Immunodetection of *Ostertagia ostertagi* in Cattle Milk Samples in Wasit Province

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Abstract

The aim of present study was to detect the seroprevalence of *Ostertagia ostertagi* specific antibodies in cattle milk samples in Wasit province, by using the indirect ELISA test for first time in Iraq. For this purpose, an overall 368 dairies cow was submitted for study and the results were revealed that 51 (13.86 %) of tested cows were positive, and the mean optic density ratios (ODRs) of ELISA test values in seropositive cattle were 0.58. Also, this study aimed to investigate an association of seropositive results with some epidemiological risk factors. Hence, the positive results, according to these factors, were as follow: in milk production factor, 6.32 % for ≥18 liters/day group, 14.29 % for ≥10-18 liters/day group, and 25 % for < 10 liters/day group; in age factor, 13.41% for ≥3-6 years group, and 14.75% for >6 years group; in breed factor, 18.27% for local breed group, 12.17% for cross-breed group, and 12% for pure breed group; in farm management factor, 21.35% for bad management group, and 5.68% for good management group; and in herd size factor, 11.59% for <25 (cow/herd) group, and 17.78% for ≥ 25 (cow/herd) group.

Statistically, the significant differences (P ≤ 0.05) were observed among related groups of milk production, breed, husbandry management, and herd size factors; while it’s not reported among groups of age factor.

**Keywords:** *Ostertagia ostertagi*, Immunodetection, Cattle, Milk samples, Wasit province
تشخيص المناعي في عينات حليب أبقار محافظة Ostertagia ostertagi
واسط

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الخلاصة

هدفت الدراسة الحالية إلى تحديد مدى الانتتشار المصلي للإلكام المضادة الخاصة في عينات حليب الأبقار في محافظة واسط في العراق. بالإضافة إلى ذلك، خضعت هذه الدراسة بالاجمال 863 بقرة مدرة لمحميب، واظهرت النتائج بأن 51% (13.86%) من الإبقار المفحصة كانت موجبة وكان معدل نسبة الكثافة البصرية (ODRs) لقيم اختبار *(Ostertagia* ostertagi في الإبقار الموجبة 0.3.1. كما هدفت هذه الدراسة إلى كشف العلاقة بين الإبقار الموجبة، وبعض عوامل الخطر الوبائية، هدفت هذه الدراسة إلى Directory اسماء عوامل الخطر، كالتالي: في عامل إنتاج الحليب، 6.86% لمجموعة ≤ 63 لتر/يوم، و62.61% لمجموعة 60 – 63 لتر/يوم، و6.86% لمجموعة > 63 لتر/يوم. في عامل العمر، 68.26% لمجموعة ≤ 6 سنوات، و62.4. في عامل السلالة، 63.64% لمجموعة السلالة المحمية، و66.64% لمجموعة السلالة الهجينة، و66% لمجموعة السلالة النقية. في عامل إدارة القطيع، 66.8% لمجموعة الإدارة السيئة، و63% لمجموعة الإدارة الجيدة. في عامل حجم القطيع، 66.1% لمجموعة > 6(بقرة/قطيع)، و64.43% لمجموعات ≤ 6(بقرة/قطيع).

الكمات المفتاحية: *Ostertagia ostertagi*، تصخيص مناعي، إبقار، عينات حليب، محافظة واسط

احصائياً، شهدت الاختلافات المعنوية عند مستوى P<0.05 بين المجاميع التابعة لوعائل انتاج الحليب، السلالات، إدارة الحقل، حجم القطيع، في حين لم يتم تسجيلها بين مجموعتي عامل العمر.

الكلمات المفتاحية: *Ostertagia ostertagi*, تشخيص المناعي، أبقار، عينات حليب، محافظة واسط
Introduction

*Ostertagia ostertagi* is one of the main important gastrointestinal roundworm nematodes of cattle livestock and other domestic animals, around the world, to resulting in an ostertagiasis disease (1). *O. ostertagi* is counted to be a high damaging internal parasite that has a direct life cycle with two different phases, the first as free-living phase on pasture and the second as a host-parasitism in the abomasum (2). As well as, the parasite is considered as a predominant cause for parasite-induced production losses in cattle throughout tropical and semi-tropical areas of the world (3). Although, these losses are most commonly seen in young cattle; the subclinical infections and some occasional outbreaks of disease can be contributed with total cost production in older cattle (4).

However, an indirect enzyme-linked immunosorbent assay (iELISA) test is applied for detection of specific antibodies against *O. ostertagi* in serum and milk samples by using of a crude adult worm as an antigen (5). In most milk immunoassays, this technique is used to detect the levels of parasitism when a suitable immunodominant proteinaceous antigen has persisted, this antigen can be used to capture of the parasitic specific-antibodies (6). Hence, indirect ELISA was developed and used for measuring of specific *O. ostertagi* antibodies. Also, the normalized results of this assay, referred to it as an optical density ratio (ODR), are quantify the levels of infestation in cows and the relationship between milk production and ODR values (7). The levels of milk antibody against ostertagiasis are considered to be promising parameter to identify the losses in production due to gastrointestinal nematodes, especially, in dairy cows (8).

The main objective of this study was to detect the seropositive prevalence of *O. ostertagi* IgG-antibodies in cattle of Wasit province / Iraq, by using an iELISA test. As well as, it’s aimed to study the association between seropositive cattle and some epidemiological related factors included milk production, age, breed, husbandry management, and grazing system.
Materials and Methods

Study’s area and Samples

From some herds and fields located in Wasit province / Iraq, a totally of 368 adult dairy cows were submitted for this study during the period from October / 2016 to January / 2017. About 25 ml of milk sample was collected from each cow into a free-preservative plastic container, transported to the laboratory, and centrifuged at 4000 rpm for 15 minute. The milk fat was skimmed off and the supernatant collected and centrifuged again under the same conditions to ensure that all milk fat was removed as recommended by the test manufacturer, and the supernatant kept in 1 ml eppendorfs and stored at -20°C until tested (9). Also, the required data about some epidemiological risk factors (milk production, age, breed, farm management, and herd size) were received from the owners, and each factor was subdivided into groups. Hence, three groups were persist in milk production factor included {<10, 10-18, and ≥ 18 liters / day}, two groups in age factor {≥ 3-6, and > 6 years}, three groups in breed factor {local, cross-breed, and pure (Friesian-Holstein) breeds}, two groups in farm management factor {bad and good managements}, and two groups for herd size factor {< 25 and ≥ 25 cows / herd}.

Immunological Test

The study’s test kit was an indirect enzyme-linked immunosorbent assay (iELISA) (SVANOVIR® / Sweden), which developed to detect O. ostertagi specific IgG-antibodies in milk samples. The kit’s method is based on an indirect solid-phase ELISA, and the milk samples were exposed to the non-infectious antigens of O. ostertagi in the wells of the microtiter bar. The positive results were indicated by developing of a blue-green color due to conversion of substrate solution by the conjugate. The reaction was stopped by adding of stop-solution, and the results were read by using a microplate spectrophotometer and the optical density (OD) was measured at 405 nm.

Data Analysis

All received data were tabled and analyzed by application of a computerized Microsoft office Excel (2013) and an IBM/SPSS (v.23) programs. Also, the seropositive results were compared between the groups related to each one of
Results

In (Table 2): An overall 368 adult dairy cattle were submitted for an immunological indirect ELISA test to detect an *O. ostertagi* IgG-antibodies in cows milk samples. The results revealed that 51 (13.86 %) of tested cattle were seropositives to IgG antibodies, while, 317 (86.14 %) were seronegatives.

<table>
<thead>
<tr>
<th>Immunological Test</th>
<th>Total No.</th>
<th>Seropositive Results</th>
<th>Seronegative Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect ELISA</td>
<td>368</td>
<td>51</td>
<td>317</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.86 %</td>
<td>86.14 %</td>
</tr>
</tbody>
</table>

In (Table 3): An association of *O. ostertagi* seropositive results with some epidemiological risk factors was discussed in figures (1, 2, 3, 4, and 5).

<table>
<thead>
<tr>
<th>Epidemiological Factors</th>
<th>Groups</th>
<th>No.</th>
<th>Seropositive Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Milk Production</td>
<td>≥ 18</td>
<td>95</td>
<td>6 (6.32 %)</td>
</tr>
<tr>
<td></td>
<td>≥ 10 -18</td>
<td>217</td>
<td>31 (14.29 %)</td>
</tr>
<tr>
<td></td>
<td>&lt; 10</td>
<td>56</td>
<td>14 (25 %)</td>
</tr>
<tr>
<td>2 Age</td>
<td>≥ 3-6</td>
<td>246</td>
<td>33 (13.41 %)</td>
</tr>
<tr>
<td></td>
<td>&gt; 6</td>
<td>122</td>
<td>18 (14.75 %)</td>
</tr>
<tr>
<td>3 Breed</td>
<td>Local</td>
<td>104</td>
<td>19 (18.27 %)</td>
</tr>
<tr>
<td></td>
<td>Cross-Breed</td>
<td>189</td>
<td>23 (12.17 %)</td>
</tr>
<tr>
<td></td>
<td>Pure</td>
<td>75</td>
<td>9 (12 %)</td>
</tr>
<tr>
<td>4 Farm Management</td>
<td>Bad</td>
<td>192</td>
<td>41 (21.35 %)</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>176</td>
<td>10 (5.68 %)</td>
</tr>
<tr>
<td>5 Herd Size</td>
<td>&lt; 25</td>
<td>233</td>
<td>27 (11.59 %)</td>
</tr>
<tr>
<td></td>
<td>≥ 25</td>
<td>135</td>
<td>24 (17.78 %)</td>
</tr>
</tbody>
</table>

Differences in small letters, vertically, between groups of each factor referred to significant differences at \( P \leq 0.05 \)
In (Figure 1): The milk production factor had been subdivided into three groups that included (< 10, ≥10-18, and ≥ 18 liters/day), and the seropositive results of these groups were 6/95 (6.32 %), 31/217 (14.29 %), and 14/56 (25 %), respectively.

In (Figure 2): In both age factor groups {≥ (3-6) and > 6 years}, the study showed that the seropositive results were 33/246 (13.41 %) and 18/122 (14.75 %), respectively.
In (Figure 3): The seropositive results in related-groups of breed factor were 19/104 (18.27 %) for local breed, 23/189 (12.17 %) for cross-breed, and 9/75 (12 %) for pure Friesian-Holstein breed.

In (Figure 4): In accordance to bad and good groups of farm management, this study had been reported 41/192 (21.35 %) and 10/176 (5.68 %), as positive results for these group.
In (Figure 5) that dealt with herd size factor, the seropositive results that reported for both, < 25 and ≥ 25 cows per herd, groups were 27/233 (11.59 %) and 24/135 (17.78 %), respectively.

**Discussion**

*Ostertagia ostertagi* is an economically important cattle’s parasite to which acquired resistance is believed to be an immune-mediated. In comparison to other common bovine parasites, several previous studies reported the occurrence of accepted levels of immunity to other helminthes \(^{(11, 12)}\). In the present study, 13.86 % was the result of an overall seropositive prevalence of *O. ostertagi* in cattle of Wasit province /Iraq. Also, the mean ODRs of ELISA values were moderately low-level (mean ODR=0.58), and this results was similar, statistically, with those reported in Spain (0.56) by \(^{(9)}\), Sweden (0.52) by \(^{(13)}\), and Poland (0.53) by \(^{(14)}\). Whereas, the mean ODRs was higher than those reported Italy (0.31) and Netherlands (0.45) by \(^{(15)}\); it’s lower than the results detected in Ireland (0.80), United Kingdom (0.82) and Belgium (0.83) \(^{(13)}\). Variation in mean ODRs between previous studies could be attributed to the differences of management/production system, environmental and local factors, parasite burden of a pasture, and the factors that interact with the level of parasite exposure \(^{(9)}\). The recent advances in detection of subclinical infestation’s levels have been, mainly, obtained by several studies worldwide by recognition of the anti-*Ostertagia ostertagi* antibody level in milk samples, which quantified by using an ELISA test, and identification of animals or herd /fields where the infection induces a loss in milk.
production (16, 17). The test development begins with the identification of parasite specific antigens that elicit a strong immune response in the host (18). The major advantage in application of this milk-dependence test was that it’s a high safety, acceptable cost, highly in sensitivity and susceptibility to estimate the average of cattle exposure to parasite, time preserved test. Also, the results of ELISA was very accurate and might be influenced, slightly, by the storage method, length of storage, and process of milk-de-fattening, and the differences was minimal and had little effect on the interpretation of results (6,7). Although, the significant negative impact on milk production, treatment responses to anthelmintics can vary amongst different studies and herds (15). In fact, the possible explanation in responses variability to anthelmintic treatment was that not only do individual cows in a herd carry worm burdens of different magnitudes, but also that dairy herds could be grazing pastures with different levels of exposure to infective larvae of O. ostertagi (19). However, the distribution of different ODR values in individual cows, disregarding on low or high milk production, might provide additional insights on the Ostertagia status in a tested herd (20). Hence, this could be used during preference an appropriate treatment program, and limiting of drug-resistance due to a random anthelmintics usage (21).

In accordance to epidemiological risk factors, the most consistent associations were be found, in this study, between the groups of milk production, farm management, herd size, and to less extent in breed factors, while the negative association was found between groups of age factor. In related to higher milk production with lower seropositivity, the dairy infested cattle with Ostertagia tend to reduce their annual rates of milk production in about (0.9-1.2) kg/cow daily. In addition, this factor was related to grazing management, especially, the length of time and grazing season (22). Although, the seropositive prevalence of ostertagiasis was distributed in both groups of age factor in examined animal, it’s appeared that the genetic background diversity and the degree of infectious exposure were play an important role as factors in terms to the intensity of protective immunity (11). In pertained to breed factor, the significant high increasing in seropositivity was detected, only, in local dairy breed more than cross-breed and pure Holstein-Frisian breed, and this might belonging to that local breed was produced lower levels of milk or to the little attention concerning with feeding and anthelmintics medication (23). The significant association to farm management was reported by this study. The cattle under grazing production systems were considered to be at high risk for suffering from negative impact of ostertagiasis than cattle from none or less-pastured herd;
and this correlation suggested that the suitable pasture management could increase the milk production and hence reduce the infection levels (24, 25). Whereas, in herd size factor, the study showed that the increasing of seropositive prevalence and levels of antibody (rising in mean ODR value) was accompanied by a significant decrease in number of dairy cows in each herd, and this could contributed to the fact that the larger and more specialized production herds have high attention, high concentrated nutrition, continuous medication and more parasite control (26, 27).

In conclusion, this study carried out for first time in Iraq, and reported an efficacy of an indirect ELISA in detection of specific Ostertagia ostertagi IgG-antibody in milk samples of cattle. Also, the study showed an association of seropositive results with some selected epidemiological risk factors (milk production, age, breed, husbandry management, and herd size).

References


