washed 3 times with approximately 300 µl of the Wash Solution. Drying of the wells between washings was avoided. The Conjugate 1X by diluting the concentrated conjugate 10X to 1/10 in dilution buffer 3 was prepared. 100µl of the Conjugate was added 1X to each well and incubated for 30 min ± 3 min at 21°C (±5°C). The wells were emptied. Each well was washed 3 times with approximately 300 µl of the Wash Solution. Drying of the wells between washings was avoided. 100 µl of the substrate solution to each well was added and incubated for 15 min ±2 min at 21°c (±5°C) in the dark.

100µl of the stop solution to each well in order to stop the reaction was added. The optical densities at 450nm was read and recorded.

Interpretation (Serum)

For each sample, calculate the S/P percentage (S/P %): sample value (OD_{sample}) divided by the mean Positive control value (OD_{PC}) multipliedby100:

$$S/P\% = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

Samples with S/P%:

Serum/Plasma	
Results	**Status**
S/P % ≤ 40 %	Negative
40 % <S/P % < 50 %	Doubtful
S/P % ≥ 50 %	Positive

Hematological parameters

One of aims this study are hematological changes revealed that routinely used laboratory findings such as [hematocrit (Ht or HCT, British English spelling haematocrit), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF)] (Purves William *et al.*, 2004), hemoglobin (HB), Total leukocytes count (TLC), even red blood cell count (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) can provide a diagnostic clue in infected animal with neosporosis (acute or chronic), thus enhancing prompt initiation of treatment and response of treatment for counting all this cellular blood components used blood analyzer.

Moreover, prepare blood smears then stained with Giemsa stain and examined under 100x oil immersion for White Blood Cell Differential Count (neutrophils, eosinophils, basophiles, lymphocytes and monocytes) according to Coles (1986).

Results and Discussion

Serological results (iELISA)

Overall seroprevalence according to breeds and areas

In this present study, we identified the overall prevalence as 5.6% (6/106) in four different areas of Wasit province.

Mean seroprevalences were 8 %, 0 %, 0 %, 7.5 %, and 7.1% in Al-Mazak, Karrada, Al-Nufaishiyah, Entakia, and Sialaareas, respectively. In addition, Seroprevalence rates in local and Shami breeds were 5.3 % and 8.3 %, respectively (Table 1). No statistical difference was found between Shami and Local breeds, also between different areas (P≥0.05). Results of the study are summarized in table (Figure 1).

As per our knowledge, there is limited serological data about caprine neosporosis (Anderson *et al.,* 2000; Dubey, 2003; Dubey and schares, 2006). Abo-Shehada and Abu-Halaweh (2010) studied 302 goats from 62 flocks in northern Jordan, and found the prevalence to be 12% at flock level and 2% at the individual level, respectively, Al-Majali *et al.* in (2008) reported individual level and flock level seroprevalence as 5.7% and 48.7% in 300 goats from 24 flocks in southern Jordan, Czopowicz *et al.* (2011) tested 1060 sera for antibodies against *N. caninum* and determined the true herd level prevalence as 9.0% in Poland 2011. Uzeda *et al.* (2007) found the prevalence as 15% in 385 goats in Bahia, Brazil 2007, Faria *et al.* (2007) found the prevalence as 3.3% in 306 goats in the north-east region of Brazil (2007), other researchers reported the seroprevalance rates as 7.0% (3/486) in Sri Lanka (Naguleswaran *et al.,* 2004) and 0% (0/24) in Taiwan, Different diagnostic tests such as ELISA, indirect fluorescence antibody test (IFAT) and enzyme immuno-assay (EIA) were used in the reported studies (Ooi *et al.,* 2000).

Seroprevalences of *N. caninum* were determined to be 25.9% (47/181) in Nigde, and 5% (9/180) in the Sanliurfa provinces of Turkey (Sevgili *et al.,* 2003; Cayvaz and Karatepe, 2011). Total prevalence as 10.2% (13/128) in three different provinces of Turkey (Utuk *et al.,* 2011).

Most of these previous studies findings have been either similar to our study results or larger.

These differences may be explained by the use of different serological tests, survey periods and sample sizes. Climatic factors may also affect the abundance of viable parasitic stages in the environment for definitive and intermediate hosts and influence on total prevalence (Faria *et al.,* 2007).

Seroprevalence according to gender, age, season and risk factors

With respect to age, one out of 12 (8.3%), none out of 60, and five out of 36 (13.8%) samples were seropositive in the young (less than to one year), adult (one to less than four year), and more than or equal four year groups, respectively. With significant differences were showed for age at (P≤0.05).

The seasonal prevalence observed was 5 out of 92 samples (5.4%) and one out of 14 samples (7.1%) for (October-December)months and warm (January-March) months, respectively.

With respect to gender, one out of 19 (5.2%), and 5 out of 87 (5.7%) samples were seropositive in the male and female, respectively.

Table 2 shows the seroprevalence of neosporosis in does with a history of abortions (6.1 %) and in does with no history of abortions 4 %).

Seroprevalence of neosporosis in caprine PU that either have or do not have cattle were 5.6% and 0%, respectively (Table 2).

Table 2 shows the seroprevalence of neosporosis in goats that live together with dogs/ wild canids (5.6 %) and in goats that do not live with dogs/ wild canids (0 %).

No statistically significant differences were observed for seasons, gender and other risk factors at ($P \geq 0.05$).

High temperature and humidity favour faster sporulation and enhanced survival of *N. caninum* oocysts in the environment. This increases the risk of postnatal infection (Thurmond and Hietala, 1995; Dubey *et al.*, 2007). Our study has several limitations. An accurate assessment of seasonal prevalence was difficult because antibodies against *N. caninum* can persist for several months (Jung *et al.*, 2014**)**.

Chartier *et al.* (2000) pointed out that neosporosis does not seem to be a major factor causing abortions in goats, which might be coincident with the findings of the present study, since the owners of the goats mentioned that only few of the females selected had a history of abortions; however, this information can not be confirmed because of the lack of productive records of the goats at the PU. Nonetheless, due to the low seroprevalence (4.5 %), abortions might be unlikely.

This is opposite to what was indicated by McAllister *et al.* (1998) that the disease causes abortions during the fourth to seventh month of gestation in cattle; this would suggest that the goats are rather a carrier of the protozoan, and that their coexistence with cattle is a risk factor for the occurrence of abortions.

Chartier *et al.* in 2000 have suggested the possibility that the goats are carriers or *N. caninum*, and therefore their coexistence with cattle represents a risk for the transmission of the infection and for the occurrence of abortions in this species

Another important risk factor pointed out in the present study was direct contact between goats and dogs. On small farms, goats are usually housed together with other animals, like dogs, which can contaminate the environment with *T. gondii*and *N. caninum* oocysts (Bartova and Sedlak, 2012). Horizontal transmission of *N. caninum* occurs through ingestion of tissue cysts, which is more likely to be relevant for carnivores, and through intake of food and water contaminated with sporulated oocysts (Dubey *et al.*, 2007).

The prevalence in our present study was lower or equal than that reported for other countries. This might be attributed to differences in the following factors: the number of definitive hosts (dogs and/or other canids in the study area), climate, age, frequency of dogs defecating in the study area, farm management systems, and/or regional ecology (Hobson *et al.*, 2005; Dubey *et al.*, 2007). Thus, it is possible to conclude that neosporosis is present, even at low serological levels, in the goat herds of Wasit province, Iraq; and is associated with some important risk factors.

Table.1 Overall seroprevalence of *N.caninumin* different areas of Wasit province and according to breeds of the goats

Location	Examined (n)			Infected (%)					
	Shami	Local	Total	Shami		Local		Total	
				n	%	n	%	n	%
Al-Mazak	0	25	25	0	0	2	8	2	8
Karrada	0	15	15	0	0	0	0	0	0
Al-Nufaishiyah	0	12	12	0	0	0	0	0	0
Entakia	12	28	40	1	8.3	2	7.1	3	7.5
Saila	0	14	14	0	0	1	7.1	1	7.1
Total	**12**	**94**	**106**	**1**	**8.3**	**5**	**5.3**	**6**	**5.6**

-No significant differences (P≥0.05) neither among infected & uninfected nor areas

Table.2 Distribution of goat seropositive for *Neospora caninum* according to gender, age, season and risk factors in Wasit province, Iraq, 2015

Variables	Caprine		
	Total	Infected	Ratio(%)
Gender			
Male	19	1	5.2
Female	87	5	5.7
Age			
5 to <12 months	10	1	10
1 to <4 years	60	0	0
≥4 years	36	5	13.8
Season			
October-December (2014)	92	5	5.4
January-March (2015)	14	1	7.1
Herds with abortion problems			
Yes	81	5	6.1
No	25	1	4
communal pastures between goat and sheep/cattle			
Yes	106	6	5.6
No	0	0	0
Contact between dogs and goat			
Yes	106	6	5.6
No	0	0	0
Presence of wild canids			
Yes	106	6	5.6
No	0	0	0

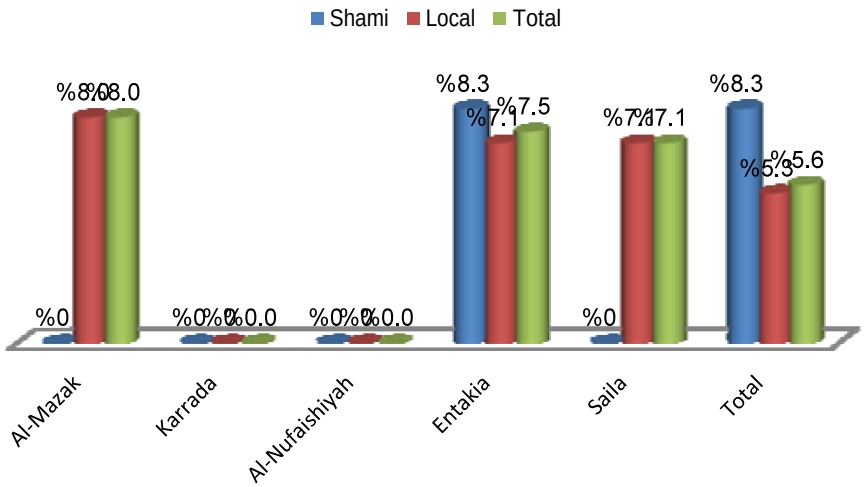
No significant differences (P≥0.05) in gender, seasonandriskfactors, but there is significant differences at (P≤0.05) in age.

Table.3 Haematologic parameters (Mean ± SE) of the non infected and infected goats

Parameters	Negative animals	Positive animals
RBC ($x10^6/\mu L$)	9.98 ± 0.18	11.06 ± 0.89
HB (g/dL)	8.26 ± 0.13	8.62 ± 0.67
PCV (%)	25.95 ± 0.43	25.22 ± 2.51
WBC ($x10^6/\mu L$)	6.70 ± 0.22 B	8.38 ± 1.64 A
MCV (fL)	26.19 ± 0.41	22.7 ± 1.30
MCH (pg)	8.35 ± 0.14	7.84 ± 0.51
MCHC (g/dL)	31.93 ± 0.28 B	34.64 ± 1.99 A
Lymphocytes($10^6/\mu L$)	41.66 ± 1.49	35.3 ± 7.48
Monocytes($10^6/\mu L$)	4.69 ± 0.18	5.20 ± 0.51
Neutrophils($10^6/\mu L$)	52.16 ± 1.46	55.8 ± 7.47
Eosinophils($10^6/\mu L$)	3.39 ± 0.16 B	5.50 ± 0.71 A
Basophils($10^6/\mu L$)	0	0

Differences A, B are significant ($P \leq 0.05$) to comparison rows

Figure.1 Organization chart for percentages of infected goats with *N. caninum* in different areas of Wasit province



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