DEDICATION

To my teachers in all fields throughout my life

&

To the soul of my father: Professor Ahmed Ali Ismail

With great

Appreciation

I dedicate this work

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ABSTRACT

Toxoplasma gondii and Neospora caninum are closely related intracellular parasites which cause reproductive failure in man and animals worldwide. The aim of this study was to determine the seroepidemiology of these parasites in dairy cows, and the co-herded camel, sheep and goats in the Sudan. Serological survey to detect antibodies against these parasites was carried out using different serological tests. The possible potential risk factors and the association between the seropositivity of these parasites and *B. abortus* infection were also analyzed using questionnaire and the available data of brucellosis from the concurrent research project. The present study is the first large scale report on serological evidence of both *T. gondii* and *N. caninum* infection associated with B. abortus infection in dairy farms from the Sudan as well as the risk factors associated with their seropositivity. The study revealed that, the overall seroprevalence of T. gondii infection at herd level of dairy animals in the State was 92.7%. The within herd seroprevalence was ranging from 8% up to 100% with mean of 51.3±24.3% in different herds of different dairy animals species in the State. The differences between the three districts, the seven localities and the four animal species were statistically highly significant (p<0.01). The overall sero-prevalence of *T. gondii* infection in dairy animals –at individual level- was 45.3%. Sheep scored the highest seroprevalence rate (75.0%) followed by goats (64.0%), camels (54.1%) and cattle (40.9%) with high statistically significant differences (p<0.01). The highest level of antibody titration (1:128) was reported in sheep and goats. The LAT and ELISA tests detected relatively similar proportion of Toxoplasma positive serum samples of dairy cows. The level of agreement between the two tests as well as the area under the ROC curve was found to be fair

and associated with the level of antibody titration recorded by LAT. The univariate analysis included region, herd type, source of fodder, source of water, neosporosis, keeping cats, stray cats, keeping both dogs and cats and presence of both stray dogs and cats as risk factors associate with positive status of T. gondii infection. However, the multivariate analysis indicated region (Omdurman, p=0.000 and Bahri, p=0.044), Animal species (sheep, p=0.006) as the significant (p<0.05) risk factors of T. gondii seropositivity.

The overall seroprevalence of *N. caninum* at herd and individual level of dairy animals in the present work was 32.2% (56/174) and 8.8% (80/906) respectively. The within herd seroprevalence was ranging from 7% up to 75% with mean of 27.2±15.4 in different herds of different dairy animals species. The highest percent inhibition (pi) recorded was 93% with mean of 38.7±12.3 and 39.3±13.9 at herd and individual level respectively. The highest prevalence rate, frequency distribution of the prevalence rate and the pi was reported in cattle at both herd and individual level followed by camel, goats and sheep respectively. Interestingly, camel was relatively similar to cattle in the occurrence of *N. caninum* infection in this study. There were no significant differences in the seroprevalence of N. caninum among the three districts, the seven localities and the four animal species (p>0.05). The univariate analysis included Production of concentrate, keeping dogs, other diseases system, source (Toxoplasmosis) as risk factors associated with cELISA positive status of *N. caninum* infection. The multivariate analysis indicated only production system (Intensive, p=0.019) and source of concentrate (Readymade, p=0.007) as the statistically significant (p<0.05) risk factors of being Neospora cELISA positive.

Mix-infection was observed in 176 heads (19.7%) out of all 895 seropositive animals and in 149 (47.9%) out of the 311 *B. abortus*

seropositive ones. Out of these 9 (6.04%) animals harbour the antibodies of the three abortifacient agents (T. gondii, N. caninum and B. abortus). Additionally 39 (48.8%) animals out of the 80 N. caninum seropositive animals have mix-infection with T. gondii. The univariate analysis showed no significant (p>0.05) association between B. abortus seropositivity and the two protozoal abortifacients. However, significant (p=0.041) association was observed between N. caninum seropositivity and T. gondii infection. Increasing odds ratios without significant (p>0.05) associations were observed in the multivariate analysis.

Different reproductive problems (abortion and repeated abortion, repeat breeding, stillbirth and neonatal mortalities were reported during interview with the owners of the investigated dairy herds. Interestingly, 58% of the interviewed farmers send their dairy animals with reproduction problems to slaughter houses. With the exception of *B*. abortus the other causes of reproduction failure were neglected. Generally, this is the first comprehensive data explaining the association between N. caninum, T. gondii, their risk factors and B. abortus seropositivity in dairy animals from the Sudan. In addition, this study documents for the first time the existence of antibody against *N. caninum* in camels, sheep and goats in the Sudan. In conclusion, the results obtained in this study confirm the wide spread nature of *T. gondii* and *N*. caninum exposure together with B. abortus among dairy animals in the Sudan. This indicates the need for further work to identify appropriate bio-security measures to prevent transmission to human and industrial animals, thus reducing the economical consequences of these abortifacient agents in the country.

المستخلص

تناولت هذه الدراسه طفيليين متشابهين هما التوكسوبلازما والنيوسبورا (and T. gondii N. caninum) ويسببان فشل الولاده في الإنسان والحيوان. وعليه صمم هذا البحث لدراسة وبائية هذين الطفيليين في أمصال أبقار مزارع الألبان والحيوانات الأخرى (إبل؛ ضأن؛ ماعز) المتواجده في هذه المزارع بإستخدام أنواع مختلفه من الإختبارات المصليه. ناقشت الدراسه أيضاً العوامل التي يمكن أن يكون لها إرتباط بحدوث الإصابه بالإضافه الى علاقتهما بوجود إصابه بالبروسيلا. الدراسه الحاليه هي الأوسع والأولى من نوعها عندما جمعت بين حدوث الإصابه بهذين الطفيليين مع الإصابه بمرض البروسيلا زائداً العوامل المؤثره على الإصابه في مزارع الألبان. بلغت مجمل نسبة الإصابه بالتوكسوبلازما في القطعان المفحوصه 92.7% بمعدلات مختلفه تراوحت من 8% الى 100% (متوسط 51.3±24.3%) في المزارع المختلفه بالولايه. سجلت الدراسه فروقات إحصائيه ذات دلاله معنويه عاليه (p<0.01) بين مناطق الولايه الثلاث ومحليات الولايه المختلفه وكذلك بين أنواع الحيوانات المختلفه. أما مجمل نسبة الإصابه بالتوكسوبلازما في جميع الحيوانات المفحوصه فبلغت 45.3% و كان الضأن الأكثر إصابةً(75.0%) بهذا الطفيل يليه الماعز (64.0%) فالإبل (54.1%) ثم الأبقار (40.9%) بإختلاف إحصائي معنوي عالى (p<0.01). كما أن معايرة الأجسام المضاده الأعلى (1:128) فسجلت في مصل الضأن والماعز. نسبة الإصابه باستخدام الاختبارين المصليين المتاحين أو الأكثر استعمالاً في البلاد (LAT and ELISA) كانت متساويه تقريباً؛ خاصةً وأن دقة وحساسية وتوافق الاختبارين كانت معقوله ولها علاقه بالتخفيفات المختلفه لاختبار التلازن (LAT). التحليل الأولى أدخل المنطقه؛ نوع القطيع؛ مصادر الأعلاف والماء؛ وجود القطط والكلاب؛ والإصابه بالنيوسبورا كعوامل مرتبطه بالإصابه بالتوكسوبلازما (p<0.05). أما التحليل اللوجستى فقد أثنى على إختلاف المنطقه ونوع الحيوان كمؤثرات مرتبطه بالإصابه وبدرجه معنويه (p<0.05) إحصائياً.

أما مجمل نسبة الإصابه بالنيوسبورا في القطعان المفحوصه فبلغت 32.2% و في جميع الحيوانات 8.8% بمعدلات مختلفه تراوحت من 7% الى 75% (متوسط % 5527.2) في المزارع المختلفه بالولايه. أعلى نسبة تثبيط (pi) في الإختبار

المناعي المرتبط بالانزيم التنافسي (cELISA) كانت 93% بمتوسط 39.3 \pm 13.9 في الحيوانات المختلفه. الأبقار هي الأكثر إصابةً و الأكثر قابليةً للإصابة بالنيوسبورا يليها الإبل ثم الماعز فالضأن. ليس هناك إختلافات إحصائيه معنويه (p>0.05) بين مناطق الولايه الثلاث ومحليات الولايه و أنواع الحيوانات المختلفه. التحليل الأولي أدخل نمط الرعايه؛ مصادر المركزات؛ وجود الكلاب؛ والإصابه بالتوكسوبلازما كعوامل مرتبطه بالإصابه بالنيوسبورا (p<0.05). أما التحليل اللوجستي فقد أثنى على النمط المكثف والمركزات الجاهزه كمؤثرات معنويه (p<0.05) إحصائياً.

من الملاحظ إزدواجية الإصابه بواقع 19.7%؛ كما أن 48.8% من الحيوانات المصابه بالنيوسبورا تحمل أيضاً أجسام مضاده للتوكسوبلازما و 47.9% من الحيوانات المصابه بالبروسيلا تحمل أيضاً أجسام مضاده لأحد أو لهذين الطفيليين. الجدير بالذكر أن 6.04% من الحيوانات تحمل أجسام مضاده لمسببات الإجهاض الثلاث (توكسوبلازما؛ نيوسبورا؛ وبروسيلا). لم يظهر التحليل أي إرتباط إحصائي معنوي (p>0.05) بين الإصابه بالطفيليين ووجود الإصابه بالبروسيلا. إلا أنه ظهر إرتباط إحصائي معنوي (p>0.05) بين الإصابه بالنيوسبورا ووجود الإصابه بالتوكسوبلازما.

خلال المقابلات (الإستبيان) تلاحظ كثرة مشاكل الخصوبه والإجهاض وتكرار الإجهاض بالإضافه الى موت المواليد في مزارع الألبان. أكثر من نصف الأبقار الفاشله في الإنتاج والولاده أو متدنية الخصوبه (58%) يتم بيعها للجزارين (السلخانه). أغلبية المربين يجهلون مسببات الإجهاض؛ والقليل منهم ذكر البروسيلا كمسبب للإجهاض. عموماً فإن هذه الدراسه هي الأولى والأوسع التي تشرح العلاقه بين طفيلي التوكسوبلازما والنيوسبورا والبروسيلا ؛ والعوامل المرتبطه بحدوثهما. كما أنها الشاهد الأول لوجود الأجسام المضاده لطفيل النيوسبورا في الإبل والماعز والضأن بالسودان.

هذا وقد خلصت هذه الدراسه إلى أن تعرض حيوانات المزرعه لطفيلي التوكسوبلازما والنيوسبورا كثير الإنتشار وبنفس مستوى إنتشار البروسيلا في مزارع الألبان. وعليه فإن الأمر يتطلب إجراء مزيد من الدراسات في مجال السلامه الحيويه لوقاية الإنسان والحيوان مما يقلل من الأثار الصحيه والإقتصاديه لمسببات الإجهاض في إنسان وحيوان السودان.

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