Serological surveillance of bluetongue virus in cattle in central Iran

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Bluetongue virus,
Cattle,
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Summary
The aim of this study was to evaluate the seroprevalence and distribution of antibodies to the bluetongue virus (BTV) among dairy Holstein cattle of central Iran. From September 2010 to August 2011, 892 blood samples from Holstein dairy cattle were collected from healthy animals. Blood samples were divided according to type of farm (industrial and non-industrial), season (warm and cold), location (North, South, East, and West), cattle production groups (calf, heifer, dairy and dry) and age groups (under 6 months, 6 months-2 years and over 2 years). The sera were screened using a commercially competitive enzyme-linked immunosorbent assay (c-ELISA) kit. Twenty-four sera (2.69 %) were found to be positive for BTV. Bluetongue virus seroprevalence was significantly higher ($\chi^2 = 8.29$, df = 3, $p < 0.05$) in cattle in southern locations as compared to those in other locations. Older animals (> 2 years) showed a relatively higher seroprevalence, but the difference was not statistically significant ($p = 0.06$). No statistically significant difference in BTV seroprevalence was noted between farming systems, seasons and cattle production groups ($p > 0.05$). The results demonstrate that the seroprevalence of BTV is low in cattle from the Isfahan province, central Iran. Further studies are needed to determine the serotypes and vectors of BTV in the central region of Iran.

Sorveglianza sierologica del virus della Bluetongue (BTV) in bovine Holstein nell'Iran Centrale

Parole chiave
Bovino,
ELISA competitiva,
Iran,
Sieroprevalenza,
Virus della Bluetongue.

Riassunto
Il presente studio è stato effettuato con l'obiettivo di valutare la sieroprevalenza e la distribuzione di anticorpi per il virus della Bluetongue (BTV) in bovine da latte Holstein nella provincia di Esfahan, situata nella zona centrale dell'Iran. Tra settembre 2010 e agosto 2011 sono stati prelevati campioni di sangue da 892 animali sani. I campioni sono stati suddivisi in base alla tipologia di allevamento, alla stagione, alla zona di provenienza, al periodo produttivo e all'età dell'animale. Lo screening dei sieri è stato effettuato con kit commerciale c-ELISA. Ventiquattro sieri (2,69%) sono risultati positivi per BTV. La sieroprevalenza è risultata molto più elevata negli animali testati nelle zone meridionali ($\chi^2 = 8,29$, $p<0,05$). Negli animali di età superiore ai 2 anni la sieroprevalenza è risultata più alta ma la differenza non è stata statisticamente significativa ($p=0,06$). I diversi tipi di allevamento, le stagioni e i diversi stadi del periodo produttivo non hanno permesso di rilevare differenze statisticamente significative ($p>0,05$). In conclusione i risultati hanno dimostrato bassi valori di sieroprevalanza nei bovini testati. Gli autori indicano la necessità di ulteriori studi per determinare sierotipi e vettori di BTV nella regione centrale dell'Iran.

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Introduction

Bluetongue (BT) is a non-contagious, infectious viral disease of ruminants. Bluetongue viruses (BTV) are arthropod-borne and comprise the type species of the genus *Orbivirus*, family *Reoviridae*. To date, a total of 26 BTV serotypes have been recognised worldwide (8, 11, 23, 17, 21). Bluetongue is responsible for substantial economic losses in ruminants and, because of this economic impact, it has been included in a list of notifiable diseases by the World Organisation for Animal Health (*Office International des Épizooties*: OIE) (23). The infection occurs throughout tropical and subtropical regions as well as in some temperate regions of the world (between latitudes 34°S and 53°N) (5, 6, 12, 18), where many populations of the adult *Culicoides* vectors reproduce and can affect domestic and wild ruminants (17, 21).

Based on virological and serological studies, BTV is widely present in sheep and goats in Iran (1, 3, 9, 13, 16). However, only limited information is available on BTV in cattle in Iran (14).

The clinical signs of BT disease in cattle range from a mild febrile illness to extensive erosions of the oral mucosa, at times such symptoms are confused with the symptoms of foot and mouth disease (FMD) (19). To our knowledge, there is no confirmed report of BT in Iran, although some field veterinarians have previously reported suspected BT-like disease in cattle. Similarly, there have been reports of FMD-like disease in cattle from central Iran that had previously been immunised against FMD. Therefore, the aim of this study was to evaluate the seroprevalence and distribution of antibodies to BTV among dairy Holstein cattle in central Iran.

Material and methods

The study was carried out on industrial and non-industrial Holstein dairy cattle farm around Isfahan (latitude 30° 43'-34° 27' N, longitude 49° 36'-55° 31' E), central Iran.

From 26 September 2010 to 25 August 2011, a total of 892 blood samples were collected via the jugular vein from healthy cattle from 24 randomly selected Holstein dairy farms in the North, South, East and West of Isfahan. According to the farm records, clinical symptoms related to BTV had not been observed in the animals. Cattle were grouped according to the type of farm (industrial: 621 and non-industrial: 271), season when the samples were collected (warm: 512 and cold: 380), location (North: 199; South: 211; East: 226; and West: 256), production (calf: 123; heifer: 122; in milk production: 514; and during the dry period: 133), and age (under 6 months: 124; 6 months-2 years: 128; and over 2 years: 640). The samples were transported to the laboratory in iceboxes. After proper clotting, the blood samples were centrifuged at 5,000 rpm for 10 min and the serum samples were stored at –20°C until analysis.

The sera were screened using a commercially c-ELISA kit (IDEXX/Institut Pourquier, Montpellier, France) following the manufacturer's instructions. This test is based on the detection of antibodies specific to the VP7 protein of BTV, and is designed to detect antibodies against any serotype of BTV. One positive and two negative controls provided by the manufacturer were included in each test. Results were expressed as a percentage of the S/N ratio, calculated as % S/N = 100 × sample absorbance/ negative control mean absorbance. According to the manufacturer's specifications, a sample is considered to be positive if its S/N% value is less than or equal to 70%.

Chi square (χ^2) test (SAS software, version 8.2) was used to assess the association between the seroprevalence of BTV and farming systems, seasons, locations, cattle production groups and age groups (20). Results were considered statistically significant for $p < 0.05$.

Results

The overall seroprevalence was 2.69% (95% confidence interval [CI], 1.82-3.97). Bluetongue virus seroprevalence was significantly higher ($\chi^2 = 8.29$, $p < 0.05$) in cattle from southern locations as compared to those in other locations (Table I).

Older animals (> 2 years) showed a relatively higher seroprevalence as compared to other age groups, but the difference was not statistically significant ($p = 0.06$). Similarly, no statistically significant difference in BTV seroprevalence was noted between farming systems ($p = 0.44$), seasons ($p = 0.45$) and cattle production groups ($p = 0.11$).

Discussion

Iran is considered a favourable country for presence and abundance of BTV vectors, because of its latitude and climate (15). Several studies have been performed in different parts of the country for BTV and seropositive sheep and goats have been extensively reported in the West, South-West, North-West and Central provinces of Iran (1, 3, 9, 13, 16).

This study reports for the first time the presence of BTV antibodies in cattle from Isfahan province, central Iran. Interestingly, the value of the seroprevalence was similar to the one found in cattle in South-East Iran (14), but it was much lower

Table I. *Distribution of bluetongue virus seroprevalence according to farming system, season, location, cattle production groups and age.*

Category	Level	Number tested	Positive	Seroprevalence (%) (95% confidence interval)
All animals	All animals	892	24	2.69 (1.82-3.97)
Farming system	Industrial	621	15	2.42 (1.48-3.95)
	Non-industrial	271	9	3.32 (1.76-6.19)
Season	Warm	512	12	2.34 (1.34-4.05)
	Cold	380	12	3.16 (1.82-5.44)
Location	North	199	4	2.01 (0.78-5.05)
	South	211	11	5.21 (2.93-9.09)
	East	226	2	0.88 (0.24-3.16)
	West	256	7	2.73 (1.33-5.53)
Cattle production group	Calf	123	0	0
	Heifer	122	2	1.64 (0.45-5.78)
	Dry	133	3	2.26 (0.77-6.43)
	Dairy	514	19	3.7 (2.38-5.71)
Age group	Under 6 months	124	0	0
	6 months-2 years	128	2	1.56 (0.43-5.51)
	Over 2 years	640	22	3.44 (2.28-5.15)

than the values recorded in sheep (53%) and goats (49%) from the same province (16). The value of the seroprevalence was also different from the one reported in neighboring countries where high BTV seroprevalences were detected both in cattle and in sheep and goats (7, 10). Since the test used in this study (c-ELISA) had 100% sensitivity and 98.0% specificity, and the sample size was sufficient for the study purpose, the apparent low seroprevalence in cattle in our study might reflect that either the vector does not bite cattle or that cattle only rarely come into contact with infected vectors.

Species of *Culicoides* from Iran have been previously reported (15), but there is no information on the *Culicoides* vectors of BTV in Iran (9). BTV seroprevalence was significantly ($p < 0.05$) higher in cattle in southern locations as compared to those in other locations. This can probably be due to their proximity to the Zayandeh-Rood River (16); this river plays an effective role on the climatic conditions and the humidity of the region, and provides excellent opportunity for reproduction, proliferation and propagation of vectors (15).

As expected, older animals were found to have a higher seroprevalence than younger animals. As described by Singer *et al.* 1998 (22), the higher seroprevalence in older animals is probably due to a longer time of exposure to the vector.

Although cattle-housing hygiene level (manure, pond, and stagnant water management) in non-industrial farms is lower than in industrial farms and the environmental conditions in non-industrial farms is suitable for vectors activity, contrary to expectations, no significant difference in BTV seroprevalence was shown between cattle kept in industrial and non-industrial farms.

BTV is highly seasonal and antibody levels can be more easily detected after the vector season (4). However, no statistically significant difference in BTV seroprevalence was found between cold and warm seasons. This observation is in contrast with the results reported by Noaman *et al.* (16), who observed higher BTV seroprevalence in sheep and goats during the warm season. This finding might indicate the absence of new infection.

The results of this study suggest that the clinical symptoms observed by some field veterinarians in cattle in this region were probably not related to BTV infection. However, further investigations on BTV epidemiology, including studies on BTV serotypes and vectors, should be planned in Iran.

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