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## Seroprevalence of *Neospora caninum*, infection among cattle in the Eastern Province of Saudi Arabia

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### Abstract

Neosporosis caused by the protozoan parasite *Neospora caninum*, is economically important disease. It causes abortion, stillbirth, low milk yield, reduced weight gain and premature culling in cattle. In this paper a seroprevalence study of *Neospora caninum*, infection among cattle was conducted in the Eastern Province of Saudi Arabia. A total of 1095 sera samples were collected from different geographical regions of the Eastern province of the Kingdom Saudi Arabia. Al-Dammam samples included Al-Garoudia, Al-Katif, Bou-Hedria and Masehat. Al-Hasa samples included Al-Shakik, Al-Omran, Al-Mahdoud and Al-Aboud. The overall prevalence was 3.4 % from which 4 %, 2.9 %, 2.8 %, and 2.6 % were recorded as prevalence in fully automated dairy farms (dairy industry), smallholders, abattoirs and semi-automated old dairy farm, respectively. Within the samples obtained from the dairy industry, the prevalence of *Neospora caninum* serum antibodies was higher in imported animals than the animals born in the farm ( 4.3% and 2%, respectively) but the relation between the two was not statistically significant ( $P = 0.29$ ). The sero-prevalence of neospora serum antibodies according to breed were 3.7 %, 2.1 % and 0 % in Holstein, mixed and local breed of dairy cattle, respectively. The prevalence of *Neospora caninum* serum antibodies was higher in animals with history of abortion (5.6% and 3.5%, respectively) but the relation was not statistically significant ( $P = 0.38$ ). The finding of the prevalence of *Neospora caninum* antibodies in cattle in the present study was reported for the first time in KSA. Deep research is needed to clarify the epidemiological and molecular situation of *Neospora caninum* infection in cattle in the KSA.

**Key words:** (*Neospora caninum*, Seroprevalence, ELISA, KSA)

### Introduction:

*Neospora caninum* is a protozoan parasite of animals. Until 1988, it was misdiagnosed as *Toxoplasma gondii* (Dubey et al., 1988). Since its first recognition in 1984 among dogs in Norway (Bjerkas et al., 1984) and the description of the new genus and species *Neospora caninum* by Dubey et al. (1988). Neosporosis had emerged as a serious disease of cattle and dogs worldwide. Abortions and neonatal mortality are a major problem in livestock operations, and neosporosis is a major cause of abortion in cattle. The life cycle is typified by the three known infectious stages: tachyzoites, tissue cysts, and oocysts. Tachyzoites and tissue cysts are the stages found in intermediate hosts, and they occurs intracellular (Dubey et al., 2002). The environmentally resistant stage of the parasite, the oocyst, is excreted in the feces of dogs and coyotes in an unsporulated stage (Gondim et al., 2004, Lindsay et al., 1999, McAllister et al., 1998). Oocysts sporulate outside the host in as few as 24 hours (Lindsay et al., 1999). All three infectious stages of *N. caninum* (tachyzoites,

bradyzoites, and oocysts) are involved in the transmission of the parasite *caninum* can be transmitted postnatally (horizontally, laterally) by ingestive tissues infected with tachyzoites or tissue cysts, or it can be transplacentally (vertically, congenitally) from an infected dam to her fetus during pregnancy. Recently, the terms "exogenous transplacental transmission" and "endogenous transplacental transmission" have been proposed to describe precisely the origin of the transplacental infection of the fetus (Trees et al., 2005).

Exogenous transplacental transmission occurs after a primary, oocyst-derived infection of a pregnant dam, while endogenous transplacental transmission occurs in a persistently infected dam after reactivation (recrudescence) of infection during pregnancy. The major economic loss due to neosporosis is reproductive failure in cattle in many countries. In addition to the direct costs involved in fetal loss, indirect costs include professional help and expenses associated with establishing a diagnosis, rebreeding, possible loss of milk yield, and replacement costs if aborted cows are culled. The diagnosis of neosporosis associated abortion is difficult and expensive (Dubey et al., 2006, Ortega-Moreno et al., 2006).

Seropositive cows are more likely to abort than are seronegative cows as demonstrated in a large number of studies, including retrospective and prospective cohort studies (García-Vázquez et al., 2005, Koiwai et al., 2002, Vaclavek et al., 2003, Waldner 2005, Weston et al., 2005). The strength of the association between seropositivity and abortion in a single group of animals varies considerably if different serological assays are used or if for the same assay different cutoff values are applied (Shares et al., 1999, Waldner et al., 1998). Consequently the estimates for odds ratios or relative risks may vary in relation to the serological test applied. There are a number of case control and cross-sectional studies that have observed that a high *N. caninum* seroprevalence in herds is associated with an increased risk of abortion at the herd level (Hobson et al., 2005, Shares et al., 2004). This is explained by an increased abortion risk in latently infected as well as in recently infected individual dams. However, not all herds with a high seroprevalence suffer from *N. caninum*-associated abortion (Jensen et al., 1999, Pare et al., 1998, Shrestha et al., 2004). Therefore, the aim of the present work is planned to fulfill the following:

Estimation of the seroprevalence of *Neospora caninum* infection among cattle in the eastern Province of Kingdom Saudi Arabia for the first time that provides information about the infection status to study the effect of breed in *Neospora caninum* seropositive animals, to illustrate the relation between seropositive animals and abortion and finally to know the current epidemiological status of *Neosporosis* in cattle in the eastern region of the KSA with special reference to the source of infection.

## **Materials and methods:**

### **Sampling:**

A total of 1095 sera samples were collected from different geographical regions of the eastern province of the Kingdom Saudi Arabia. Al-Dammam samples include Al-Garoudia, Al-Katif, Bou-Hedria and Masehat. Al-Hasa samples

include Al-Shakik, Al-Omran, Al-Mahdoud and Al-Aboud. The samples were categorized into 4 groups according to type of population as illustrated in table (1).

Table (1): Classification of samples according to management system

Animal Group	Description	Number of sera collected
Group 1	Dairy cattle farms	526
Group 2	Small holders	420
Group 3	Slaughterhouse	71
Group 4	Old semi-automated dairy farm	78
Total		1095

#### Detection of antibodies to *Neospora caninum*:

HerdChick is antineospora enzyme immunoassay Kit for the detection of antibody to *Neospora caninum* in bovine serum produced by DEEXX Laboratories, Drive, Westbrook, Maine, USA. The procedures were followed according to manufacture.

#### Interpretation of results:

1) Calculation of Negative control mean (NC mean)

$$A1 A (650) + A2 A (650)$$

$$NC \text{ mean} = \frac{\text{-----}}{2}$$

This means the sum of optical density of the negative control wells (A 1&2) divided by 2.

2) Calculation of Positive control mean (PC mean)

$$A3 A (650) + A4 A (650)$$

$$NC \text{ mean} = \frac{\text{-----}}{2}$$

This means the sum of optical density of the positive control wells (A3&4) divided by 2.

3) Calculation of S/P Ratio:

$$\text{Sample A (650- NC mean)}$$

$$S/P \text{ Ratio} = \frac{\text{-----}}{\text{PC mean} - \text{Nc mean}}$$

This means that the SP ratio equals the optical density of the sample minus the NC mean divided by the difference between the PC mean and the NC mean.

#### Conclusion of the results:

- 1) Serum samples with SP ratio of less than 0.50 are classified as NEGATIVE for the neospora antibodies.
- 2) Serum samples with SP ratio greater than or equal to 0.50 are classified as POSITIVE for the neospora antibodies.

#### Results and discussion

The results of the prevalence of *Neospora caninum* serum antibodies according to the source of the samples are illustrated in table (2). The overall prevalence was 3.4 % from which 4 %, 2.9 %, 2.8 %, and 2.6 % were recorded as prevalence in fully automated dairy farms (dairy industry), smallholders, abattoirs and semi-automated old dairy farm, respectively. The statistical analysis

concerning the effect of the source of the sample tested on the prevalence of *Neospora caninum* antibodies in sera of cattle is non-significant. Within the samples obtained from the dairy industry, the prevalence of neospora serum antibodies was higher in imported animals than animals born in the farm (4.3% and 2%, respectively) but the relation was not statistically significant ( $P = 0.29$ ).

A similar seroprevalence of *Neospora caninum* antibodies in sera of dairy cattle were recorded in Canada (5.6 %) by Vanleeuwen et al. (2005), in Czech Republic (3.9 %) by Vaclavek et al. (2003), in France (5.6 %) by Klein et al. (2000), in Sweden (1.3 %) by Bartels et al. (2006), in Thailand (5.5 %) by Kyaw et al. (2000) and in Vietnam (5.5) by Huong et al. (1998). A relatively higher overall seroprevalence of 13.3 % was reported in China (Wang et al., 2009); in the other hand a lowest seroprevalence of 0.7 % was reported in Norway (Klevar et al., 2010).

These results supports the seroprevalence of *Neospora caninum* antibodies in cattle in the eastern Province KSA presented in the present study as they also used an ELISA test from IDEXX or other companies. Other studies have reported a significantly higher seroprevalence. For instance a seroprevalence as high as 25.5%, 26 and 60.6 have been recorded in Canada (Vanleeuwen, et al., 2002) France (Pitel, et al., 2000) and United State (Pare et al., 1997), respectively.

The relatively low seroprevalence observed in this study compared with other countries, is not surprising. Dairy cattle in Saudi Arabia are mainly reared belong two distinctive ways: intensive dairy farming systems and smallholders (traditional) systems. The majority of dairy production is originated from the specialized intensive systems, while cattle rearing were considered as a secondary activity in the smallholders systems (Alnaeem, 2003). However, in both systems, the housing method do not permit dogs to roam in or around cattle houses which prevent the infection cycle of the *neospora caninum* to take place.

Table (2): Prevalence of *Neospora caninum* serum antibodies according to the source of the samples

Source	No. Tested	No. Positive	Prevalence
Dairy Industry	526	21	4%
Smallholder	420	12	2.9%
Abattoirs	71	2	2.8%
Semi-automated old dairy farm	78	2	2.6%
Overall	1095	37	3.4%

Table (3) illustrated the prevalence of *Neospora caninum* serum antibodies according to breed (based on subset of 640 samples with known breed). A seroprevalence of 3.7 %, 2.1 % and 0 % were recorded in Holstein, mixed and local breed of dairy cattle, respectively. No significant difference in the *Neospora* seroprevalence was seen between the Holstein and the mixed breeds ( $P = 0.4$ ). In West Africa, Kamga-Waladjo (2009) reported a significant difference in the prevalence of neosporosis among different breeds (53.3% in local breeds, 13.4% in exotic breeds and 25% in crossbreeds). There were indications from several countries that *N. caninum* seroprevalences differ according to the cattle

breed (Bartels et al., 2006). However, these results must be interpreted with caution, because the differences observed might have been caused by differences in the production systems used for the different breeds and not by differences in breed-related susceptibility to infection. For example, native Spanish breeds were less likely to be seropositive than Holstein Friesian, Rubia Gallega, or mixed breeds. This was explained by differences in the intensity of management (Bartels et al., 2006): in contrast to Holstein Friesian and Rubia Gallega cattle, which in Spain are more intensively managed, native breeds, are predominately located on highland pastures with very low stocking densities.

Table (3): Prevalence of *Neospora caninum* serum antibodies according to

Breed	No. Tested	No. Positive	Prevalence
Holstein	543	20	3.70%
Mixed	97	2	2.10%
Local	1	0	0.0%

breed (based on subset of 640 samples with known breed)

Table (5) illustrates the prevalence of *Neospora caninum* serum antibodies according to abortion history (based on subset of 544 samples with known reproduction history). The prevalence of *Neospora caninum* serum antibodies was higher in animals with abortion history (5.6% and 3.5%, respectively); but the relation was not statistically significant ( $P = 0.38$ ). It is clear that abortion may be caused by several bacterial, viral, fungal or protozoal agents. Factors having an effect on the occurrence of epidemic abortion outbreaks may completely differ from those influencing the risk of endemic abortions. Risk factor analyses often have the disadvantage that there is no information regarding the context (epidemic or endemic) in which the abortions occurred. Consequently, it is not possible to assign the risk or protective factors identified in epidemiological studies to the occurrence of epidemic or endemic abortions. Seropositive cows were more likely to abort than the seronegative cows, as demonstrated in a large number of studies, including retrospective and prospective cohort studies (García-Vázquez et al., 2005, Koiwai et al., 2005, Vaclavek et al., 2003, Waldner et al., 2005, Weston et al., 2005). The strength of the association between seropositivity and abortion in a single group of animals may vary considerably if different serological assays are used or if for the same assay different cutoffs values are applied (Shares et al., 1999, Waldner et al., 1998). Consequently the estimates for odds ratios or relative risks may vary in relation to the serological test applied. The abortion risk increases with increasing levels of *N. caninum*-specific antibodies in individual animals (Kashiwazaki et al., 2004, López-Gatius et al., 2005 and Waldner et al., 2005). Thus, it might be possible to use information on individual *N. caninum*-specific antibody levels or antibody titers (and not only seropositivity) as a predictive tool for identifying animals with a high risk of abortion in herds with high seroprevalence for *N. caninum* (Quintanilla-Gozalo et al; 2000).

Table (5): Prevalence of *Neospora caninum* serum antibodies according to abortion history (based on subset of 544 samples with known reproductive history)

History of Abortion	Number of tested	Number of Positive	Percentage of Positive
No	490	18	3.5
Yes	34	2	5.6

One of the findings is a positive case (heifer) just arrived from Australia. This finding may help to document a possible source of infection is the importation of infected cattle and the dissemination of infection may be vertical in the heifer. It can be concluded that the management system in the full-automated dairy industry as well as in smallholders did not allow the spread through dogs. In other words the life cycle of *Neospora Caninum* can not be completed to a large extent by the horizontal transmission of the parasite. The finding of the prevalence of *Neospora Caninum* antibodies in cattle in the KSA is recorded for the first time. In depth, research is needed to clarify the epidemiological and molecular situation of *Neospora caninum* infection in cattle in the KSA.

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## سيرولوجي للعدوى بطفيل النيوسبورا الكلابية في الأبقار في المنطقة الشرقية بالمملكة السعودية

بهد المنعم زغواة- فاضل بن محمد هوساوي- عبد المحسن بن عبد الله النعيم  
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بطفيل النيوسبورا الكلابية في الأبقار من الأمراض ذات الأهمية الاقتصادية حيث انها من مسببات  
ض و تؤثر علي الكفاءة التناسلية للابقار و تسبب قلة في إنتاج اللبن و قلة في معدل النمو. في هذا البحث تم  
سح سيرولوجي للعدوى بطفيل النيوسبورا الكلابية في الأبقار في المنطقة الشرقية بالمملكة العربية السعودية  
رة. تم تجميع عينات بعدد إجمالي ١٠٩٥ عينة من الدمام و شملت الجارودية و القطيف و بوحدرية و  
ت و كذلك عينات من الاحساء و شملت الشقيق و العمران و المحدود و العبود. كانت نسبة العدوي الكلية  
٣ % حيث كانت نسبة العدوي في المزارع الكبيرة ٤ % و في الحالات الفردية و المزارع الصغيرة هي  
% و في العينات التي تم تجميعها من المسالخ هي ٢,٨ % و في مزرعة قديمة نصف البية هي ٢,٦ % . عند  
نتائج مزارع الالبان الكبيرة وجد ان نسبة الحيوانات الايجابية المستوردة هي ٤,٣ % بالمقارنة بنسبة  
ات الايجابية المولودة داخل المزرعة ٢ % و لكن لا توجد فروق معنوية احصائية في نسب هذه  
فات. عند تحليل نتائج تأثير السلالة علي العدوي وجد ان النسبة الايجابية في سلالة الهولشتين ٣,٧ %  
ة بالخليط ٢,١ % و لكن لا توجد فروق معنوية احصائية في نسب هذه الاختلافات. في محاولة لوجود  
بين الحيوانات المجهضة و الايجابية للعدوي بطفيل النيوسبورا الكلابية وجد ان نسبة الحيوانات الايجابية هي  
% بالمقارنة بالنسبة في الحيوانات غير المجهضة ٣,٥ % و لكن لا توجد فروق معنوية احصائية لهذه  
ات. يسجل هذا البحث لأول مرة نسبة العدوي بطفيل النيوسبورا الكلابية في الأبقار لأول مرة بالمملكة العربية  
ية و هناك حاجة لتكملة الدراسات الوبائية و الجزيئية للوقوف علي الوضع الوبائي و تحديد مدى الجدوي من  
ة توفر برنامج وقائي للحد من الخسائر الاقتصادية للمرض.