Prevalence of Bluetongue virus serotype 4 in cattle in the State of Sao Paulo, Brazil

Adriana Hellmeister de Campos Nogueira[1], Eliana De Stefano[1], Maira de Souza Nunes Martins[1], Liria Hiromi Okuda[1], Michele dos Santos Lima[1], Thais da Silva Garcia[1], Otto Heinz Hellwig[2], José Eduardo Alves de Lima[2], Giovanni Savini[3] and Edviges Maristela Pituco[1*]

[1] *Instituto Biológico, Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Laboratório de Viroses de Bovídeos, Avenida Conselheiro Rodrigues Alves 1252, Sao Paulo, 04014-900, Brazil.*
[2] *Coordenadoria de Defesa Agropecuária do Estado de Sao Paulo, Av. Brasil 2340, Campinas, Sao Paulo, 13070-178, Brazil.*
[3] *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Campo Boario, 64100 Teramo, Italy.*

[*] *Corresponding author at: Instituto Biológico, Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Laboratório de Viroses de Bovídeos, Avenida Conselheiro Rodrigues Alves 1252, Sao Paulo, 04014-900, Brazil. Tel.: +55 11 5087 1786, e-mail: pituco@biologico.sp.gov.br.*

Veterinaria Italiana 2016, **52** (3-4), 319-323. doi: 10.12834/VetIt.570.2721.1
Accepted: 09.07.2016 | Available on line: 30.09.2016

IV International Conference on *Bluetongue and Related Orbiviruses*. November 5-7, 2014 - Rome, Italy - Selected papers

Keywords
Bluetongue virus,
Competitive ELISA,
Orbiviruses,
Serotype 4,
South America,
Virus neutralization.

Summary
Bluetongue (BT) is considered endemic in several regions of Brazil. The State of Sao Paulo was divided into 7 cattle production regions (circuits) according the different systems of breeding, operational and logistical capacity of the state veterinary service. At least 1 animal from each property (a total of 1,716 farms) was tested by competitive ELISA for the presence of antibodies against BTV. Sero-positive sera were subsequently also tested by virus neutralization tests (VNT) using serial dilutions from 1:10 (cutoff) up to 1:640 (in MEM). BTV-4 neutralizing antibodies were detected in 86% (1,483/1,716) of the animals tested. These results show that BTV-4 is endemic and widespread in the State of San Paulo and indirectly confirm that in the State there are favourable conditions for the multiplication of competent vectors. However, as no clinical signs have ever been reported in cattle in the region, BTV-4 infection is likely to occur silently in the State of Sao Paulo.

Prevalenza del sierotipo 4 del virus della Bluetongue nei bovini dello stato di San Paolo, Brasile

Parole chiave
Bluetongue,
Brasile,
Virus neutralizzazione,
Orbivirus,
ELISA competitiva,
Sierotipo 4 del virus della Bluetongue,
Sud America.

Riassunto
La Bluetongue (BT) è endemica in diverse regioni del Brasile. Lo Stato di San Paolo è stato diviso in 7 regioni (circuiti) in relazione ai diversi sistemi di allevamento bovino e alle capacità logistiche e operative del servizio veterinario statale. Almeno un animale per ogni allevamento (per un totale di 1.716 aziende) è stato esaminato con test ELISA competitivo per la presenza di anticorpi anti-BTV. Gli antisieri sieropositivi sono stati testati attraverso il test di neutralizzazione virale (VNT) utilizzando diluizioni seriali da 1:10 (limite massimo) fino a 1:640 (diluzioni in terreno di coltura MEM). Anticorpi neutralizzanti BTV-4 sono stati osservati nell'86% (1.483/1.716) degli animali testati. I risultati ottenuti oltre a suggerire che BTV-4 è endemico e diffuso nello Stato di San Paolo confermano indirettamente che in questo stato esistono condizioni favorevoli per la riproduzione di vettori competenti. Tuttavia, visto che non sono mai stati notificati casi clinici nei bovini della regione, è verosimile che l'infezione da BTV-4 nello Stato di San Paolo sia silente.

Introduction

Bluetongue (BT) is an infectious disease caused by the Bluetongue virus (BTV), which is transmitted by adult *Culicoides* biting midges (Tabachnick 2004). The Bluetongue virus is classified as the 'type species' of the genus *Orbivirus*, within the family *Reoviridae*. There are 27 BTV serotypes that have been recognized so far, including recently discovered serotypes: BTV-25, Toggenburg Orbivirus (TOV) isolated in Switzerland (Hofmann *et al.* 2008); BTV-26 isolated in Kuwait (Maan *et al.* 2011), and BTV-27, isolated in Corsica (Jenckel *et al.* 2015).

Bluetongue is classified as a 'notifiable disease' by the World Organization for Animal Health (OIE 2014) and has important socioeconomic impacts that affect international trade in animals and their products. The disease was first described in South Africa in the late 18th century and was called epizootic malignant catarrhal fever of sheep (Hutchen 1902). In 1905 the name 'bluetongue' was proposed (Ronderos *et al.* 2003) due to the inflammation and cyanotic appearance of the tongue and oral mucosa, (MacLachlan 1994). In 1906, it was demonstrated that the disease was caused by a virus, when blood of sick sheep was injected into susceptible animals, thereby reproducing clinical disease (Lobato 1999). According to Cunha and colleagues (Cunha *et al.* 1987, Cunha *et al.* 1988), BT emerged in Brazil due to the importation of infected animals.

The disease affects ruminants of economic interest, including sheep, goats, cattle, buffalo, and deer, although clinical signs are most often observed in sheep and certain deer species. The most commonly observed clinical signs are facial oedema, erosion and ulceration of the gastrointestinal tract, coronitis lameness, and fever, with a high rate of mortality, reproductive problems (Lobato 1999).

Differential diagnosis of BT is of great importance, due to similar clinical signs with foot and mouth disease, malignant catarrhal fever, contagious pustular dermatitis, poxvirus infections, border disease, and 'foot root' Actinobacillosis.

As BT is transmitted by insects of the genus *Culicoides*, knowledge of the competent vector species in different ecosystems is important, in order to understand the epidemiology of the disease. The ability of adult *Culicoides* to transmit BTV varies with the species and is markedly influenced by climate, temperature, air humidity, and the rainfall (Mellor 1996, Mellor 2000).

Two BTV outbreaks have been reported in Brazil. The first occurred in 1980, when the virus was isolated from 4 cattle exported to the USA (Groocock and Campbell 1982). The second one was reported in 2002, when BTV was isolated in 12 sheep during an outbreak in the State of Paraná (Clavijo *et al.* 2002).

Bluetongue is considered endemic in several regions of Brazil, due to the climate and temperature favourable to *Culicoides*. Several serological surveys show a high prevalence, ranging from 10% to 100%, but without clinical disease in cattle, indicating that BTV can spread around the country 'silently'. To date, little is known about the existing BTV serotypes and about the species of *Culicoides* involved in disease transmission.

Materials and methods

In Brazil, the number of cattle in commercial management in 2012 was 211,279,082, of which 10,757,383 belong to Sao Paulo[1]. This number represents approximately 5.1% of the national herd. According to the Köppen-Geiger climate classification, the State of San Paulo has 7 climate types, the one covering the entire central part of the state is the dominant one, and it is characterized by a highland tropical climate, with rain in Summer dry Winters, with an average temperature above 22°C in the warmer months.

The State of San Paulo was divided into 7 cattle production regions (Figure 1), according to the different systems of breedings, operational, and logistical capacity of the state veterinary service. For detection of BTV-specific antibodies, a screening test was performed by competitive ELISA solid phase (ELISA CFS) supplied by Panaftosa (Rio de Janeiro). One positive animal from each property (1,716 samples) was selected to evaluate the presence of antibodies against BTV serotype 4 (BTV-4) by virus neutralization test (VNT). A volume of 50 µl of each serial serum dilution (from 1:10 to 1:640 in MEM) was added to

[1] Instituto Brasileiro de Geografia e Estatística (IBGE). 2012. Sistema IBGE de Recuperação Automática - SIDRA. http://www.sidra.ibge.gov.br/.

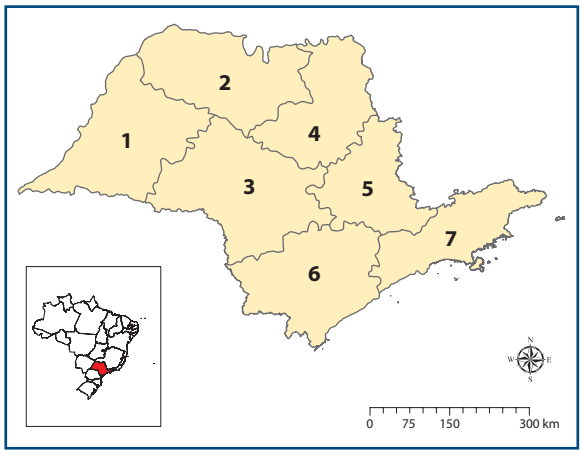

Figure 1. *Map of State of Sao Paulo and division in seven circuits.* In detail, the locate of São Paulo state in relation to Brazil (Dias *et al.* 2009.)

Veterinaria Italiana 2016, **52** (3), 319-323. doi: 10.12834/VetIt.570.2721.1

Table I. *Number and percentage of cows not reactors and reactors according to antibodies titers by virus neutralization assay to BTV-4 in the 7 Circuits of State of São Paulo, in 2011.*

Titers express in aritmetic numbers	Circuits														Total
	1		2		3		4		5		6		7		
	N*	%	N*	%	N*	%	N*	%	N*	%	N*	%	N*	%	
NR	19	7.8	79	30	28	10.9	33	14.2	26	10.6	17	7.5	31	12.6	233
10	23	9.5	66	25.1	59	22.9	72	31	76	30.9	42	18.5	53	21.5	391
20	27	11.1	57	21.7	50	19.4	66	28.4	60	24.4	58	25.6	61	24.7	379
40	55	22.6	48	18.3	67	26	36	15.5	49	19.9	55	24.2	52	21.1	362
80	46	18.9	9	3.4	36	14	16	6.9	26	10.6	29	12.8	32	13	194
160	42	17.3	1	0.4	13	5	6	2.6	8	3.3	19	8.4	13	5.3	102
320	20	8.2	3	1.1	4	1.6	2	0.9	0	0	3	1.3	2	0.8	34
640	11	4.5	0	0	1	0.4	1	0.4	1	0.4	4	1.8	3	1.2	21
Total of animals analyzed	243		263		258		232		246		227		247		1,716

each well of a flat bottom microtitre plate, with an equal volume of BTV-4 containing 200 $TCID_{50}$/50 µl. The plates were incubated at 37°C in 5% CO_2. After 1 hour, approximately 50 µl of VERO cells containing 3 x 10^5cells/ml were added to each well and the plates, were incubated as above for 96 hours. The presence of neutralizing antibodies was determined by a lack of cytopathic effect (cpe), observed using an inverted light microscope. The neutralizing antibody titre was calculated as the inverse of the greatest dilution at which neutralization occurred. Samples with titers ≥ 10 were considered sero-positive reactors.

The investigated farms for BT were 'georeferenced' using latitude and longitude, GCS-WGS84. Distribution maps for BTV were developed using ESRI® ArcGIS Desktop 10.1.

Results

Since all sampled cows were older than 24 months, all tested positive to the ELISA assay. The samples were subsequently submitted to virus neutralization tests against BTV-4. In this assay 86% (1,483/1,716) of the tested animals were positive, with titers ranging from 10 to ≥ 640 [95% confidence interval (CI) lower and upper limit 84.7 to 88.0]. Table I shows the number and percentage of cows not reactors and reactors according to neutralising antibodies titers to BTV-4 in the 7 Circuits of State of San Paulo. Figure 2 shows the spatial distribution of farms with BTV-4 positive cattle (red in different intensity) according to neutralising titers and BTV-4 negative cattle (green) in State of San Paulo in 2011.

Discussion

The results revealed a high prevalence of antibodies against BTV-4 in cows. Although no clinical signs of BTV were observed during the study, the presence of antibodies indicates that there was viral circulation in the study area.

The high BT sero-prevalence is consistent with other reports of national herds (Cunha *et al.* 1987, Castro *et al.* 1992, Melo *et al.* 2000, Bernardes 2011), which present similar results in regions with favourable climate conditions to complete the BTV transmission and infection cycle. In 2009, Teomichi and colleagues (Teomichi *et al.* 2009) found 42% (92/219) of seropositivity in bovines in Pantanal Sul mato-grossense. In the same year, in Corumbá, Brazil, it was found 51.3% (181/353) of seropositivity using the agar gel immune-diffusion (AGID) technique. In the State of Rio Grande do Sul, Costa and colleagues (Costa *et al.* 2006) verified a low sero-prevalence of BTV in cattle (0.6%). Although there were also some infected *Culicoides*, the climate conditions were unfavourable for vector multiplication. In regions with arid and semi-arid climate topology, as in the Sertao in the State of Paraíba (Alves *et al.* 2008), the prevalence of BTV in sheep was 8.4%. In the micro-region of Juazeiro in the State of Bahia, Souza and colleagues reported 0.43% of animals with antibodies against BTV (Souza *et al.* 2010). This low sero-prevalence may be due to temperature and humidity conditions in the region, which make vector multiplication more difficult.

One of the risk factors for BTV infection may be the intensive indoor farming of livestock, which may increase the probability of bites by vector insects. According to Cunha and colleagues (Cunha *et al.* 1988), these animals are most susceptible to vector bites, perhaps due to the higher concentration of animals or because of the characteristics of the facilities, such as high humidity, standing water, and organic material, which favour the appearance and multiplication the vector midges. In this study the high prevalence of the infection may also be partially explained by the sampling of 2 year old

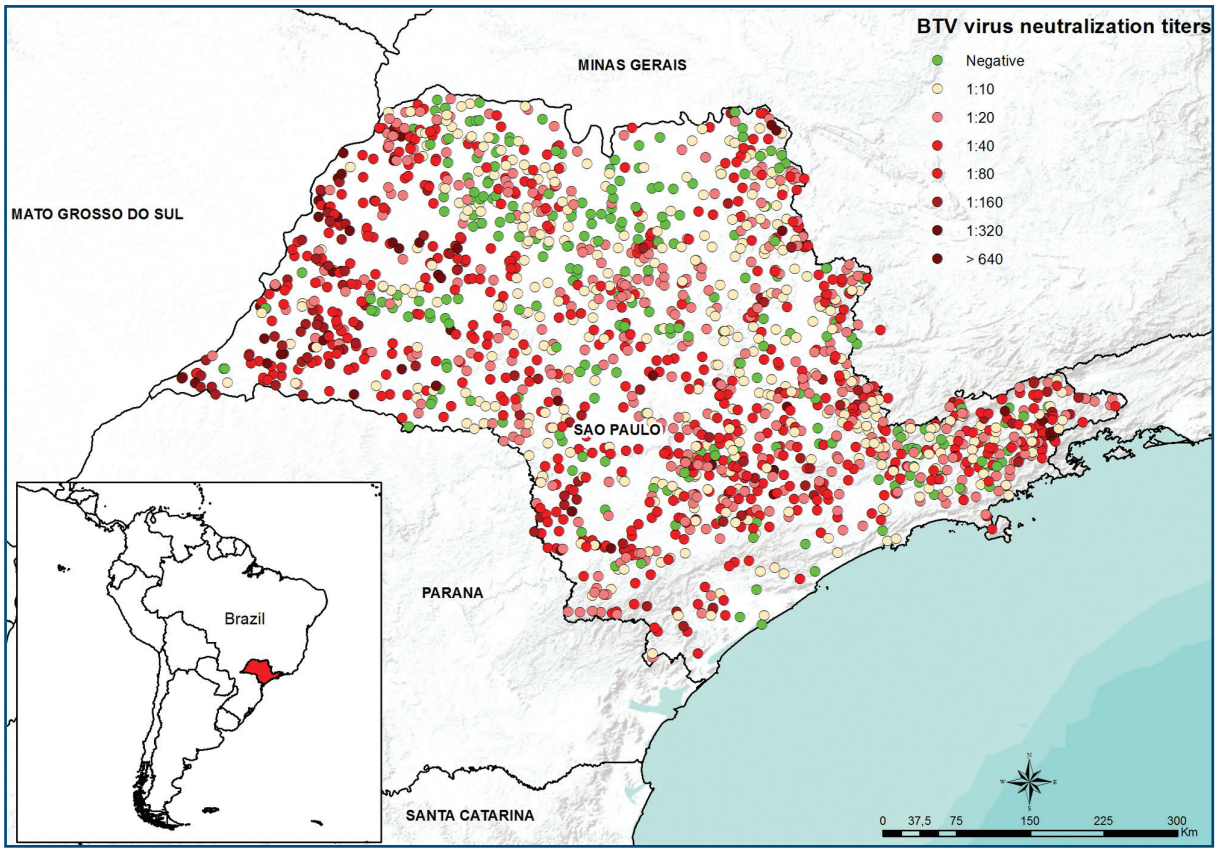

Figure 2. *Spatial distribution of properties with cattle reactors (red in different intensity) according to antibodies titers by virus neutralization assay and no reactors (green) for BTV-4 in State of Sao Paulo in 2011.*

females, which have a higher probability of having suffered a previous/multiple infections.

The results confirmed that BTV infection occurs silently in cattle in the 7 circuits of the State of San Paulo. Although the clinical signs of BTV infection are usually less severe in cattle than in sheep, the lack of apparent clinical signs may reflect circulation of a low virulence strain or adaptation of the animals to the strain(s) that are circulating in Brazil.

The results also indicate that the temperature and humidity conditions in the State of San Paulo are favourable for the multiplication and maintenance of competent vector midge species, supporting endemic circulation of BTV. These data agree with those provided by Groocock and Campbell (Groocock and Campbell 1982) concerning the circulation of BTV-4 in Brazil.

The diagnosis and confirmation of BT disease lead to the imposition of restrictions of animals movement and trade in their products, affecting both animal health and agribusiness. However, the lack of accurate data concerning the distribution of BTV-4 in the State of San Paulo has more difficult to make decisions concerning prevention and infection-control strategies.

In conclusion, a high prevalence of BTV-4 was detected in cattle in all circuits of the State of San Paulo, demonstrating virus circulation in many regions. Notably, in region 2 (Figure 2), on the border with the state of Minas Gerais, there were animals positive to ELISA and negative to BTV-4 VNT (possible cross-reaction), suggesting the presence of another serotype of BTV in that region.

Veterinaria Italiana 2016, **52** (3), 319-323. doi: 10.12834/VetIt.570.2721.1

References

Alves F.A.L., Alves C.J., Azevedo S.S., Silva W.W., Silva M.L.C.R., Lobato Z.I.P. & Clementino I.J. 2009. Soroprevalência e fatores de risco para a língua azul em carneiros das mesorregiões do Sertão e da Borborema, semiárido do estado da Paraíba. *Ciencia Rural*, **32**, 484-489.

Bernardes N.T.C.G. 2011 Soroprevalência da língua azul em bovinos do Estado de São Paulo, Brasil, Instituto Biológico de São Paulo, São Paulo.

Castro R.S., Leite R.C., Abreu J.J., Lage A.P., Ferraz I.B., Lobato Z.I.P. & Balsamão S.L.E. 1992. Prevalence of antibodies to selected viruses in bovine embryo donors and recioients from Brazil, and its implications in international embryo trade. *Trop Anim Health Prod*, **24**, 173-176.

Clavijo A., Sepulveda L., Riva J., Pessoa Silva M., Tailor Ruthes A. & Lopez J.W. 2002. Isolation of bluetongue virus serotype 12 from an outbreak of the disease in South America. *Vet Rec*, **7**, 301-302.

Costa J.R.R., Lobato Z.I.P., Herrmann G.P., Leite R.C. & Haddad J.P.A. 2006. Prevalência de anticorpos contra o vírus da língua azul em bovinos e ovinos do Sudoeste e Sudeste do Rio Grande do Sul. *Arq Bras Med Vet Zootec*, **58**, 273-275.

Cunha R.G., Souza D.M. & Passos W.S. 1987. Anticorpos para o vírus da Língua Azul em soros de bovinos do Estado de São Paulo e Região Sul do Brasil. *Revista Brasileira de Medicina Veterinária*, **9**, 121-124.

Cunha R.G., Souza D.M. & Teixeira A.C. 1988. Incidência de anticorpos para o vírus da língua azul em soros de caprinos e ovinos do estado do Rio de Janeiro. *Arquivo Fluminense de Medicina Veterinária*, **3**, 53-56.

Dias R.A., Gonçalves V.S.P., Figueiredo V.C.F., Lôbo J.R., Lima Z.M.B., Paulin I.M.S., Gunnewiek M.F.K., Amaku M., Ferreira Nero J.S. & Ferreira F. 2009. Situação epidemiológica da brucelose bovina no Estado de São Paulo. *Arq Bras Med Vet Zootec*, **61**, 118-125.

Groocock C.M. & Campbell C.H. 1982. Isolation of an exotic serotype of bluetongue virus from imported cattle in quarantine. *Can J Comp Med*, **46**, 160-164.

Hofmann M.A., Renzullo S., Mader M., Chaignat V., Worwa G. & Thuer B. 2008. Genetic characterization of Toggenburg orbivirus, a new bluetongue virus, from goats, Switzerland. *Emerg Infect Dis*, **14**, 1855-1861.

Hutchen D. 1902. Malarial catarrhal fever of sheep. *Vet Rec*, **14**, 629.

Jenckel M., Bréard E., Schulz C., Sailleau C., Viarouge C., Hoffmann B., Höper D., Beer M. & Zientara S. 2015. Complete coding genome sequence of putative novel bluetongue virus serotype 27. *Genome Announc*, **3** (2), pii: e00016-15.

Lobato Z.I.P. 1999. Língua Azul: a doença nos bovinos. *Rev Bras Reprod Anim*, **23**, 515-523.

MacLachlan N.J., Nunamaker R.A., Katz J.B., Sawyer M.M., Akita G.Y., Osburn B.I. & Tabachnick W.J. 1994. Detection of bluetongue virus in the blood of inoculated calves: comparison of virus isolation, PCR assay, and *in vitro* feeding of *Culicoides variipennis*. *Arch Virol*, **136**, 1-8.

Maan S., Maan N.S., Nomikou K., Batten C., Antony F., Belaganahalli M.N., Samy A.M., Reda A.A., Al-Rashid S.A., El Batel M., Oura C.A. & Mertens P.P. 2011 Novel bluetongue virus serotype from Kuwait. *Emerg Infect Dis*, **17**, 886-889.

Melo C.B., Oliveira A.M., Azevedo E.O., Lobato Z.I.P. & Leite R.C. 2000. Anticorpos contra o vírus da língua azul em bovinos do sertão da Paraíba. *Arq Bras Med Vet Zootec*, **52**, 19-20.

Mellor P.S. 1996. *Culicoides*: vectors, climate change and disease risk. *Veterinary Bulletin*, **66**, 301-306.

Mellor P.S. 2000. Replication of arboviruses in insect vectors. *J Comp Path*, **123**, 140-146.

Ronderos M.M., Spinelli G.R., Lager I. & Diaz F. 2003. La importância sanitária de los jejenes del gênero *Culicoides* (Díptera:Ceratopogonidae) em la Argentina. *Entomologia y Vectores*, **10**, 601-612.

Souza T.S, Costa J.N., Martinez P.M., Costa Neto A.O. & Pinheiro R.R. 2010. Anticorpos contra o vírus da língua azul em rebanhos ovinos da microrregião de Juazeiro, Bahia. *Arq Inst Biol*, **77**, 419-427.

Tabachnick W.J. 2004. *Culicoides* and the global epidemiology of bluetongue virus infection. *Vet Ital*, **40**, 145-150.

TomichI R.G.P., Nogueira M.F., Lacerda A.C.R, Campos F.S., Tomas W.M., Herrera H.M., Lima-Borges P.A., Pellegrin A.O., Lobato Z.I.P., Silva R.A.M.S., Pellegrin L.A. & Barbosa-Stancioli E.F. 2009 Sorologia para o vírus da língua azul em bovinos de corte, ovinos e veados campeiros no Pantanal sul-mato-grossense. *Arq Bras Med Vet Zootec,* **61**, 1222-1226.

TomichI R.G.P., Serra C.V., Bomfim M.R.Q., Campos F.S., Lobato Z.I.P., Pellegrin A.O., Pellegrin L.A. & Barbosa-Stancioli E.F. 2009. Sorodiagnóstico de doenças da reprodução em rebanhos de bovinos leiteiros de assentamentos rurais de Corumbá, MS. *Arq Bras Med Vet Zootec*, **61**, 986-991.

World Organization for Animal Health (OIE). 2014. Manual of diagnostic tests and vaccines for terrestrial animals. Paris, OIE.