The current situation of Bluetongue virus Disease in some Egyptian governorates during 2018-2019 Nadia, M.H. Danial; Hala, K. Abdelmegeed; Omnia, M. Kattab; Morcos, Ibrahim Yanni; M.H. Ali; Rabab, T. Hassanien; Eman, M. AboHatab; Mervat, I.I.; Habashi, A.R.; Hanan, A. Fahmy; Essam, I.; Momtaz, A. Shaheen and Abd-ELhakam M.

Virology Research Dept. Animal Health Research Institute (AHRI), Agriculture Research Center (ARC)

Received in 22/09/2019 Accepted in 19/10/2019

Abstract

Bluetongue (BTV) is a notifiable multiple species non-contagious vector born viral disease of domesticated and wild ruminants. An active surveillance study was conducted to understand the current situation of BTV in some Egyptian governorates in period between 2018-2019, the present seroprevalence in sample population of 5219 animals comprising 2366 cattle, 2683 sheep and 170 goat covering line agro-climatic zone (Delta, East, West, Central and Upper Egypt). The antibody prevalence was highest in goat (49.4%) followed by sheep (48.4%) and cattle (41.5%). The Upper Egypt district had the highest prevalence of BTV (65.5%) while Delta district had the lowest prevalence (37.3%) respectively. Also, the highest percentage of positivity for BTV antibodies was (56.9%) observed in autumn and (35.3%) in winter. Our results showed that attention and quarantine measures should be established to investigate clinical suspected cases to obtain real information about the situation of possible circulating BTV disease in Egypt by periodical diagnosis using suitable diagnostic techniques which act as a safeguard for our livestock.

Keywords: Current situation, Bluetongue, Egypt 2018-2019

Introduction

Bluetongue (BT) is an infectious, vector-borne viral disease that affects wild and domestic ruminants. The disease is caused by bluetongue virus (BTV), a member of the genus *Orbivirus* within the family *Reoviridae*. Up to now, 27 serotypes of BTV are officially recognized (Maan *et al.* 2011a, b; Zientara *et al.* 2014). The virus Horizontal transmission has been demonstrated for BTV serotypes 26 and 27 (Batten *et al.* 2014; Bréard *et al.* 2017). BTV can exert major effects on animal health

especially ruminant populations, restricting

international trade in Livestock. It is listed as a notifiable disease by the office international des Epizooties (OIE) (Gibbs and Greiner, 1994).

In warmer African climates BT outbreaks can occur throughout the year. In cooler climates like in the southern parts of South Africa the clinical disease usually becomes apparent in late summer and autumn and disappears with the first winter frosts (**Coetzee** *et al*, 2012).

BTV can infect ruminants and camelids, but clinical disease is more pronounced in sheep (De Koeijer and Elbers, 2007). The major economic impact of BT is that it is a reportable disease by almost all regulatory authorities along with at least 14 other diseases that are considered to have major adverse economic and societal ramification (Mc Vey *et al.*, 2013).

BTV is classified into 27 serotypes based on the genetic and antigenic feature of the neutralizing protein VP₂. VP₇ protein is a major determinant of serogroup specificity and most serological assays to detect BTV are based on detection of anti VP₇ antibodies (Mertens *et al.*, **2005**).

Laboratory diagnosis depends on the isolation and identification of the causative virus as well as demonstration of rising antibody titer in serum samples collected during acute and convalescent stages of the disease (Thomas, 1980).

In Egypt: the first documented outbreak of (BTV) infection occurred in imported Marino sheep (Hafez and Ozawa, 1973). The reported (BTV) serotypes in subsequent outbreak were 1, 2, 10, 12 and 16 (Ismail *et al.*, 1987).

The BTV initially has been considered as an endemic disease in Africa and Cyprus, but recently, it was identified in many regions of the world such as Australia, the USA, Israel, and European countries (Kyriakis *et al.* 2015).

The present study was carried out to clarify the disease status in some Egyptian governorates in different districts during 2018, 2019.

Materials and Methods

During 2018–2019 samples were collected during the vector breeding season in Autumn and winter seasons, a total of 5219 serum samples were collected of 2366 cattle, 2683 sheep and 170 goats from different provinces distributed on (Delta, East, West, Central and Upper Egypt) at least two governorates from each district, the collected blood from livestock was conducted solely for the purposes of this study and it was performed aseptically by veterinarian who was one of the investigators (OIE, 2011).

1-Samples:

Preparation of blood samples

A total of 5219 blood samples were collected from cattle, sheep and goats from different Egyptian governorates. Ten ml of blood from each animal were collected in clean sterile tubes.

The clean serum was obtained using sterile Pasteur pipettes from the blood samples were left in tightly closed tubes overnight at 4°C and then centrifuged at 3000r pm for 10 min to separate the sera.

The collected samples from animals may contain live virus and appropriate inactivation steps should be put in place. Heat inactivation has been described (Van Vuren & Paweska, 2010).

2-Serological detection of BTV antibodies using competitive ELISA

Serum samples were stored at -20° C till the serological tests were performed by commercial ID Vet, (France) BTV competitive ELISA kit was used for detection of BTV specific antibodies against VP₇ protein in sera samples according to manufacturer instruction. (Tittarelli, 2014).

-The wells were coated with the VP7 protein.

The samples to be tested and the controls were added to the microwells. The anti- VP_7 antibodies, if present form an antibody-antigen complex which masks the VP_7 epitopes.

- -An anti VP₇ peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free VP₇ epitopes forming an antigen-conjugate-HRP complex.
- -After washing to eliminate the excess conjugate, the substrate solution (TMB) is added.
- -The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested.
- -In the absence of antibodies, a blue solution appears which becomes yellow after the addition of the stop solution.
- -In the presence of antibodies, no colouration appears.
- -The Microplate was read at 450 nm.

Results

In the present study, the serological survey for BTV in different Egyptian governorates revealed that the virus was distributed in different localities in Egypt the overall seroprevalence of BTV in cattle and small ruminants was 2365 out of 5219 serum samples in percentage 45.3 % were positive for BTV antibodies and the Upper Egypt district showed higher percentage of positivity than other districts and seasonal variation have important role in viral infection where the highest percentage of positive samples was 56.9% in autumn as in tables (1 and 2)

Table (<u>(1)</u> .	Results	ofELISA	test for	detection	Bluetongue	antibodi	es in	different	Districts
I abit (1.	Results	ULLIDA	1051 101	ucicciion	Diuciongue	annooui	cs m	uniterent	Districts

District	No. of sample	Positive +ve	Percentage %
Delta	2445	912	37.3%
East	791	286	36.2%
West	1056	624	59.1%
Central	672	376	56.0%
Upper Egypt	255	167	65.5%
Total	5219	2365	45.3%

Table (2). Result of ELISA Test for Detection of Bluetongue BTV antibodies in Autumn and Winter seasons:

Season	No. of sample	Positive +ve	Percentage %	
Autumn	2406	1370	56.9%	
Winter	2813	995	35.3%	

In cattle 983 out of 2366 in percentage, 41.5% were positive for BTV antibodies detecting ELISA and The higher percentage was in Upper Egypt (Luxor – Aswan– Sohag and Qena) as provided in **Table (3).**

Table (3). Result of ELISA Test for BTV antibodies in cattle serum samples in different Egyptian governorates:

District	No. of sample	Positive +ve	Percentage %	
Delta	1328	459	34.6%	
East	460	166	36.1%	
West	90	44	48.9%	
Central	352	208	59.1%	
Upper Egypt	136	106	77.9%	
Total	2366	983	41.5%	

In sheep 1298 samples out of 2683 in percentage 48.4% were positive for BTV antibodies detection. The west district (Marsa Matrouh and El -Wadi El -Gadid) was found to be the highest positive area which constituted 60.6% (as shown in Table 4).

District	No. of sample	Positive +ve	Percentage %
Delta	1065	426	40%
East	310	115	37.1%
West	881	534	60.6%
Central	320	168	52.5%
Upper Egypt	107	55	51.4%
Total	2683	1298	48.4%

Table (4) Result of ELISA Test for BTV antibodies in sheep serum in different Egyptian governorates

Concerning goat, our results showed that 84 out of 170 in percentage 49.4% where the West is the highest district ((Marsa Matruh and El-Wadi El-Gadid) (table 5)

Table (5) Result of ELISA Test for BTV antibodies in goat serum in different Egyptian governorates

District	No. of sample	Positive +ve	Percentage %	
Delta	52 27		51.9%	
East	21	5	23.8%	
West	85	46	54.1%	
Central	-	-	-	
Upper Egypt	12	6	50%	
Total	170	84	49.4%	

Discussion

In cattle, goats and sheep, BTV causes an acute disease with high morbidity and mortality, BTV also infects goats, cattle and other domestic animals as well as wild ruminants (**Roy, 2008**). Multivariate logistic regression analysis would have probably given a more appropriate answer on the influence on the BTV prevalence of each of the variables considered BTV disease is a highly infectious non -contagious disease of ruminants and listed as a transboundary animal disease (TADs) that can spread rapidly across countries borders (Sohail *et al.*, 2019). In this study, a seroprevalence for the high number of serum samples from different localities in Egypt found (2365) out of (5219) in percentage (45.3%) were positive for BTV antibodies **(Table 1).**

Among several ELISAs that have recently been developed, the Competitive ELISA in which a group-specific Mab (monoclonal antibodies) to BTV is used, has proved to be the most sensitive and specific assay for detection of antibodies to BTV. Following extensive national and international validation, the competitive ELISA is gradually replacing the AGID as a universal test to certify ruminants for trade purposes and to diagnose BT infection in domestic and wild animals. (Abduslam Mahmoud et al., 2019). In this study five districts, the overall seroprevalence was not uniformly distributed and significantly. Higher seroprevalence in cattle was recorded in upper Egypt (Southern region) (77.9%); conversely, the other small ruminant showed the highest prevalence values were found in (Western region) (60.6% in sheep and 54.1% in goat respectively in (Tables 4 and 5) and these results were in agreement with (Abduslam Mahmoud et al., 2019) who mentioned that similar overall and species seroprevalence in Similarly, The differences were found between small and large ruminant prevalence values in Libyan provinces. The higher percentage in cattle was recorded in upper Egypt and the Southern part was observed where animal movements across the borders either transitional are recurrent thus, related to the risk of importing animals infected with different pathogens from the Southern borders is high (Dayhum et al. 2018).

Moreover, seasonal variation have important role in viral infection as in (Table 2) where percentage of positive serum samples to antibodies against BTV sample were 56.9% in autumn (early winter) than in winter the percentage decrease to 35.3% as recorded by (Purse *et al.*, 2005) that BTV occurrence is seasonally in the affected Mediterranean countries, subsiding when temperatures drop and hard frosts kill the adult midge vectors. Viral survival and vector longevity are seen during milder winters (International Society, 2007). Free livestock movements or pastoralist activities present and common in the Southern regions might have facilitated the introduction of the emerging serotype which already reported in Sudan (Elfatih *et al.* 1987).

Conclusion

We recommended Northern African authorities collaborate in organizing common surveillance programmer to early detect novel strains or emerging serotypes in order to set up proper preventive measures, and, in case, develop specific vaccines and plan coordinated vaccination campaigns. That our results gave attention to quarantine measures should be investigated suspected cases repeatedly to obtain the updated about the situation of the disease in Egypt and periodical diagnosis for the evidence of disease using suitable diagnostic techniques and vector control should be done in the suspected area.

References

- Abduslam S. Mahmoud; Giovanni Savini; Massimo Spedicato; Federica Monaco; Irene Carmine; Alessio Lorusso; Tolari Francesco; Maurizio Mazzei; Mario Forzan; Ibrahim Eldaghayes and Abdunaser Dayhum (2019). Exploiting serological data to understand the epidemiology of bluetongue virus serotypes circulating in Libya Vet Med Sci Feb; 5(1): 79–86.
- Bréard, E.; Schulz, C.; Sailleau, C.; Bernelin-Cottet, C.; Viarouge, C. and Vitour, D. (2017). Bluetongue virus serotype 27: experimental infection of goats, sheep and cattle with three BTV-27 variants reveal atypical characteristics and likely direct contact transmission BTV-27 between goats. Transboundary and Emerging Diseases 65, e251– e263.
- Coetzee, P.; Stock Stad, M.; Venter, E.H.; Myrmel, M. and Van Vuuren, M. (2012). Bluetongue: a historical and epidemiological perspective with the emphasis on South Africa. Viro1 J. 9(1): 198.
- Dayhum, A.; Sharif, M.; Eldaghayes, I.;
 Kammon, A.; Calistri, P. and Danzetta,
 M.L. (2018). Sero-prevalence and epidemiology of peste des petits ruminants in Libya. Transboundary and Emerging Diseases

Journal 65, e48–e54.

- De Koeijer, A.A.; Elbers, A.R.W. and Knols, G.J. (2007). Modelling of vector – borne disease and transmission of bluetongue virus in North-West Europe. In: Takkeneditors. Emerging Pests and Vector Born Disease in Europe, Wageningen: Wageningen Academic publisher; 2007, PP.99-112.
- Elfatih, M.; Mohammed, H. and Taylor, W.P. (1987). Infection with bluetongue and related orbiviruses in the Sudan detected by the study of sentinel calf herds. Epidemiology and Infection 99, 533–545.
- Gibbs, E.P. and Greiner, E.C. (1994). The epidemiology of bluetongue Comp Immunol Microbial infect Dis. 17: 207-220.
- Hafez, S.M. and Ozawa, Y. (1973). Serological survey of bluetongue in Egypt. Bull. Epiz. Dis. Afr., 21(3): 297- 304.
- International Society for Infectious Diseases Bluetongue (2007) – Europe (51)".. 2007-10-30.
- Ismail, J.M.; Martin Jeggo and Nazmi Ayoub (1987). Bluetongue neutralization test with different virus under variable condition Agr. Res. Rev. Egypt. 65(5): 867-872.
- Kyriakis, C.S.; Billinis, C.; Papadopoulos, E.; Vasileiou, N.G.; Athanasiou, L.V. and Fthenakis, G.C. (2015). Bluetongue in small ruminants: an opinionated review, with a brief appraisal of the 2014 outbreak of the disease in Greece and the south-east Europe. Vet Microbial 181: 66–74.
- Maan, S.; Maan, N.S.; Nomikou, K.; Batten, C.; Antony, F. and Belaganahalli,
 M.N. (2011a). Novel bluetongue virus serotype from Kuwait. Emerging Infectious Diseases 17, 886–889.
- Maan, S.; Maan, N.S.; Nomikou, K.; Veronesi, E.; Bachanek-Bankowska, K. and Belagana-halli, M.N. (2011b). Complete genome characterization of a novel 26th blue tongue virus serotype from Kuwait. PLoS ONE 6, e26147.
- Mc Vey, D.S.; Kennedy, M. and Chengappa, M.M. (2013). Veterinary Microbiology. 3rd edition, Wiley-Blackwell. Mellor Ps, Baylis M, Mertens PP. Bluetongue, London, Academic Press; 2009.

- Mertens, P.; Maan, N.S.; Samuel, A.R.; Attoul, H. Orbiviruses in fauquet C.M.; Mayo, M.A.; Manilooff, J.; Desselberger, U. and Ball, L.A. (2005). editors, virus Toxonomy. Classification and Nomenclature of viruses, Amsterdam, Nether land; Elsevier Academic press; 2005, 466-483.
- **OIE Terrestrial Animal Health, (2011).** cod twentieth edition; ISBN 978-92-9044-825-9
- Purse, Bethan V.; Mellor, Philip S.; Rogers, David J.; Samuel, Alan R.; Mertens, Peter
 P. C.; Baylis, Matthew (February 2005).
 "Climate change and the recent emergence of bluetongue in Europe". Nature Reviews Microbiology. 3 (2): 171–181.
- Roy, P. (2008). "Molecular Dissection of Blue tongue Virus". *Animal Viruses: Molecular Biology*. Caister Academic Press. pp. 305– 354. ISBN 978-1-904455-22-6.
- Sohail, T.; Yaqub, T.; Abbas, T.; Rabbani, M.; Nazir, J.; Maqbool, S.M.; Yaqub, S.; Habib, M.; Mukhtar, N. and Shahbaz, M. (2019). Seroprevalence of Bluetongue virus in small and large ruminants in Punjab province, Pakistan. Acta tropica2019; 189: 22-29.
- **Thomas, E.W. (1980).** The diagnosis and control bluetongue. Report, 2II XL VIII general session of OIE Committee.
- **Tittarelli, M. (2014).** Standardized and validated for the detection of antibodies against the BTV in ruminants serum by Competitive Enzyme Linked Immunosorbent Assay. IZS, TE.
- Van Vuren, P.J. and Paweska, J.T. (2010). Comparison of enzyme-linked immunosorbent assay-based techniques for the detection of antibody to Rift Valley fever virus in thermos-chemically inactivated sheep sera. Vector Borne Zoonotic Dis., 10, 697–699.
- Zientara, S.; Viarouge, C.; Höper, D.; Beer, M.; Jenckel, M. and Hoffmann
 B. (2014). Novel bluetongue virus in goats, Corsica, France. Emerging Infectious Diseases 20, 2123–2125., Vol. 20, No. 12.