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Paper

Serosurvey of Bluetongue virus in small ruminants in Egypt and its associated risk factors

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Abstract

Bluetongue is an emerging, non-contagious, vector-borne disease that affects both domestic and wild ruminants. This study aimed to determine the seroprevalence of Bluetongue virus (BTV) in four Egyptian governorates and to evaluate the associated risk factors. A total of 740 serum samples were collected from 380 sheep and 360 goats and tested using a commercial competitive ELISA (cELISA). The overall BTV seroprevalence was 16.2%, with 17.1% in sheep and 15.3% in goats. Although the seroprevalence did not differ significantly across the studied regions, the highest prevalence was recorded in Kafr El-Sheikh (20.7%). Univariable analysis revealed a significant association between BTV seropositivity and several factors, including sex, age, presence of vectors, history of abortion, and contact with cattle. According to the multivariable logistic regression model, females, animals older than 2 years, and those with a history of abortion were respectively 2.3, 2.6, and 1.6 times more likely to be seropositive. Furthermore, the presence of insect vectors and close contact with cattle increased the risk of BTV infection by 1.6 and 2.1 times, respectively. This study highlights the significant risk factors associated with BTV seropositivity, with a slightly higher prevalence observed in sheep compared to goats. These findings underscore the need for effective disease surveillance, management, and control strategies targeting both sheep and goat populations.

Keywords

Bluetongue, cELISA, serosurvey, risk factors, sheep, goats, Egypt

Introduction

Bluetongue (BT) is a non-contagious, vector-borne disease transmitted by *Culicoides* spp., affecting both domestic and wild ruminants (Kramer et al., 1985; Radwan et al., 2022). The causative agent, Bluetongue virus (BTV), belongs to the genus *Orbivirus* within the family *Sedoreoviridae* (formerly *Reoviridae*) (Worwa et al., 2010). The viral genome consists of ten segments of double-stranded RNA (dsRNA), seven of which encode structural proteins (VP1–VP7), while the remaining segments encode five non-structural proteins (NS1, NS2, NS3/3a, NS4, and NS5) (Maan et al., 2007).

BT is endemic in Africa, the Middle East (including Egypt and Israel), and parts of Asia (Tabachnick, 2010; Maclachlan, 2011). The World Organization for Animal Health (WOAH) currently recognizes 27 notifiable serotypes of BTV. Including atypical strains, a total of 36 serotypes (BTV-1 to BTV-36) have been identified to date (Maan et al., 2007; Maan et al., 2011; Caixeta et al., 2024).

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BTV infection can be particularly severe in small ruminants, with mortality rates reaching up to 70%, depending on factors such as host susceptibility, environmental conditions, and viral strain (Malik et al., 2018; Singh et al., 2021). Clinical manifestations in sheep and goats occur in three forms: acute, chronic, and subclinical. The acute form is characterized by viremia, facial edema, ulceration and hemorrhages in the oral mucosa, cyanosis of the tongue, excessive salivation, and coronitis (Backx et al., 2007; Khezri and Azimi, 2013a; Katsoulos et al., 2016; Ahmad et al., 2024). BT can result in both direct and indirect economic losses. Direct losses include reduced productivity (e.g., abortion, decreased milk and meat production, and mortality), vaccination expenses, and disease control costs. Indirect losses are primarily associated with trade restrictions that limit market access (Rushton and Lyons, 2015; Gethmann et al., 2020).

Several serological techniques are used for BTV diagnosis, including agar gel immunodiffusion (AGID), competitive ELISA (c-ELISA), indirect ELISA, antigen capture ELISA (ac-ELISA), immunofluorescence (IF), virus neutralization (VN), and immunoperoxidase (IP) tests. Among these, c-ELISA is highly specific (99.6%) and more sensitive than other ELISA formats due to the use of monoclonal antibodies, which minimize cross-reactivity (Rojas et al., 2019). The OIE Manual of Diagnostic Tests and Vaccines recommends AGID and c-ELISA, highlighting the latter as a rapid and reliable method capable of detecting antibodies as early as six days post-infection (Breard et al., 2004).

The first report of BTV in Egypt was in Merino sheep (Hafez and Ozawa, 1973), followed by the identification of multiple serotypes (1–4, 10, 12, and 16) (Ismail et al., 1987). More recently, BTV has been serologically detected in cattle (Selim et al., 2023), camels (Selim et al., 2022), and small ruminants (Mahmoud et al., 2017). Currently, there is no vaccination program for small ruminants against BTV in Egypt. Therefore, continuous monitoring and updating of the disease's epidemiological status are essential, particularly in regions with high animal density.

The aim of this study was to estimate the seroprevalence of BTV and to identify potential risk factors associated with BTV seropositivity in small ruminants across four Egyptian governorates.

Materials and Methods

Ethical statement

The study protocol was conducted in accordance with ethical standards and was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Benha University, Egypt. Prior to sample collection, informed consent was obtained from all animal owners after explaining the objectives of the study and ensuring they would be informed of the test results. The study was conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines

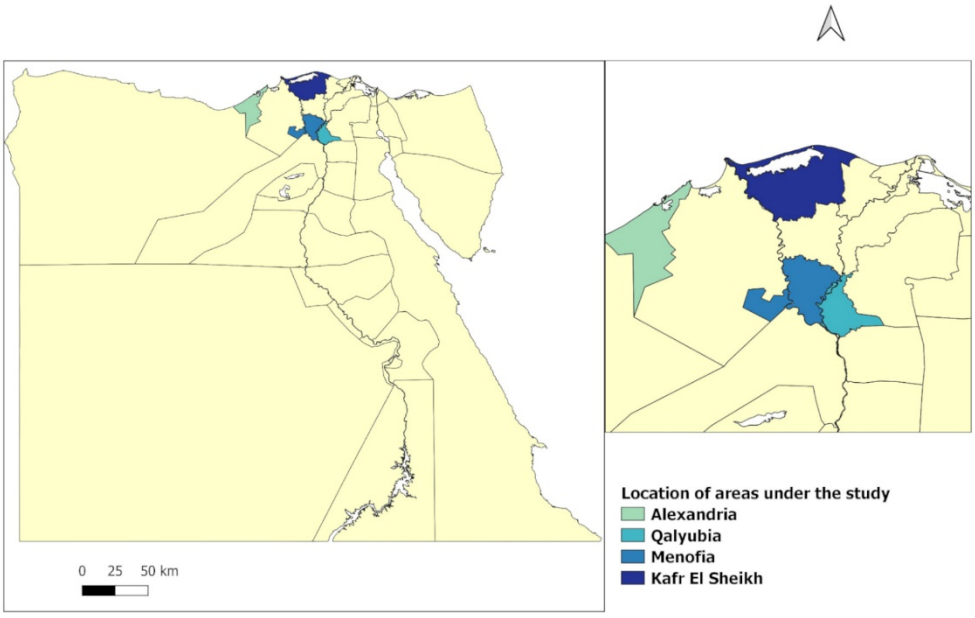

Figure 1. MAP showed location of governorates under the study (MAP generated by QGIS software).

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Study area

The study was conducted in four Egyptian governorates—Kafr El-Sheikh, Qalyubia, Menofia, and Alexandria—during the period from January to December 2023. The selected sites are geographically located at the following coordinates: Kafr El-Sheikh (31°06′42″N, 30°56′45″E), Qalyubia (30.41°N, 31.21°E), Menofia (30.52°N, 30.99°E), and Alexandria (31°10′N, 29°53′E) (Figure 1).

The climate in the study area is classified as tropical, characterized by hot summers and mild winters. The annual average temperature is approximately 25 °C, with minimal precipitation during the winter season. These climatic conditions, combined with the region's agricultural systems, create a favorable environment for the proliferation of arthropod vectors, including ticks, mosquitoes, and *Culicoides* spp., which are known to transmit bluetongue virus.

Sample size and sampling

The sample size was determined according to using following formula: $n = Z^2 P(1 - P)/d^2$

where n is the required sample size, Z is the value for 95% confidence level (1.96), P is the expected prevalence (16.9%) based on a previous study in small ruminants in Egypt (Mahmoud and Khafagi, 2014), and d is the accepted absolute precision (5%). The minimum calculated sample size was 216 animals; however, to improve the accuracy and precision of the estimated prevalence, the sample size was increased to 740, including 380 sheep and 360 goats.

Animals were randomly selected from different flocks distributed across the four studied governorates. All animals were apparently healthy at the time of sampling.

Questionnaire

All animals included in the study were assessed using a standardized questionnaire administered to the animal owners. The questionnaire was designed to collect data on both individual and management-related risk factors potentially associated with Bluetongue virus (BTV) infection. The recorded variables included:

Locality: Kafr El-Sheikh, Qalyubia, Menofia, or Alexandria

Species: Sheep or goat

Age group: <1 year, 1–2 years, or >2 years

Sex: Male or female

Presence of insect vectors (e.g., Culicoidesspp.): Yes or No

History of abortion: Yes or No

Contact with cattle: Yes or No

These data were used for subsequent statistical analysis to identify risk factors significantly associated with BTV seropositivity.

ELISA assay

All serum samples were screened for Bluetongue virus (BTV) group-specific IgG antibodies against the VP7 protein using a commercial competitive ELISA (cELISA) kit (ID Screen® Bluetongue Competition, Grables, France), following the manufacturer's instructions. The optical density (OD) was measured at 450 nm using a microplate reader (AMR 100, AllSheng, China).

The results were expressed as a percentage of the signal-to-noise (S/N) ratio, calculated using the following formula: $S/N\% = OD_{Sample} / OD_{NC} \times 100$). Samples with an S/N ratio **≥ 40%** were considered **negative**, while those with an S/N ratio **< 40%** were considered **positive** for antibodies against BTV, regardless of serotype.

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Statistical analysis

Statistical analysis was performed using SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to summarize the serological results and associated variables, including locality, species, age, sex, presence of insect vectors, history of abortion, and contact with cattle.

The chi-square test (χ^2) was applied to assess the association between each individual risk factor and BTV seroprevalence in the univariable analysis. Variables with a *P*-value < 0.2 in the univariable analysis were subsequently included in a multivariable logistic regression model to identify independent predictors of BTV seropositivity.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each variable in the final model. A *P*-value < 0.05 was considered statistically significant.

Results

A total of 740 serum samples collected from sheep (n = 380) and goats (n = 360) were screened for antibodies against Bluetongue virus (BTV) using a competitive ELISA (c-ELISA). Out of the 740 samples, 120 (16.2%) tested positive for BTV-specific antibodies, with a 95% confidence interval (CI) of 13.74–19.05% (Table I).

According to univariable analysis, the variables locality and species showed no statistically significant association with BTV seropositivity (P > 0.05). In contrast, age, sex, presence of insect vectors, history of abortion, and contact with cattle were significantly associated with BTV seroprevalence (P < 0.05) (Table I).

The highest seroprevalence was recorded in Kafr El-Sheikh (20.7%). Regarding species, sheep showed a higher seroprevalence (18.2%) compared to goats (14.2%).

The seroprevalence was also higher among:

•Females (18.4%) compared to males,

•Animals older than two years (21.4%),

•Animals exposed to vectors (18.1%),

•Animals with a history of abortion (23.3%),

•Animals in contact with cattle (19.2%) (Table I).

Multivariate logistic regression analysis confirmed that:

•Females (OR = 2.3) and

•Animals older than two years (OR = 2.6)

were more likely to be BTV seropositive.

Additionally, three risk factors were significantly associated with increased odds of BTV infection:

•Contact with cattle (OR = 2.1, 95% CI: 1.27–3.35),

•History of abortion (OR = 1.6, 95% CI: 1.04–2.57),

•Presence of insect vectors (OR = 1.6, 95% CI: 0.97–2.53) (Table II).

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Variable	Total examined animals	No of positive	No of negative	% of positive	95% CI	Statistic
locality						
Kafr ElSheikh	208	43	165	20.7	15.72-26.68	$\chi2$=7.211 df=3 P=0.065
Qalyubia	164	26	138	15.9	11.05-22.21	
Menofia	175	30	145	17.1	12.28-23.41	
Alexandria	193	21	172	10.9	7.23-16.06	
Species						
Sheep	380	65	315	17.1	14.61-22.35	$\chi2$=0.454 df=1 P=0.500
Goats	360	55	305	15.3	10.94-18.15	
Sex						
Male	158	13	145	8.2	4.87-13.57	$\chi2$=9.436 df=1 P=0.002*
Female	582	107	475	18.4	15.44-21.73	
Age (years)						
<1	188	17	171	9.0	5.72-14	$\chi2$=12.873 df=2 P=0.002*
1-2	262	41	221	15.6	11.75-20.54	
>2	290	62	228	21.4	17.05-26.46	
Presence of vector						
Yes	518	94	424	18.1	15.07-21.7	$\chi2$=4.736 df=1 P=0.030*
No	222	26	196	11.7	8.12-16.61	
History of abortion						
Yes	382	89	293	23.3	19.34-27.79	$\chi2$=4.878 df=1 P=0.027*
No	200	31	169	15.5	11.14-21.16	
Contact with cattle						
Yes	500	96	404	19.2	15.99-22.88	$\chi2$=10.102 df=1 P=0.001*
No	240	24	216	10.0	6.81-14.45	
Total	740	120	620	16.2	13.74-19.05	

*The result was significant if P value less than 0.05

Table I. Seroprevalence of Bluetongue Virus in Small Ruminants in Relation to Different Risk Factors.

Variable	B	S.E.	OR	95% CI for OR		P value
				Lower	Upper	
Sex						
Female	0.845	0.313	2.3	1.26	4.30	0.007
Age						
1-2	0.582	0.311	1.8	0.97	3.29	0.031
>2	0.951	0.297	2.6	1.45	4.63	0.001
Presence of Vector						
Yes	0.450	0.244	1.6	0.97	2.53	0.015
History of abortion						
Yes	0.492	0.230	1.6	1.04	2.57	0.032
Contact with cattle						
Yes	0.722	0.248	2.1	1.27	3.35	0.004

B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval

Table III. Multivariate Logistic Regression Analysis of Risk Factors Associated with Bluetongue Virus (BTV) Seropositivity in Small Ruminants.

Discussion

Bluetongue virus (BTV) infects both domestic and wild ruminants, particularly sheep, and is transmitted by biting midges of the genus *Culicoides*. The disease was first identified in South African Merino sheep in the late 18th century (Gerdes, 2004; Kyriakis et al., 2015), and has since been reported in many parts of the world, including the Americas, Asia, Africa, the Middle East, Australia, and several European countries (Yousef et al., 2012; Kyriakis et al., 2015; Sbizera et al., 2019). Although BTV has been detected in multiple animal species in different regions of Egypt, studies investigating the associated risk factors for seropositivity have been lacking. Therefore, this study aimed to determine the seroprevalence of BTV in small ruminants in selected Egyptian governorates and to assess relevant epidemiological risk factors.

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In the present study, 120 out of 740 animals (16.2%) tested positive for BTV-specific antibodies. This finding aligns closely with the results of Mahmoud and Khafagi (2014), who reported a 16.9% seroprevalence in small ruminants in Egypt. However, the prevalence observed here was lower than that reported in other countries: 41.17% in southern Ethiopia (Yilma and Mekonnen, 2015), 78.4% in Grenada (Sharma et al., 2016), and 45.7% in India (Sreenivasulu et al., 2004). Conversely, our results indicate a higher seroprevalence than that observed in other studies, such as 6.57% in sheep in southeast Iran (Khezri and Azimi, 2013b), 6.96% (13.7% in goats and 5.7% in sheep) in Algeria (Kardjadj et al., 2016), and 2.63% in Kerala, India (Ravishankar et al., 2005). These regional differences may be attributed to variations in geographic location, sampling methods, immunological status of the animals, age, species, and especially the local abundance and distribution of *Culicoides* vectors (Abera et al., 2018; Sohail et al., 2018).

In this study, Kafr El-Sheikh governorate had the highest seroprevalence rate, likely due to its agricultural profile and favorable environmental conditions that support the survival and reproduction of *Culicoides* midges (Meiswinkel et al., 2007; Carpenter et al., 2009). Although sheep showed a slightly higher seroprevalence than goats, the difference was not statistically significant. This is consistent with Mahmoud and Khafagi (2014). Sheep are known to be more susceptible to BTV and may exhibit more severe clinical symptoms, while goats often have subclinical infections and greater resistance (Yilma and Mekonnen, 2015). However, other studies have reported higher seroprevalence rates in goats compared to sheep (Elmahi et al., 2020), suggesting that species-specific exposure patterns and immune responses may vary by region and management system.

A significantly higher seroprevalence was observed among female animals ($P = 0.002$), consistent with the findings of Elmahi et al. (2020) and Abera et al. (2018). Although the exact reason for this remains unclear, it may be due to sampling bias, as females typically remain longer in flocks for breeding and milk production, leading to greater cumulative exposure to vectors over time.

Age was also significantly associated with BTV seropositivity, with older animals (>2 years) being more likely to test positive. This finding is in agreement with Mohammadi et al. (2012), and can be attributed to increased lifetime exposure to infected *Culicoides* midges. Moreover, younger animals are often kept indoors or more closely monitored, reducing their risk of exposure (Gaire et al., 2014; Elmahi et al., 2020). The presence of insect vectors was strongly associated with increased seropositivity, supporting previous findings by Malik et al. (2018). Vector density and distribution are directly influenced by environmental conditions such as temperature, humidity, and rainfall, which in turn affect the transmission dynamics of BTV (Maclachlan, 2010).

Animals raised in contact with cattle showed significantly higher BTV seroprevalence, consistent with findings from Gaire et al. (2014). This may be due to the fact that cattle, owing to their larger size and body heat, attract greater numbers of *Culicoides* midges, increasing vector density in mixed-species farming systems (Miranda, 2018; Mullins, 2024). Consequently, small ruminants in proximity to cattle are at higher risk of exposure. A notable finding of this study was the significantly higher seroprevalence among animals with a history of abortion, in agreement with previous studies (Gaire et al., 2014). BTV is known to cause reproductive disorders such as early embryonic death, abortion, fetal mummification, stillbirths, and congenital abnormalities (Saminathan et al., 2020). Formenty et al. (1994) and Bumbarov et al. (2012) also reported higher BTV seroprevalence among animals with reproductive losses, highlighting the importance of considering BTV as a differential diagnosis in abortion cases in Egypt (Mahmoud et al., 2021).

This study has several limitations. Selection bias may have occurred if sampled animals were not representative of the general population, especially in relation to geographic distribution and management practices. The cross-sectional nature of the study and the use of serological testing alone limit the ability to distinguish between current and past infections. Additionally, the absence of virus isolation or molecular identification of circulating BTV serotypes prevents detailed epidemiological characterization, which is essential for designing targeted control measures and vaccination strategies.

Conclusion

The present study confirmed the presence of antibodies against Bluetongue virus (BTV) in small ruminants within the investigated Egyptian governorates. Multivariate logistic regression analysis identified several significant risk factors associated with BTV seropositivity, including age, sex, presence of insect vectors, history of abortion, and contact with cattle. These findings contribute to the field of veterinary epidemiology by providing valuable insights for the development of effective surveillance, prevention, and control strategies, ultimately supporting the protection of livestock health and productivity. Further studies are warranted to identify the circulating BTV serotypes and to assess the epidemiological situation on a broader geographical scale across Egypt.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-original draft preparation, A.S., H.S.G., M.M. and M.A.A.; writing-review and editing, A.S., H.S.G., M.M. and M.A.A.; project administration, M.M.; funding acquisition, A.S., H.S.G., M.M. and M.A.A. All authors have read and agreed to the published version of the manuscript.

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