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SPATIO-TEMPORAL CHARACTERISTICS OF THE BLUETONGUE EPIZOOTY IN THE BALKAN PENINSULA FROM 2014 TO FEBRUARY 2015

Zharko Stojmanovski[1], Blagojcho Tabakovski[2]

[1]*Food and Veterinary Agency, Inspection Department, 11 Oktomvri str. bb,
Branch Office, 1300 Kumanovo, Republic of Macedonia*
[2]*Food and Veterinary Agency, Animal Health and Welfare Department, 3-ta Makedonska
Brigada 20, 1000 Skopje, Republic of Macedonia*

ABSTRACT

Starting in May 2014 an emerging Bluetongue (BT) serotype 4 (BTV-4) epizooty has affected the ruminant population of eleven countries from the Balkan Peninsula. Consequently, the veterinary services implemented various bio-security measures and a considerable discussion has been raised if future BTV surveillance and preventive measures should be taken in risk based zones and periods. Therefore, the objective of this work was to describe the spatial and temporal characteristics of the BTV-4 epizooty in the Balkan Peninsula from May 2014 to February 2015. We used the space-time permutation model of the scan statistic to identify the space-time disease clusters. The scan statistic was parameterized to a maximum temporal length of 150 days (duration of the epizooty in the Balkans in 2014) and a radius of 100 km as a maximum spatial cluster size (protection zone for BT). Results were significant ($p < 0.05$) to the maximum spatial size defined for the clusters. From the 6295 BT outbreaks the scan statistics identified 33 disease clusters in nine Balkan countries. The highest number of outbreaks occurred from September to November 2014. The earliest cluster was detected in Greece in July 2014 with a radius of 56 km. The latest cluster was detected in Croatia in February 2015 with a radius of 99,8 km. These results are a first description of the spatial and temporal characteristics of the 2014-February 2015 BT epizooty in the Balkans.

Key words: bluetongue, space-time clustering, scan statistic, Balkans, surveillance, epizooty

INTRODUCTION

Bluetongue (BT) is a a non-contagious, vector-borne disease in ruminants caused by a bluetongue virus (BTV) and transmitted by the biting midges of the *Culicoides* spp. Currently, there are 26 known BTV serotypes (1). Hosts of the infection are domestic and wild ruminants, mainly sheep, followed by goats and cattle (2).

The first BT outbreaks (serotype 4, BTV-4) in South-East Europe were reported in 1979 on several Greek islands near the Anatolian Turkish coast (3). From then on, surveillance and control measures were applied in the Greek islands in east Aegean Sea (from 1980 to 1991), and until summer 1998 there were no reports of clinical disease in these islands (4). However, from 1998 to 2005 at least six BTV strains belonging to five serotypes (BTV-1, BTV-2, BTV-4, BTV-9, and BTV-16) were found to circulate in the Mediterranean Basin (5, 6). During this period, the Balkan countries reported at least 2000 BT outbreaks with however, two main epizooties; the first epizooty in 1999 in Greece (BTV-9/4/16) and Bulgaria (BTV-9); and the second epizooty in 2001, affecting Greece, Bulgaria (BTV-1/9) and Macedonia, Serbia and Croatia (BTV-9) (7). BTV outbreaks were reported by Bulgaria in 2006. Greece reported BTV-16 outbreaks in 2008 (7). Additionally, from 2009 to 2012, Greece was the only country in the Balkans which reported BTV-1/4/8/16 outbreaks (8).

In 2014, the emergence of the BTV-4 epizooty dramatically spread among 11 Balkan countries, leaving a devastating effect on the animal health

and the economies of the countries. These countries cover 838 680 km² area and hold a livestock population of more than 27 million sheep, 7 million goats and 7 million cattle (9).

The circulation of numerous BTV serotypes in the Mediterranean Basin of South-East Europe, as well as the extent of the spread of BTV-4 from 2014 to February 2015 in the Balkan Peninsula - shows the necessity of understanding the risk areas and time periods for an early alerting of BTV emergence (re-emergence).

Therefore, this work is the first extensive description of the BTV-4 epizooty from 2014 to February 2015 in the Balkan Peninsula. Using the date of observation of the clinical signs of BT and the space-time permutation model of the scan statistic, this work presents the main risk zones and periods of BT occurrence in the Balkans.

MATERIAL AND METHODS

Study area and study period

From 29/05/2014 to 28/02/2015, BTV-4 outbreaks were reported by 11 Balkan countries, i.e. Greece, the European part of Turkey, Bulgaria, Macedonia, Albania, Serbia, Montenegro, Croatia, Bosnia and Herzegovina, Romania and Hungary.

Data sources

In order to identify the space-time clusters, we collected the data for the BT outbreaks reported to the World Animal Health Information System (WAHIS) of the World Animal Health Organization (OIE) (10). We freely downloaded the data regarding the country, administrative region, and location, date of observation of the first signs and the date of reporting of each of the BT outbreaks.

From a total of 6295 outbreaks, 3651 outbreaks had no information about the date of observation of the first signs of BTV-4. In such cases, we used the reporting date as a reference date. Eight outbreaks in Montenegro had no information about the BTV serotype and therefore were excluded from analysis. Overall, in this work we used all available data for the BTV-4 outbreaks, i.e. 42% of the outbreaks with known observation date and 58% of the outbreaks with a known reporting date.

Data analysis

Developed in 2001 by Kulldorff (11), the space-time cluster analysis has been used for a variety of health issues, including outbreaks of emerging and vector-borne diseases, such as Avian Influenza, West Nile virus, Malaria, and Dengue fever (11). Space-time scan statistics are suitable for temporal historical data, in particular for a large amount of data, accumulated in space and time. Additionally, the outputs are easy to understand and interpret (12).

The space-time cluster analysis requires only outbreak data, with information about the spatial location and time for each outbreak. The number of observed outbreaks in a cluster is compared to what would have been expected if the spatial and temporal locations of all outbreaks were independent of each other, so that there is no space-time interaction. This is so, because there is a cluster in a geographical area if, during a specific time period, that area has a higher proportion of its outbreaks in that time period compared to the remaining geographical areas. This allows the space-time cluster analysis to identify not only the centre and the radius of a primary cluster (hotspot), but also specifies a time window when the clusters of new outbreaks are present (11).

The space-time cluster analysis requires several parameters:
i) a time aggregation period, which we have set to a 7-day time aggregation, because once an initial disease outbreak is reported to the OIE, countries send weekly reports to the OIE;
ii) a maximum size of the temporal window, which we have set up to 150 days, which was the maximum duration of continued detection of outbreaks notified to the OIE in 2014;
iii) a maximum extension of the spatial window, which we have set up to a radius of 100 km, assuming to represent a cluster of outbreaks due to local, rather than long distance, disease transmission and spreading (13).

Space-time cluster analysis (permutation model) was performed with R SaTScan package which provides an automatic link to the SaTScan software. The statistical analysis was done using Statistica 7 software (14). Data visualisation was made with Q-GIS (15). The values of p<0.05 were considered as statistically significant.

RESULTS

Bluetongue epizooty

From May 2014 to February 2015, a total of 6295 BTV-4 outbreaks were reported in the study area with 108.300 cases. Greece reported 2698 (42.8%) of all outbreaks, while Bosnia and Herzegovina only five BTV-4 outbreaks (0.08%) (Fig. 1 and 2).
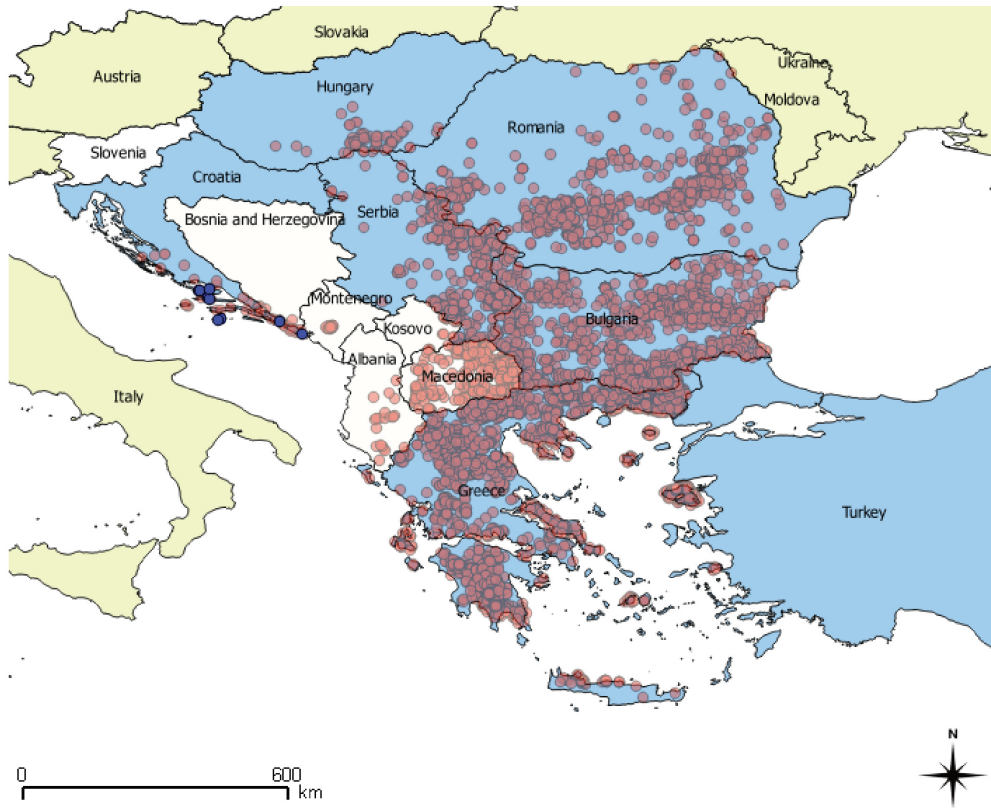
Figure 1. Spatial distribution of the BT outbreaks in 2014 (red points) and January-February 2015 (blue dots). The countries in blue refer to the countries which performed vaccination (either mandatory or voluntary) in 2015, Balkans countries with white colour refer to the countries with no-vaccination against BT

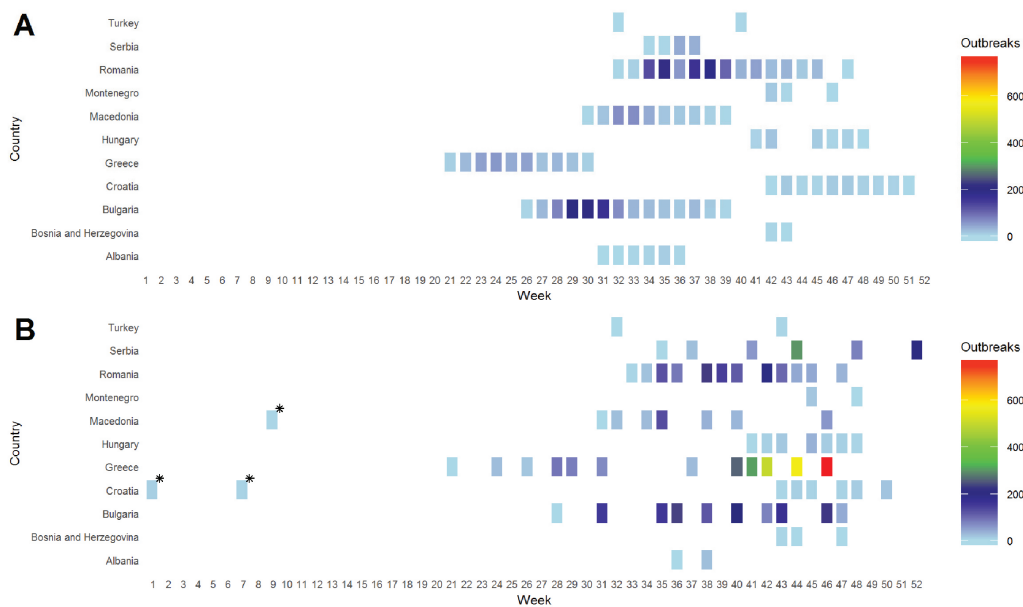**Figure 2.** Number of outbreaks per week of observation of the clinical signs during the epizooty in 2014 **(A)**. The number of outbreaks per week of reporting to the OIE. The stars indicate the outbreaks that were reported till February 2015 **(B).**

The peak of the outbreaks was November 2014, with 2078 (32.9%) reported and/or observed BT outbreaks (Fig. 2).

Cluster identification

During the BTV-4 epizootic in the Balkans, we identified in total 33 space-time clusters, among which 32 were statistically significant (p<0.05) and non-significant cluster number 15 (p=0.23) (Table 1, Fig. 3).

Thirty two clusters were identified in 2014 and only one in February 2015 in the coast of Croatia (cluster number 33). The earliest BT disease cluster was identified in July 2014 in Peloponnese, Greece (cluster number 1) (Table 1, Fig. 3).

Table 1. Chronological order of BT space-time disease clusters in Balkans epizooty from 2014 to February 2015. The observed (O) and expected (E) number of outbreaks per cluster and the ratio O/E are indicated

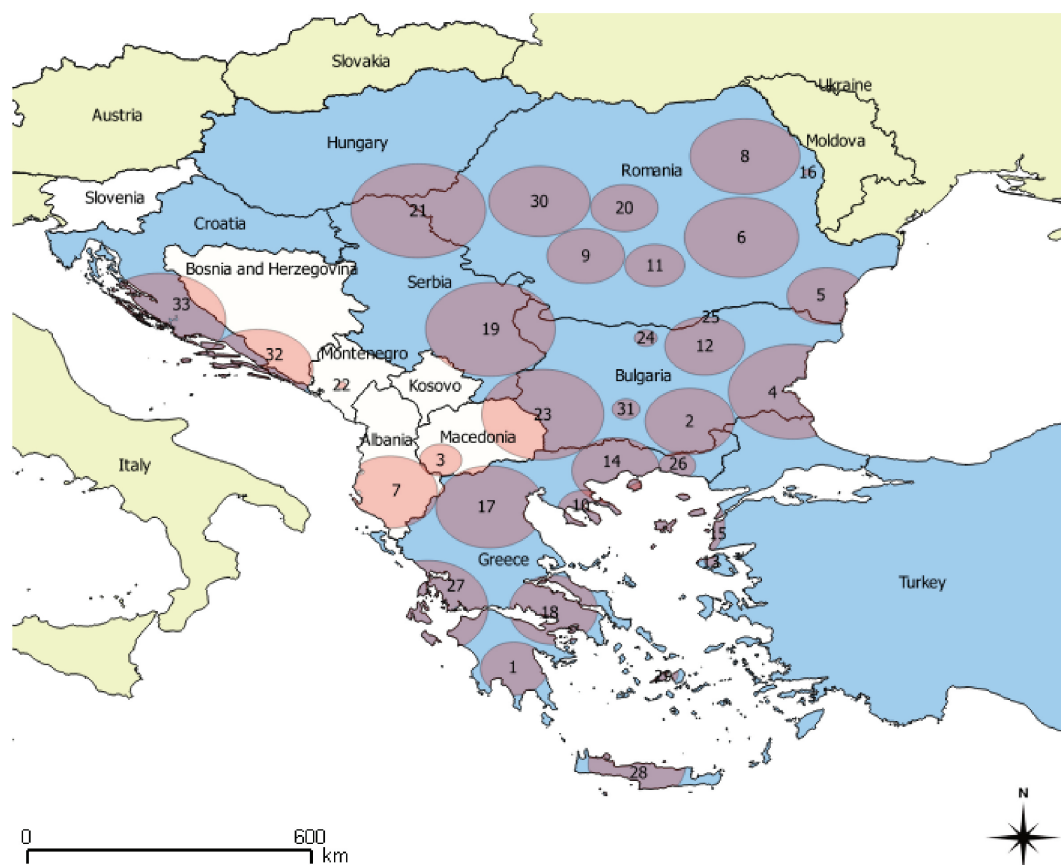| Cluster number	Country	Radis (km)	Start date	End date	Observed/ Expected	O/E	Duration (days)
1	Greece	56	15.07.14	16.07.14	86/3,9	22,1	2
2	Bulgaria, Greece, Turkey	69,8	07.08.14	08.08.14	124/11,5	10,6	2
3	Macedonia	33,4	01.09.14	05.09.14	33/2,4	13,8	5
4	Bulgaria, Turkey	99,7	08.09.14	08.09.14	50/7	7,1	1
5	Romania	59,4	09.09.14	09.09.14	3/0,004	750	1
6	Romania	84,4	22.09.14	23.09.14	151/14,7	10,3	2
7	Albania	75,7	24.09.14	24.09.14	19/0,5	38	1
8	Romania	79,1	29.09.14	29.09.14	7/0,03	233,3	1
9	Romania	57,7	30.09.14	03.10.14	109/6,5	16,9	4
10	Greece	41,4	06.10.14	06.10.14	20/3,5	5,7	1
11	Romania	44,6	08.10.14	08.10.14	32/0,4	80	1
12	Bulgaria, Romania	61,2	10.10.14	10.10.14	90/6,5	13,8	1
13	Greece-island of Lesbos	10,9	13.10.14	13.10.14	58/4,2	13,8	1
14	Greece	69,8	13.10.14	13.10.14	94/15,5	6,1	1
15	Greece-island of Lemnos, Turkey	97,5	20.10.14	20.10.14	14/2,7	5,2	1
16	Romania	6,4	22.10.14	22.10.14	5/0,07	71,4	1
17	Greece	86,7	03.11.14	03.11.14	335/61	5,5	1
18	Greece	72,8	03.11.14	03.11.14	51/10	5,1	1
19	Serbia, Bulgaria	98,9	04.11.14	04.11.14	191/24,3	7,9	1
20	Romania	49,3	04.11.14	04.11.14	25/1,6	15,6	1
21	Serbia, Romania, Hungary	98,7	10.11.14	13.11.14	44/1,3	33,9	4
22	Montenegro	7,6	14.11.14	14.11.14	15/0,06	250	1
23	Macedonia, Bulgaria, Greece, Serbia	95,3	17.11.14	17.11.14	106/8,01	13,3	1
24	Bulgaria	17,4	18.11.14	18.11.14	9/0,03	300	1
25	Bulgaria	0,00	20.11.14	20.11.14	25/0,1	250	1
26	Greece	29	21.11.14	21.11.14	165/32	5,2	1
27	Greece	99,9	21.11.14	21.11.14	78/13,2	5,9	1
28	Cyprus	83,4	21.11.14	21.11.14	33/6,1	5,4	1
29	Greece-island	27,1	21.11.14	21.11.14	21/4	5,25	1
30	Romania	74	25.11.14	25.11.14	7/0,01	700	1
31	Bulgaria	21,6	26.11.14	26.11.14	26/0,2	130	1
32	Croatia, Bosnia and Herzegovina	84,2	19.12.14	19.12.14	14/0,1	140	1
33	Croatia, Bosnia and Herzegovina	99,8	13.02.15	13.02.15	5/0,01	500	1

Figure 3. BT disease clusters (red circles) in the Balkans, detected from 2014 to February 2015 by use of the permutation model of the time- space scan statistic. The numbers refer to the designation of cluster presented in Table 1

In all detected clusters there occurred 3815 outbreaks, which represents 60.6% of the overall analysed 6295 outbreaks. The highest number of outbreaks was identified in the north-west of Greece, cluster number 17 (n=466, 12.2%) radius 86.7 km, which was identified in November 2014 and the least in municipality of Ruse in Bulgaria, cluster number 25 (n=1, 0.03%) radius less than 1 km, which was identified also in November 2014.

In July and August were charaterised by the detection of one disease cluster, September and October by seven, while the monthly peak was identified in November (n=15, 45%), with one cluster in December and the last one in February 2015. The median O/E value was 250 with minimum O/E =5.1 and maximum O/E =700 values (Table 1).

The mean duration of the identified BTV-4 clusters was 1.39 days (range 1 to 5 days, median of 1 day). Twenty-seven of the clusters (82%), were with a duration of 1 day (Table 1).

The Pearson`s coefficient of correlation showed weak negative linear correlation between the radius

of the disease cluster and the duration in days (r = -0.12; p = 0.51), and a weak positive linear correlation between the radius of the disease cluster and the observed cases (r = 0.28; p = 0.12).

DISCUSSION

Spread of the Bluetongue virus

Our results show that the 2014 BTV-4 epizooty started at the south of Greece and spread rapidly through the Balkan countries. In contrast to the current BTV epizooty, the BTV-9 epizooty in September 2001 started in the prefecture Ioannina in western Greece, and then spread to Albania, Macedonia, Kosovo, Bulgaria and Croatia (16).

Our results also suggest that the south-east parts of the Balkan Peninsula had a higher number of BTV-4 outbreaks compared to the north-west (17). In June 1999, the BTV-9 epizooty started in south-east Bulgaria, near the coast of the Black Sea

and then spread to the west, and south in Greece (Rodopi and Evros regions) (16).

Our findings suggest that the epizooty started at the end of May 2014 in Greece and continued to spread in July in Bulgaria and Macedonia, in August in Serbia and Romania and in October in Croatia and Hungary.

Most of the clusters were identified from September to November 2014, which affirms that most of the BT infections occurred in late summer and autumn 2014 (September-November). However, due to the fact that for 58% of the outbreaks the observation date was unknown, these results might be biased, as a result of delayed reporting and missing information. A study in Serbia which investigated the BTV-4 outbreaks at a national scale, showed similarly that the peak of the outbreak was in September and October 2014 (18). In Macedonia, the last BT outbreak was reported at the start of November 2014 and no BTV viral circulation has been detected up to the end of study period (19).

Compared to the epidemiological analysis conducted by EFSA (20), which showed two peaks of case notification (August and October) in the 2006 BTV-6 epizooty, the BTV-4 epizooty in the Balkans had only one peak.

These results re-affirm the seasonality of BT in this part of Europe, which starts in spring and might extend by February of the following year. For comparison, the BTV-8 epizooty in north-western Europe started in August 2006 in the Netherlands; the first occurrence of BTV-8 in the United Kingdom was in September 2007; identical to Sweden which declared its first outbreaks of BTV-8 in September 2008 (21, 22, 23).

In Europe, BT is characterized by strong seasonal variations in incidence, related to the seasonality of the vector population (24). The infections usually occur in late summer and autumn (25), largely because of environmental factors, such as humidity and temperature (26). Wind was also a significant contributor to the spread of the infection (27, 28). Our future works will be focused on modelling the influence of the animal demographic and bioclimatic conditions on the spread of BT in the Balkans.

Bluetongue clusters

Our findings suggest that BT clustered in 32 statistically significant hotspot areas, compared with the two clusters in North-West Europe during the BTV-8 epizooty in 2006. First disease cluster number 1 was identified in Greece in July 2014 with a radius of 56 km.

During the 2006 BTV-8 epizooty in the northern Europe, the first cluster (Maastricht cluster in The Netherlands) was identified in August at a radius of 35 km which extended to 75 km by December 2006. The second cluster was identified in the North-West Belgium (Ghent cluster) in the middle of October with a maximum size of 21.6 km (20). In our study, the second cluster was identified in Bulgaria, 3 weeks after the first cluster in Greece. This cluster was identified in August, 2014 with a radius of 69.8 km, and included both parts of Greece and Turkey.

In comparison, the four detected clusters in 2014 match with administrative areas affected by the BTV outbreaks in 1998 and 2002. Overlapping occurred in Bulgaria (1999, Burgas region) - cluster number 4, Greece-mainland (1999, Evros region) cluster number 2 and the Greek island Lesbos (October 1979) cluster number 13, Macedonia (2001, North-Eastern region-municipality Kriva Palanka) cluster number 23, Albania (2002, Librazhd-Elbasan area) cluster number 7, and Croatia (2001, Dubrovnik-Neretva region) cluster number 32 (29). In September 1999, BTV-4 clusters in Greece were identified in the prefecture of Chalkidiki (16), which is similar to our findings (cluster 10 which was identified in October 2014).

It is an interesting fact that in 2013, there were no declared BT outbreaks neither in mainland nor island areas in the Balkans and Asian part of Turkey. The nearest affected areas were Italy and Sicily with BTV-1/2/16 on the west and the Gaza Strip BTV-2/4 on the east (6). It can be assumed that BTV-4 was circulating at a very low prevalence rate (possibly in livestock and/or wildlife) (30).

Finally, the identified clusters do not oblige authorities to target preventive measures within certain clusters, but allow them monitoring of specific regions in high risk areas and periods (31).

CONCLUSION

Our work can serve as guidance to governmental veterinary authorities to formulate animal health strategies to mitigate the spread of the disease through, vaccination, education campaigns, increased monitoring in targeted areas and interventions to improve biosecurity on the holdings. In addition, it is important to recognize timely and joint regional country actions, as vectors do not recognise frontiers.

CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

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