Bluetongue and other orbiviruses in South America: gaps and challenges

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Veterinaria Italiana 2015, 51 (4), 253‑262. doi: 10.12834/VetIt.600.2892.1
Accepted: 21.07.2015 | Available on line: 31.12.2015

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

Keywords
Bluetongue, Bluetongue virus, Culicoides spp., Epidemiology, Orbiviruses, South America.

Summary
South America (SA) has the ideal climatic conditions for occurrence of Bluetongue virus (BTV) and other orbiviruses. Based on serological evidence of BTV circulation, the virus is widespread across SA. However, little knowledge has been acquired about BTV origin and distribution, and circulation of specific serotypes is almost non-existent. The first barrier to a better understanding of Bluetongue (BT) in SA concerns its recognition in the field, as most infections of seropositive ruminants are unapparent in endemic areas. There are few reports on BTV isolation in SA, many of them from viraemic asymptomatic animals. Among the known competent BTV vectors, Culicoides insignis is the most abundant species in SA. However, information about biological characteristics and competence of various other Culicoides species described in SA is missing. The few reports on BT outbreaks lead to an underestimation of the disease impact on the continent economy. To overcome these major gaps, it is necessary to: improve diagnostic structure and disease recognition in the field; identify BTV serotypes and their distribution in different SA areas; and to study the biology and epidemiology of Culicoides. Furthermore, it is important to have a surveillance system for BT, as well as contingency plans for possible outbreaks in SA.

Il virus della Bluetongue e altri orbivirus in Sud America: lacune e sfide

Parole chiave
Bluetongue, Culicoides spp., Epidemiologia, Orbivirus, Sud America, Virus della Bluetongue.

Riassunto
Il Sud America (SA) ha condizioni climatiche ideali per favorire la presenza del virus della Bluetongue (BTV) e di altri orbivirus. Studi sierologici mostrano che il virus della Bluetongue (BTV) è diffuso in tutta il SA. Tuttavia, non si sa molto sull’origine del virus, la sua distribuzione e la circolazione dei sierotipi specifici. Uno dei principali ostacoli che ha favorito questa carenza di informazione è la difficoltà diagnostica, in quanto nelle aree endemiche molte infezioni decorrono in forma asintomatica. I pochi casi di isolamento di BTV in questi territori derivano da animali viremici asintomatici. Culicoides insignis è la specie più abbondante tra i vettori competenti del BTV in SA. Le informazioni sulle caratteristiche biologiche e le competenze di altre specie di Culicoides presenti sono esigue. La mancanza di informazione sui focolai di Bluetongue non permette un’accurata valutazione dell’impatto della malattia sull’economia del continente. Per colmare queste lacune è necessario migliorare le strutture e le capacità diagnostiche in campo, identificare i sierotipi di BTV e la loro distribuzione nelle varie regioni del SA, conoscere la diffusione, l’abbondanza e la competenza vettoriale dei Culicoides presenti in SA. È inoltre di fondamentale importanza adottare un sistema di sorveglianza per la malattia e piani di emergenza per gestire eventuali focolai.
Bluetongue and other orbiviruses distribution in South America

South America (SA) has an extreme geographic and climatic variation, which contributes to a large number of biomes with an unparalleled number of plants and animal species. Basically, the continent can be divided in 4 climate zones:

- tropical spanning on areas close to the equator, with high daily temperatures throughout the year and an extreme amount of precipitation;
- temperate, which is located South of the Tropic of Capricorn, where it is possible to find a great range of temperature variation throughout the year, also with cold Winters;
- arid climates, which are found in deserts, coastal areas, and Northwest areas in Brazil;
- cold region extending in the Southern end of Argentina and Chile, with dry seasons and high winds. Some of these climates are extremely cold, whereas others are extremely hot but with very little precipitation (Pinto et al. 2008).

Based on vector distribution and climatic and environmental conditions, the epidemiology of Bluetongue virus (BTV) can be categorised into zones: endemic, epidemic, and incursion zones. The endemic zone lies in tropical climates where competent Culicoides spp. are actively spreading viruses throughout the year. Bluetongue disease (BT) is rarely observed in this zone. The epidemic zone is located in temperate climates, where competent Culicoides spp. appear during the warm season and disease occurrences are observed seasonally. The incursion zones are those where BT appears every decade or so, associated with climatic changes (Gibbs and Greiner 1994). So, considering the good conditions for Culicoides survival and the distribution of BTV in the world, it is possible to conclude that the majority of SA territory could harbour the disease. However, little is known about BTV and other orbiviruses in SA.

Since the first description of BTV in SA in 1978 (Silva 1978), with the only exception of Uruguay (OIE 2015), at least an indirect evidence of BTV has been found in all SA countries. Figure 1 summarises BTV serological data in domestic ruminants in SA, found in the literature of the past 30 years. Dark blue areas, indicating high BTV seroprevalence, are found in tropical and subtropical areas. As expected, serological surveys indicated a lot of seronegative animals in the temperate and cold zones in the South of Brazil, Argentina, and Chile. Another area with very low seroprevalence is located in the Northeast of Brazil, which, despite being located in a tropical zone, is a very hot and dry region of the country. Serological studies done in Ecuador and Peru indicate that BTV is present in both countries, but the small number of sampled animals does make it difficult to assess prevalence (Lopez et al. 1985, Manilla 1998, Merino Mena 2011). Regarding Bolivia and Paraguay, there are no available data. The BT status of these countries on OIE web page was “disease suspected, but not confirmed”, from 2005 to 2007 in Paraguay, and from 2008 to the first semester of 2010 in Bolivia, respectively. Between 2010 and 2011, the OIE status of Bolivia was “confirmed infection but no clinical disease” (OIE 2015). Also, in Bolivia, a serological survey was done on grey brocket deer (Mazana gouazoubira), which did not reveal any seropositive animal of the 17 sampled, (Deem et al. 2005). Table I shows a summary about BTV serosurveys in domestic ruminants in SA.

Regarding BTV serosurveys on camelids in SA, there are reports from Argentina (Karesh et al. 1998, Puntel et al. 1999, Marcoppido et al. 2010, Marcopiddo et al. 2011), Chile (Lager 2004), and Peru (Rivera et al. 1987). Seropositive alpacas (21%) were only detected in Peru (Rivera et al. 1987).

Although BTV seems to be widespread in SA, the available information about BTV serotypes distribution is very limited. Some initial data was obtained from seroneutralization tests, using AGID
positive sera. Serotypes 1, 2, 4, 6, 10, 12, 13, 14, 17, and 19 were identified in Brazil (Silva 1978, Cunha 1990), while serotypes 12, 14, and 17 (Homan et al. 1985c) were detected in Colombia. In a study conducted by Gumm and colleagues (Gumm et al. 1984), serotypes 14 and 17 were identified in Guiana, and serotypes 6, 14, and 17 were detected in Suriname.

BTV serotypes were only identified by real time polymerase chain reaction (RT-PCR) and virus isolation in Brazil, Argentina, and more recently, in French Guiana. Serotypes 4 and 12 were isolated from outbreaks affecting sheep from the South and Southeast of Brazil (Clavijo et al. 2002, Antoniassi et al. 2010, Balaro et al. 2014). Serotypes 2, 13, and 17 have been detected in symptomatic sheep and goats in the French Guiana (Viarouge et al. 2014). Furthermore, serotype 4 was isolated from asymptomatic cattle in Argentina and Brazil (Groocock and Campbell 1982, Gorsch et al. 2002, Legisa et al. 2013) and serotypes 1, 2, 10, 12, 13, 17,
and 24 were isolated from domestic ruminants in French Guiana (Viarouge et al. 2014). Serotypes 4, 8, 10, and 16 were identified in semen of 2 healthy bulls from the southeast of Brazil (Gasparini et al. unpublished data).

Only few reports are available on Epizootic haemorrhagic disease (EHD), caused by the Epizootic haemorrhagic disease virus (EHDV). Serotypes 1 and 2 were identified by seroneutralization in Colombia, Guiana, and Suriname (Gumm et al. 1984, Homan et al. 1985). The only report of an outbreak of EHD, affecting deer, is from Brazil (Favero et al. 2013). Viarouge and colleagues (Viarouge et al. 2014) reported EHDV-1 isolation and EHDV-6 genome identification from asymptomatic cattle in French Guiana. These studies indicate that EHDV circulates in domestic and wild ruminants in SA. Table II summarises the studies about EHDV identification in SA.

A new orbivirus, the Peruvian horse sickness virus (PHSV), was isolated from horses in an outbreak of neurological disease, in 1997, in Peru (Attoui et al. 2009). In the same year, Rioja virus, lately recognised as Yanan virus-1, was also isolated in Peru from an outbreak of neurological disease in donkeys, cattle, sheep, and dogs (Attoui et al. 2009). The isolation of new orbiviruses in SA brought attention to the diversity of orbivirus species and BTV serotypes that may still need to be identified in the continent.

**Occurrence of clinical disease**

Considering that much of the SA territory is located in an endemic area for the occurrence of both BT and EHD, it is expected that clinical disease is rarely observed. Moreover, considering the great variability of clinical signs and the large number of other endemic diseases that have similar clinical aspect, it is possible to assume that clinical cases are underreported. Mild clinical signs of BT were identified in sheep and goats in French Guiana (Viarouge et al. 2014), however, outbreaks of BT and EHD have been described only in Brazil (Clavijo et al. 2002, Antoniassi et al. 2010, Favero et al. 2013, Balaro et al. 2014). The few clinical reports of BT in SA are summarised as follows:

- The first report of an outbreak of BT with clinical disease occurred in Curitiba, Parana State, Brazil, in April 2001. Twenty-one sheep and 1 goat had clinical signs that were consistent with a diagnosis of BT. Two sheep and 1 goat died from the disease. Bluetongue virus was detected in tissues of some animals using RT-PCR. Virus isolation and immunoperoxidase assay confirmed the diagnostic. Virus neutralisation (VN) test was used to type the BTV isolate, and BTV-12 was identified (Clavijo et al. 2002).
- In February 2002, an outbreak affecting goats, with the death of 13 animals, occurred in Parana State. In the following month, 2 outbreaks affecting 29 sheep (18 died) and 13 goats (9 died) occurred in the same state. There is no information confirming BTV diagnosis and the serotype involved in these 3 outbreaks (Lager 2004, OIE 2015).
- In March and April of 2009, 2 outbreaks occurred in Rio Grande do Sul, in the South of Brazil, in 2 farms, affecting sheep. On the first farm, 14 sheep of both sexes and different ages showed clinical signs suggestive of BT. Eight of them died and had lesions compatible with the disease. On the second farm, in April, 2009, 1 young male showed clinical signs and lesions with 24 were isolated from domestic ruminants in French Guiana (Viarouge et al. 2014). Serotypes 4, 8, 10, and 16 wereidentified in semen of 2 healthy bulls from the southeast of Brazil (Gasparini et al. unpublished data).

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### Table II. Epizootic haemorrhagic disease virus identification in South America.

<table>
<thead>
<tr>
<th>Country</th>
<th>Region</th>
<th>Species/technique/number of samples</th>
<th>Seroprevalence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>São Paulo</td>
<td>Cattle, sheep, goat and wild ruminants/ELISA/?</td>
<td>39% (EHDV); 39% (EHDV and BTV)</td>
<td>Pandolfi et al. 1998a</td>
</tr>
<tr>
<td></td>
<td>São Paulo and Mato Grosso do Sul</td>
<td>Deer (Blastocerus dichotomus)/ELISA/81</td>
<td>74% (EHDV); 60% (EHDV and BTV)</td>
<td>Pandolfi et al. 1998b</td>
</tr>
<tr>
<td>Colombia</td>
<td>Antioquia</td>
<td>Dairy cattle/AGID and SN/86 (cows); 29 (calves and heifers); Zebu cattle/AGID and SN/49</td>
<td>Dairy cattle: 10.47% (EHDV-1); 2.33% (EHDV-2) (cows); 20.69% (calves and heifers); Zebu cattle: 6.12% (EHDV-1); 2.04% (EHDV-2)</td>
<td>Homan et al. 1985c</td>
</tr>
<tr>
<td>French Guiana</td>
<td>Saint-Laurent, Sinnamary, Matità, Cayenne, Kaw</td>
<td>Cattle and buffaloes/ELISA, RT-PCR and VI/122</td>
<td>ELISA: 60% (EHDV) and 38.52% (EHDV and BTV); RT-PCR: 40% (EHDV) and 39% (EHDV and BTV)</td>
<td>Viarouge et al. 2014</td>
</tr>
<tr>
<td>Guiana</td>
<td>Many regions</td>
<td>Cattle/SN/48 Sheep and goats/SN/NA</td>
<td>Cattle: 27.08% (EHDV-1); 4.17% (EHDV-2); 4.17% (Ibaraki virus); Sheep and goats: no seropositive animals</td>
<td>Gumm et al. 1984</td>
</tr>
</tbody>
</table>

*NA = not available data/information*
that also suggested BT. The diagnosis of BTV-12 was confirmed by RT-PCR and sequencing (Antoniassi et al. 2010).

- From January to April 2013, dairy ewes from a farm in Vassouras, Rio de Janeiro State, Brazil, presented clinical and pathological findings suggestive of BTV. BTV-4 was identified after molecular analysis.

- From January to October 2014, 7 outbreaks affecting sheep occurred in 5 towns in Rio Grande do Sul (RS), Brazil. The mortality rate varied from 1.7% to 56%. The major clinical signs observed were dyspnoea with nasal discharge and laminitis. The major lesions observed were pneumonia, haemorrhage of the pulmonary artery, and esophagitis with ulcers, all suggestive of BT. BTV-4 was identified as the involved serotype in all farms. This was the first report of BTV-4 circulation associated with clinical signs in RS state (Guimarães et al. unpublished data).

- Viarouge and colleagues (Viarouge et al. 2014) described clinical disease compatible with BT in sheep and a goat in French Guiana. Three sheep showed clinical signs such as conjunctivitis, nasal flow, ulcerative crusting on the lips, haemorrhagic ulcer on posterior cloves (crown), dry scabs around the eyes and on the head, and facial oedema. One goat showed inflammation of the vulva and crusting at the corners of the lips. The serotypes identified by RT-PCR from these animals were: BTV-2, BTV-13, and BTV-17.

- In February 2008, at a Zoo in Santa Catarina State, Brazil, 1 gray brocket deer (Mazama gouazoubira) died five days after presenting sublingual swelling, drooling, lethargy, prostration, glossitis, slight cyanosis, and blood on the perineum. Seventeen days later, a 1-year-old male pygmy brocket deer (Mazama nana) from the same zoo suddenly died. Infection by EHDV was detected by RT-PCR, and confirmed by virus isolation and immunoperoxidase assay (Favero et al. 2013).

A great number of Culicoides species has been described in SA, but the most abundant and more often reported is Culicoides insignis. Other frequently reported species in different countries of the continent are Culicoides foxi, Culicoides paraenses, Culicoides venezuelense, Culicoides lahillei, Culicoides leopoldoi and Culicoides limai. However, their role on orbiviruses transmission is still unknown. Culicoides pusillus does not seem to be as important in SA as it is in Central and North America. This species has been described together with many others that were captured in small numbers, such as Culicoides debilipalpis, Culicoides acotylus, Culicoides antunesi, Culicoides betesi, Culicoides bimaculatus, Culicoides brasilianum, Culicoides briceni, Culicoides claviesi, Culicoides crucifer, Culicoides debilipalpis, Culicoides denisae, Culicoides diabolicus, Culicoides duartei, Culicoides flavivenula, Culicoides guyanensis, Culicoides horticulta, Culicoides maruim, Culicoides lutzi, Culicoides paraghacoci, Culicoides paramarum, Culicoides pausienfuscatus, Culicoides phlebotomus, Culicoides plaumani, Culicoides recifei, Culicoides todatangae, Culicoides uniradialis, Culicoides viartei (Gorsch et al. 2002, Ronderos et al. 2003, Corrêa et al. 2007, Aybar et al. 2010, Carvalho and Silva 2014).

Figure 2 summarises orbivirus identification in SA from symptomatic and asymptomatic animals.

**Vector distribution**

Many gaps related to the information about the orbiviruses vectors in SA exist. Because Culicoides pusillus have been described as the main biological vector of orbivirus in Central America (Mo et al. 1994), they are believed to be responsible for orbiviruses transmission in SA too, although BTV and/or EHDV have never been isolated from any vector in this continent.
Orbiviruses in South America: gaps and challenges

**Susceptible host**

South America has an important role in ruminant production in the world and presents a great number of orbiviruses susceptible hosts concentrated in endemic and epidemic areas.

Ruminant species and breeds were introduced in SA originally by the first European settlers and were forced to be adapted to the climatic and vegetation conditions. In the 20th century, mainly from the 70’s to the 90’s, there was an increase in the importation of highly productive breeds of sheep and goat to ensure genetic improvement for meat and milk production in SA. In Brazil, for example, from 1987 to 2006, live small ruminants were mainly imported from many European countries, as well as from Australia, New Zealand, and North America1.

To date, there are many mixed ruminant breeds and it is necessary to know their susceptibility to BTV. In Brazil, sheep breeds reported to be susceptible to BT clinical disease are Lacaune, Texel, Hampshire Down, Corriedale, and Santa Ines, which is a Brazilian breed (Antoniassi et al. 2010, Balaro et al. 2014). In French Guiana, sheep and goat breeds affected were Martinik and Creole, respectively (Viarouge et al. 2014).

In SA, camelid species are important mainly for wool production, such as alpacas, vicunas, and guanacos. Although serological surveys tried to find BTV antibodies in these species, all of them were negative (Karesh et al. 1998, Puntel et al. 1999, Lager 2004), with the exception of a study in Peru where a seropositivity of 21% was found in alpacas (Rivera et al. 1987).

Although prevalence studies in cattle and buffalo indicate a high level of seropositivity (Cunha 1990, Lage et al. 1996, Melo et al. 2000, Konrad et al. 2003, Venditti 2009, Martins et al. 2011), clinical signs associated with BTV or EHDV infections in these species have never been reported in the continent.

**Climate changes**

There is increasing evidence that climate changes are influencing the distribution pattern of insect-borne pathogens such as BTV, as shown by its Northward expansion in Europe since the late 1990s (Purse et al. 2005, Purse et al. 2008, Wilson and Mellor 2008, Mardulyn et al. 2013). Due to their small size and poikilothermy, Culicoides are particularly susceptible to climate change (Wilson and Mellor 2008). Higher temperature reduces the time required for oogenesis and oviposition in Culicoides females, as there is a decrease in the time necessary to digest a blood meal (Mullens and Holbrook 1991). Therefore, higher temperatures may increase the biting rate, thus augmenting the possibility of BTV transmission between vector and host. Moreover, higher larval development temperatures may increase the competence of Culicoides for one or two generations, or even induce competence in otherwise non-competent species, as a consequence of the “leaky gut” phenomenon (Mellor 2000, Mellor et al. 2000). Environmental temperature also has a direct effect on BTV replication rate in the insect, as the activity of the viral RNA-polymerase is positively influenced by temperature. Bluetongue virus requires a minimum temperature between 10°C and 15°C to replicate inside the Culicoides vector (Wilson and Mellor 2009, Carpenter et al. 2011). Therefore, at higher temperatures, BTV replication rate increases, thus reducing the time required for the virus to reach the salivary glands (the extrinsic incubation period, EIP). A shorter EIP may further increase the proportion of vectors that would be able to transmit the virus to the next host (Wilson and Mellor 2008).

Climate changes occur in SA, although there is little consensus on the impact that these will have. There is a general agreement on the increase of 2-4 degrees of the average temperature in the continent until the end of this century (Nobre et al. 2004). Associated with that, a change of the precipitation pattern in some areas has also been envisaged. This may change Culicoides distribution, and consequently, BTV and related orbiviruses distribution may also change.

Temperatures in SA have been forecasted to change in the range of 0.4°C to 1.8°C by 2020 and between 1.0°C and 7.5°C by 2080 (Magrim et al. 2007). The annual precipitation average is projected to decrease in Northern SA, near the Caribbean coasts, and in large part of Brazil, Chile, and Argentina, while it is expected to increase in Colombia, Ecuador, Peru, around the equator, and in South-Eastern SA. Emerging diseases, including food-borne and vector-borne diseases, are expected to increase and have an impact on human and animal health (Pinto et al. 2008).

**Economic impact**

As important information related to the disease occurrence is missing, it is very difficult to assess precisely the economic losses caused by BTV and related orbivirus in SA. Indirect losses caused by trade restrictions imposed by importing countries for live animals, semen, embryos, and products derived from ruminants will cause a major economic impact.

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in the continent, although no precise estimate has been provided yet.

Balaro and colleagues (Balaro et al. 2013) evaluated the costs of the 2013 BTV outbreak in Rio de Janeiro state, Brazil. A longitudinal prospective case study was conducted in the affected farms from March 2013 to August 2013 to assess all treatment costs linked to illness and subsequent co-morbidities in the flock. Mortality, abortion, medication, veterinary, and additional labour services, as well as the drop of production registered in 6 months, led to a loss of US$ 6,700.

**Gaps and challenges**

In the past 10 years, excellent reviews about BT situation in SA have been published (Larger 2004, Legisa et al. 2014). But what draws attention is that, during these years, little progress on the understanding of this disease was made. Essential data to better understand the past, present, and future of BT and other animal diseases caused by orbiviruses in SA are still missing and despite the efforts of researchers, many gaps need to be filled. The following questions are particularly relevant: how many and which BTV and EHDV serotypes are present in SA? Which genotypes and topotypes are circulating? How are they distributed in the continent? What about virulence and virus biological characteristics? Are there other orbiviruses species important for animal health in the continent?

As for the vectors, more questions could be added to the previous: how many species of Culicoides are important for orbivirus transmission and how are they distributed in the continent? What are the SA Culicoides biology and ecology? Which will be the impact of climate change in vectors distribution?

In relation to susceptible hosts, there are some more questions: which SA species and breeds are susceptible to BT and other orbiviruses infection/clinical diseases? Which species of wild animals are susceptible to BT and other orbiviruses infection/clinical diseases? Do they play a role in the transmission/epidemiology of the virus to domestic species? How much are the losses caused by the disease in SA?

Considering all the gaps reported here, a lot of challenges came up to enable a better understanding of BTV and related orbiviruses in SA.

The implementation of surveillance programs for virus identification and characterisation that may be supported by SA governments should be the major challenge. As BTV and EHDV symptomatic animals seem to be a rare event in the continent, sentinel and asymptomatic animals should be considered as a target for virological surveillance. One important point is to improve the clinical surveillance for identification of BT and EHD in the field. Due to the wide variation in terms of clinical signs, it could be assumed that there are many clinical cases underreported or misdiagnosed with other endemic diseases. Veterinarians must include these diseases in differential diagnosis of diseases that affect domestic and wild ruminants. Moreover, disease reporting must be improved to enable a better understanding of its distribution and economic impact, as well as, the readiness for an early response, prevention, and intervention measures in susceptible livestock, wildlife, and vectors biology.

To support these actions, diagnostic structures must be improved, not only to detect diseases caused by orbiviruses, but also to be able to differ them from other endemic diseases. It is important to have laboratories that can offer orbiviruses serologic and etiologic diagnostic tests to identify the viruses rapidly. Molecular characterization of isolates can help with valuable epidemiologic information about virus circulation and origin and elucidate BTV and other orbiviruses epidemiology in the continent.

Vector's identification, surveillance and study of biological and behavioural characteristics are also essential. It is important to determine seasonal occurrence and abundance, which could be correlated with the risk of disease occurrence. Because arthropods are highly sensitive to environmental and seasonal temperatures, the range of vector-borne diseases can be highly affected by climate change. The impact of these changes in the distribution of viruses is unknown, so it is important to connect the orbivirus research groups with geographic/climatic factor research groups. It is important to link climate data with surveillance systems with the aim of better understand the spatial and temporal positions of pathogens, host, vectors and their interactions.

So far, only a few research groups in SA are systematically working with orbiviruses and Culicoides. In this sense, investigation and training on these topics must be stimulated. Finally, the major challenge may be to join a multidisciplinary team to work together. Farmers, veterinarians, animal health authorities, epidemiologists, entomologists, biologists, meteorologists, virologists, and economists should all contribute with their expertise to improve our overall knowledge of BTV and related orbiviruses in SA.
References


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