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Research article

Seroprevalence of bluetongue virus IgG-antibodies in cattle in some Iraqi Provinces, using of a competitive-ELISA

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Abstract

This study was performed to detect seroprevalence of BTV IgG-antibodies in apparently healthy cattle in four provinces in Iraq. An overall 468 serum samples were collected during a period of April / 2016 to May / 2017 and examined by using a competitive-ELISA. The total results revealed that 28.42% of cattle were seropositive for BTV infections. Highest seroprevalence was reported in provinces of Dhi-Qar (35.04%) and Al-Qadisiyah (30.77%), while, the lowest were in Babylon (23.08%) and Wasit (24.79%). Among breed, no statistical differences (P≤0.05) were detected between pure-breed (33.8%) and cross-breed (32.67%), which appeared with high rate for seroprevalence compared to indigenous-breed (18.49%), P>0.05. In addition, a significant increase in a prevalence of BTV-antibodies was showed in a cattle group of (>5) years (55.17%), followed by (>3-5) years (40.38%), and (1-3) years (7.61%) groups, (P>0.05). Regarding to gender, females were testified, relatively, an exposure rate for BTV (30.03%) more than males (23.48%), (P>0.05).

Keywords: Bluetongue virus, Cattle, ELISA, IgG, Prevalence, Iraq.

Introduction

Bluetongue virus (BTV) is a double stranded RNA *Orbivirus* within the family of *Reoviridae*, which infecting mainly sheep and goats as well as cattle, buffalo, camel and deer; resulting in a bluetongue (BT) disease (1, 2). A total of 27 distinct serotypes which isolated, identified, and distributed differently in all continents, worldwide, except Antarctica (3, 4). BTV is carried with an adult female for several types of biting fly particularly, *Culicoides immicola* that, actually, a main vectors in Africa Asia and Europe (5). In addition, it's shown that BTV can be transmitted by semen experimentally and naturally (6); as well as transplacentally (7). However, the disease is non-contagious occurs mostly in warmer climates during rainfall and increasing temperature, and vanish at beginning of severe chilling weathers (8). Sheep, goats and, for less degree, some wild animals can appear different signs ranged from sub-clinically, mildly, to acutely or even fatally infection; whereas, in endemic countries, cattle may develop clinical signs (9). Many indirect economic losses related to decreasing in body's weights or conditions, dropping for milk yields, and reproductive problems, which appeared with high effects than overt diseases (10). As well as, there are restrictions in movement of animals or their trade, which afflicted endemic countries as Iraq (11, 12). Usually, sera were testing for presence of BTV

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antibody to facilitate the safe international trade, serologic surveillance, and for serotype identification of field strains (13). Although, complement fixation test, fluorescent antibody, hemagglutination inhibition test, neutralization test, and indirect enzyme linked immunosorbent assay (ELISA) that employed toward BTV antibody; the OIE manual preferred recently for competitive-ELISA (14, 15). The last assay is rapid, reliable, automated, and ideally confirm an exposures for singular serotype with determining the source of transmission and spread of virus in absence of clinical signs (16). In addition, the test is based on a major, structural viral protein (*VP7*) as a highly conserved antigen that directed for detection of specific antibodies of all 27 BTV serotypes without cross-reaction with other *Orbivirus* serogroups (17). In Iraq, competitive-ELISA was applied for detection persistence the BTV-antibodies in sheep and goats but not in cattle (12). Hence, the present study was aimed for revealing a seroprevalence of BTV in apparently healthy cattle in four Iraqi provinces by using a monoclonal competitive-ELISA, for first time in Iraq (*VP7*). In addition, the association of seropositive results with some epidemiological factors (residence, breed, age and gender) discussed in this study.

Material and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 292

Study samples

In different local areas related to 4 Iraqi provinces (Al-Qadisiyah, Babylon, Dhi-Qar, and Wasit) and during a period of April / 2016 to May / 2017; a total of 468 apparently healthy cattle, from both gender with more than 1 year, were selected randomly and submitted for this study. From each animal, about 6 ml of blood samples were drew from jugular vein by using free-anticoagulant vacutainer tubes (*AFMA, Jordan*) that transported within a cold ice keeper to the laboratory and centrifuged, directly, at 4000rpm for 15 minutes to obtain sera. Sera samples were saved into numbered 1mL eppendorf microtubes (*China*) and kept at -20°C until be used. In addition, all data about epidemiological factors (locality, breed, age, and gender) of study's animals were collected or owners questioned, and documented.

Competitive-ELISA

Serological diagnosis of specific antibodies to the viral protein of the bluetongue virus (VP7) in cattle sera was conducted with competitive-ELISA (*IDEXX Montpellier, Institut Pourquier, France*). Steps of preparation and testing of sera and controls were performed according to manufacturer instruction. Results were read at 450nm of optical density (OD) by a microplate photometer reader (*BioTek, USA*), and submitted to the following formula:

$S / N \% = (OD_{\text{Serum Sample}} / OD_{\text{Negative Control}}) \times 100$.

In addition, the reaction results of control and serum samples were validated and interpreted as show in Table (1).

Table (1): Validation criteria and interpretation of study's results

Validation Criteria	Mean OD	Negative control	0.700 – 3.00
	S / N %	Positive Control	< 20 %
Samples Interpretation	S / N %	Negative result	≥ 80%
		Doubtful result	70% - 80%
		Positive result	≤ 70%

Statistical analysis

All obtained data and results were introduced, tabled and analyzed using of two computerized programs, Microsoft Office

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Excel (2007) and IBM/SPSS (V_{23}). Descriptive statistics and Chi-square (x^2) were applied to detect the significant differences between BTV seropositive and seronegative, and the associations of seropositivity within epidemiological risk factors, at a level of P≤0.05 (18).

Results

Of 468 sera samples examined by competitive-ELISA, 133 (28.42%) of cattle were totally seropositive with specific IgG-antibodies against bluetongue virus, (Table 2).

Table (2): Total seropositive prevalence of BTV-IgG antibody

Test	Total No.	Seropositive	Seronegative
Competitive-ELISA	468	133 (28.42%)	335 (71.58%)

Regarding to locality factor, the results of testing 117 cattle selected randomly from each province were 36 (30.77%), 27 (23.08%), 41 (35.04%), and 29 (24.79%) seropositive cattle in Al-Qadisiyah, Babylon, Dhi-Qar, and Wasit provinces, respectively, (Table 3).

Table (3): Distribution of BTV-IgG antibodies among locality factor

Locality		Total No.	Seropositive	Seronegative
1	Al-Qadisiyah	117	36 (30.77%) B	81 (69.23%)
2	Babylon	117	27 (23.08%) C	90 (76.92%)
3	Dhi-Qar	117	41 (35.04%) A	76 (64.96%)
4	Wasit	117	29 (24.79%) C	88 (75.21%)
Total		468	133	335

Variation in vertical large letters in seropositive refers to significant difference (P≤0.05)

Among groups of breed factor, the study comprised of 146 indigenous, 251 cross, and 71 pure (Holstein Friesian) breeds cattle; and the results showed that 37 (25.34%), 82 (32.67%), and 14 (19.72%) of cattle were seropositive in these groups, respectively, (Table 4).

Table (4): Distribution of BTV-IgG antibodies among breed factor

Breed		Total No.	Seropositive	Seronegative
1	Indigenous	146	27 (18.49%) B	119 (81.51%)
2	Cross	251	82 (32.67%) A	169 (67.33%)
3	Pure	71	24 (33.8%) A	47 (66.2%)
Total		468	133	335

Variation in vertical large letters in seropositive refers to significant difference (P≤0.05)

In depending on their ages, study's cattle were divided into three age groups involved 197 cattle in a group of (1-3) years, 213 cattle in a group of (> 3-5) years, and 58 cattle in a group of (>5) years. The prevalence of specific bluetongue virus-antibodies was 15 (7.61%), 86 (40.38%), and 32 (55.17%) seropositive cattle among age groups, respectively, (Table5).

Table (5): Distribution of BTV-IgG antibodies among age factor

Age		Total No.	Seropositive	Seronegative
1	1-3 years	197	15 (7.61%) C	182 (92.39%)
2	> 3-5 years	213	86 (40.38%) B	127 (59.62%)
3	> 5 years	58	32 (55.17%) A	26 (44.83%)
Total		468	133	335

Variation in vertical large letters in seropositive refers to significant difference (P≤0.05)

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Over 353 females and 115 males cattle tested in this study, the results were revealed on 106 (30.03%) and 27 (23.48%) seropositive animals, respectively, (Table 6).

Table (6): Distribution of BTV-IgG antibodies among gender factor

Gender		Total No.	Seropositive	Seronegative
1	Female	353	106 (30.03%) A	247 (69.97%)
2	Male	115	27 (23.48%) B	88 (76.52%)
Total		468	133	335

Variation in vertical large letters in seropositive refers to significant difference (P≤0.05)

Discussion

In Iraq, a few recent numbers of studies have been dealt with an incidence of blue tongue virus in both ovine and caprine animals, while in cattle, it is poorly defined and lack for any data. This study reported, firstly, that the total seroprevalence of specific BTV-antibodies in cattle was 28.42%, (Table 1). Worldwide, the prevalence rate of cattle BTV-antibodies were 10.7% in Egypt (19), 44.8% in Saudi Arabia (20), 67% in Sudan (21), 88% in Turkey (22), 94.7% in Venezuela (23), 21-40% in Argentina and 4-89% in Brazil (24). In last decades, the worldwide allocation and quality of BTV infections have significant variation due to the climate changes that implicated as a potentially reasons for these dramatic events (25, 26). In Iraq, there is a chance for domestic and wild animals to be infected with BTV during the movement to other areas or crossing the borders of neighboring countries. BTV becomes of great interest for diagnosticians, managers, or to dairy producers due to outbreak reoccurrence among ruminant animals in geographically areas identified in past as devoid of virus (7, 27). In addition, changes in climate through the recent years might be linked for expansion and increasing an incidence in areas considered, previously, as with low or free of disease such in Europe (10). Cattle were detected to be played very great part as a field reservoir for BTV that transferred by insect vectors to more susceptible animals, ovine and caprine (28). However, in endemic areas as Iraq, the clinically pronounced forms of infection have not been detected in naturally diseased cattle (21, 29). Competitive-ELISA, used in this study, is based on using of a viral protein 7 (*VP7*) as antigen coated a plate with 100% sensitivity and 98% specificity (29). *VP7* is highly conserved hydrophilic protein play an important role in the structural integrity of BTV core, and express group-specific antigenic determinants defining several distinct phylogenetic groups (30). As a core, *VP7* is non-infectious in different mammalian cells but it's at least 100 fold more infectious for adult *Culicoides* midges or their cell lines (17). In vitro, though the immune effectors of *VP7* is immunodominant during antibody production, but the antibodies are not neutralizing and probably not protective (31). In regarding to residence factor, seropositivity of BTV-antibodies was distributed in localities as 30.77% in Al-Qadisiyah, 23.08% in Babylon, 35.04% in Dhi-Qar, and 24.79% in Wasit, (Table3). This difference in prevalence BTV-antibodies might be attributed to development of irrigation projects and petroleum industries that concerned with liberation large quantities of water onto surroundings, presence of suitable habitats such as marshland and leftovers, and humidity that act as sources for breeding of *Culicoides* vectors in these regions (21). Whereas, the low seroprevalence of BTV-antibodies might reflect that either the vector does not bite cattle or that cattle only rarely come into contact with infected vectors (29). Although the clinical incidence of BTV in cattle is usually low, seropositive prevalence could vary with the strain of virus and breed (32). In this study, indigenous, cross, and pure

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breeds appeared with 18.49%, 32.67%, and 33.8% respective seropositive prevalence for BTV-antibodies, (Table 4). In endemic BTV areas, the susceptibility of cattle to BTV can be dependent on breed as the indigenous breed show natural resistance for infection in contrast to cross and pure breeds (33). As well as, the high seropositivity of cross and pure breeds to BTV in comparison to indigenous breed could be ascribed to genetic diversity, low resistance to infections, high stress as a result of high milk production (32, 34). Significant relationship (P>0.05) has been showed between the BTV-seropositive with age of study's animals. The seroprevalence of BTV-antibodies was 7.16%, 40.38%, and 55.17% in (1-3) years, (>3-5) years, and (>5) years age groups, respectively, (Table 5). It should be noted that all cattle, included in this study, were unvaccinated due absence of BTV-vaccination schedule in Iraq, as well as, they aged >1 year. As detected previously, maternal immunity might persist for 6 months to 1 year of age, hence, animals with positive-antibodies will indicated a natural infection with BTV (35). This gradual increasing in prevalence seropositivity with advancing of age might be concerned to the frequently expositing of old cows to *Culicoides* vector. As reported by (36), young calves in most cases were grown indoors and have more cares by its owner. However, results of this study were in compatible with previously studies detected that the older cattle were at high risk for BTV-infection (21, 37). In this study, epidemiological gender factor has a significant association (P>0.05) with BTV infection among females and males that reported, respectively, 30.03% and 23.48% of BTV-seropositivity (Table 6). This result was in contrast with those detected by (38) who reported that there were no significant differences between females and males of examined cattle, and (39) who observed that bulls have a greater risk for infection than females or castrated males. However, the results of this study could be attributed either to the fact that bulls receive a high care in comparison to females; females exposed to high stress as a result of gestation, parturition and milk production; or to low samples of examined males (40, 41). In conclusion, this study indicated a high seroprevalence of BTV-antibodies in apparently healthy cattle that may act as reservoirs and could play an effecter role for transmission of BTV to other field animals. Significant associations were showed, also, in seropositivity within related groups of study's areas, breeds, ages, and genders. However, further BTV-surveillance programs must be expanded to involve other areas in Iraq.

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