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Research Article

Amblyomma Variegatum, Hyalomma Impeltatum and Hyalomma Truncatum Anthropophilic Ticks Introduced To Gabon with Cattle Imports: A Potential Threat for Public Health

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Abstract: This survey aimed to characterize the breeds of zebus involved in the introduction of anthropophilic ticks from Cameroon to Gabon, to identify the tick species related the cattle imports, to compare the relative frequencies of these acarian, to determine the factors associated with the anthropophilic ticks introduction to Gabon and to propose a preventive treatment when cattle cross the border. 712 ticks were collected from 156 zebus between 5 to 6 years old imported from Cameroon to Gabon. Arthropods and cattle were characterized. Questionnaires were used in order to identify factors associated with the introduction of anthropophilic ticks to Gabon .The zebus Fulbe and Bororo were the breeds of cattle imported from Cameroon to Gabon. Amblyomma variegatum (92.7%), Hyalomma truncatum (6.7%) and Hyalomma impeltatum (0.6%) were the tick species associated with the cattle imports entering to Gabon. These arthropods are able to introduce to Gabon Rickettsia africae, Rickettsia aeschlimannii, the virus of Crimea- Congo hemorrhagic fever as well as the Dogbe virus that are a threat for public health. The lack of a veterinary control service at the border in Bitam and of governmental decision to create a veterinary control service; were respectively considered as health determinant and contributing factor associated to the introduction to Gabon of anthropopfhilic ticks**.**

Keywords: Determinant, Factor, Ticks, Anthropophilic, Cattle, Gabon, Cameroon.

INTRODUCTION

Several factors such as war, transhumance, migrations of animals, animal international trade and the conquest of new territories can contribute to spread ticks in the world because these arthropods move with animals when they are attached to their body. During second war world horses imported from Australia used by American armed forces introduced *Rhipicephalus* (*Boophilus*) *microplus* to New Caledonia (Fivaz B *et al*., 1992). This tick found a favorable environment for completing its life cycle; with as the consequence the loss of $ 1580 per year when the infestation intensity on animals is 10 females engorged per day (Marchal C, 2011). In this country, this arthropod is become resistant to acaricides such as deltamethrin, tactik and ethion (Chevillon C *et al*., 2007). Transhumance practiced in several West African countries is another factor that can facilitate the tick spreads because it involves the movements of 70 – 90% of cattle population on long distances (Bouslikhane M, 2015). During transhumance animals can move following the seasons from a region to another looking for food (Byaruhanga C, 2017), this could be the main factor of expansion of *Rhipicephalus microplus* from Côte d'ivoire to Benin via Mali, Burkina-faso and Togo (Coraf/Webcard, 2015). In the same way the migration of birds is an opportunity for ticks to invade new regions, for instance migratory species such as Passerines are able to disperse the Ixodes ticks through Europe, and they may play an important role in the geographic dispersal of viral encephalitis; transmitted by ticks across this continent (Waldenström J *et al*., 2007), (Klaus C *et al*., 2016). Concerning animal international trade, many species are sold around the

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world and these movements contribute to the expansion of ticks, among the continents or countries (HaysJ, 2008), as example, two horses imported from Portugal to Brazil in 1999 have introduced tick species named *Hyalomma marginatum* to Brazil (Labruna M *et al*., 2001). This arthropod transmits the virus of Crimea-Congo hemorrhagic fever (CCHF) to humans (National Institue for Communicable Diseases, 2016). The conquest of new regions is also involved in the movements of many vector borne diseases among which ticks act as vectors. Thus, during the 18 century the *Amblyomma variegatum* tick was introduced in Caribbean with cattle imports coming from Senegal. This acarian is responsible of a human Rickettsiose due to *Rickettsia africa* (Cicculli V *et al*., 2019) both in Africa and in Caribbean. Regarding Gabon, each year this country imports several thousands of cattle from Cameroon (Motta P *et al*., 2017) but there is no information concerning the anthropophilic ticks introduced in this country as well as the pathogens associated with these arthropods; therefore, the import of animals is a threat for public health because of the risks of introduction of diseases and disease vectors. In order to construct a data base related to anthropophilic ticks and to struggle effectively against the introduction of these arthropods to Gabon, the objectives of the present survey were to characterize both the breeds of cattle and the species of anthropophilic ticks related to the cattle imports, to compare the relative frequencies of these acarian; to identify the probable factors associated with the introduction of ticks by cattle imports and to propose preventive measures aiming to treat cattle when they cross the border to enter to Gabon

MATERIALS AND METHODS

Study site

This study was conducted at the slaughterhouse of the municipality of Owendo located in the south of the city of Libreville, the first town of Gabon. This locality experiences a warm and humid climate, the annual average for the, precipitations is 2503 mm per square meter, those of the temperatures and hygrometry are respectively 26°C and 85%. The main economic activities in this area are performed around his port, in the sector of fishery, in the wood industry, in small shipbuilding, in building industry, in rail activity as well as around his abattoir. No cattle breeding activities had not ever held in both municipalities in Libreville and Owendo.

Animals

This study was conducted in the slaughterhouse of Owendo from August to December 2018. 156 cattle imported from Cameroon to Gabon have been included in this survey. Their age was between 5 and 6 years old. From arrival until tick sampling, these animals received no acaricides as treatment. Data concerning, the identification of tick species as well as the cattle breeds, were noted on the file of each animal. Cattle fact sheets (Lhost P, 1969) were used in order to characterize these animals.

Sampling and Identification of the Species of Ticks

Ticks were collected manually and kept in flasks containing 10 ml of ethanol at 70%. In order to identify species of ticks, identification keys (Walker A *et al*., 2003) and a binocular loupe were used. The relative frequency of each species was determined.

Analysis Framework of the Introduction of Ticks Associated With Imports Cattle

Two questionnaires were used in order to create a health problem analysis framework (Turnock, 2009) in which both determinants and contributing factors related to the introduction of the anthropophilic ticks associated with the cattle imports from Cameroon to Gabon will be identified. This study includes also information related to Bitam, the first Gabonese town located at border from which cattle coming from Cameroon enter to Gabon. For the first questionnaire, 70 persons from Bitam or knowing that city have answered to the questions. The asked questions were: Does there a veterinary controls are performed at the border in Bitam? The response should be yes, no or I don't know. In order to confirm or to infirm the performing of a veterinary controls at Bitam, a second questionnaire was forwarded to the AGASA (Agence Gabonaise de la Sécurité Alimentaire) the national service that has in charge the veterinary control of the imports of animals in Gabon. In case veterinary controls have never been applied at border in Bitam on the animal imports, AGASA would give explainations.

Statistical Analysis

The samples of ticks collected from cattle had short size (n=12) and were not normally distributed therefore the Kruskal-Wallis (Social Science Statistics Calculators, 2019) test was used to determine the statistical significance and the association between the species of ticks and their frequencies. The interval of confidence was estimated at 95% and $P < 0.05$ was considered as the level significance of the test.

RESULTS

Cattle Breeds

The cattle Fulbe and Bororo were imported from Cameroon to Gabon. These animals belonged to the group of hump cattle, designated Bos indicus, and were infested by anthropophilic ticks.

Ticks Species and Their Prevalence

A total of, 712 anthropophilic ticks (Fig1) were collected on imported zebus from Cameroon to Gabon. *Amblyomma variegatum*, *Hyalomma truncatum* and *Hyalomma impeltatum* were; the tick species that were identified during this investigation, with prevalence of 92.7%, 6.7% and 0.6% respectively. *Amblyomma variegatum* was the most significantly numerous species (Table1) ($P < 0.05$). The least

numerous significant specie (Table1) was *H. impeltatum* (P< 0.05).

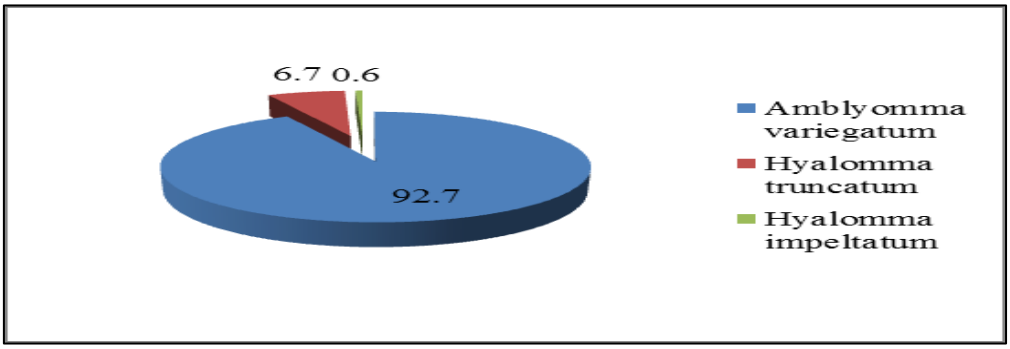

Figure 1: Relative frequencies of anthropophilic ticks collected from the cattle imports

KRUSKAL-WALIS TEST

Table1: The Kruskal-walis test used in order to compare the relative frequencies of the species of anthropophilic ticks affecting cattle imports

	Ranks		
	Species of ticks	**N**	**Mean rank**
Population of the ticks	*Amblyomma variegatum*	12	30.5
	Hyalomma truncatum	12	14.75
	Hyalomma impeltatum	12	10.25
	Total	36	

Test statistics

	Population of ticks
Chi-square	24.44
df	2
Asymp. Sig	0.005

CONCLUSION:

Asymp.sig (P-value = 0.005) < 0.05

The null hypothese (H0): The relative frequencies of the tick species were the same; was rejected

The alternative hypothese (H1): The relative frequencies of the species of ticks were significantly different; was retained

Factors Promoting the Introduction of Ticks from the Cattle Imports

The answers to the questionnaires (Table2) asked to persons from Bitam or working in that city has shown that 85.71% among them said that no veterinary controls were not performed at the border and 14.29% were not able to give any answer. AGASA has confirmed no animals was submit to any veterinary control at the border in Bitam (Table 3). According this agency (Table3), the introduction of the anthropophilic ticks to Gabon, were associated with the lack of veterinary control service at the border (health determinant) and of the governmental decision to create this service in Bitam (contributing factor). The analysis framework of the introduction of these arthropods associated with cattle imports (figure2) was deduced from the table2 and table3.

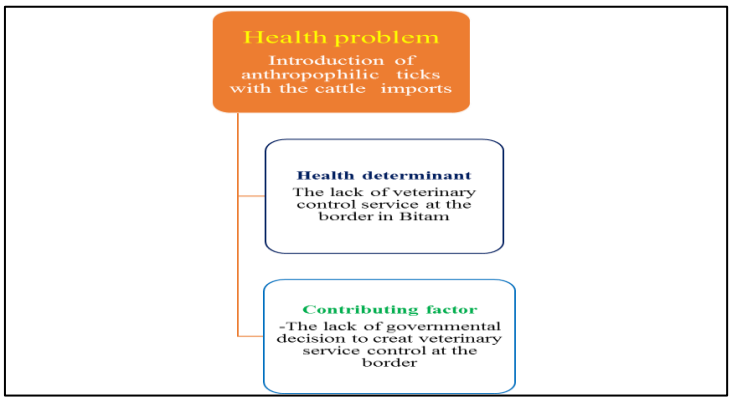

Fig2: The Framework analysis of the introduction to Gabon of ticks by the cattle imports

Table2: The rate of people who have participated to the identification of the contributing factors associated with the lack of veterinary control service at the frontier

Questions	Yes	No	I don't know	Total
Are veterinary control performed at the border in Bitam?	60 (85, 71%)		10 (14.29%)	70 (100%)

Table 3: The contribution of AGASA in the identification of the cause and the contributing factor to the lack of veterinary control service at the border (Bitam)

Questions	Answer	Cause	Contributing factors
Are veterinary controls performed at the border (Bitam)?	No	-	-
What is the cause for which veterinary controls are not performed at the border (Bitam)?	-	The lack of veterinary control service at the frontier (Bitam)	The lack of governmental decision to create a veterinary control service at the border in Bitam

DISCUSSION

This survey has shown that the zebus Bororo and Fulbe imported from Cameroon have introduced anthropophilic ticks to Gabon. These animals were from Adamaoua a northern province of Cameroon (Lhost P, 1969). With lyre-shaped horns, the first breed is able to support the long transhumances but its meat productivity is low. The last breed is a species of zebu having a fairly meat production satisfactory. Because of the size and shape of their horns, these two types of cattle were classified in the group of zebus Fulani with long horns and in the group of zebus Gudali with short-horns, respectively (Lhost P, 1969), (Maaouia A *et al*., 2017). These animals were introduced in Gabon in order to furnish meat to the populations of this country (FAO, 2017). With three types of climates (Institut National de la Statistique, 2015), Cameroon is a country that allows the development of a wide range of species of ticks (Morel PC *et al*., 1965) so it was not surprising that the climatic factors have contributed to the infestation of cattle imported from Cameroon to Gabon by the anthropophilic ticks.

Three species of anthropophilic ticks have infested the ruminant imports entering to Gabon. *Amblyomma variegatum* was one these arthropod. In this study, *A. variegatum* was the most abundant tick species which has significantly infested cattle. This arthropod is a three-host tick. The ruminants are his main hosts but this aggressive acarian can bite humans (Jensenius M *et al*., 2003) or feed; on other animals, like dogs when cattle are absent (Moubamba, 2006). This arthropod has a wide range of habitats from rain forest, highland, and savannah to steppe (Walker A *et al*., 2003). The range of suitable temperatures for this tick vary from 20° C to 30° C, with a hygrometry 75% to 95% and his comfortable interval of annual rainfall is 1000mm-2750mm (Frebling M, 2006). With a temperature around 25°C and an annual average of rainfall of 1500mm, Gabon is an excellent environment for *Amblyomma variegatum*, this was confirmed by the last study related to the tick distribution in this country which has shown the presence of arthropod in Gabon (Pourrut X *et al*., 2011). This acarian is widely distributed; in the south of Sahara (Walker A *et al*., 2003) from West Africa to Madagascar via East Africa, its southward extension on the continent is limited by the competition with *Amblyomma hebraeum* (Bournez L, 2014). Since the 18 century Amblyomma variegatum has colonized Caribbean and today he is at the gates of North America. This arthropod is a vector of Dugbe virus (Lutomiah J, 2014) and yellow fever virus (Cornet J *et al*., 1982). According medical reports, the first virus was detected in the blood and in the cerebrospinal fluid of the human patients with transient meningitis in West Africa (Burt F *et al*., 1996). Experimentally, nymphs of *Amblyomma variegatum* has shown their competency in the transmission of the virus of yellow fever to monkeys (*Cercopithecus aethiops tantalus*) (Cornet J *et al*., 1982). The success of this experiment could be a threat in public health because these animals, are considered as models in the study of certain diseases that are able to infect humans. This arthropod is also responsible of the transmission to the humans of *Rickettisa africae*, with the symptoms such as fever, headache, myalgia, eschar and rash (Ndip L *et al*., 2004). Similar pathological signs were seen in other human rickettsial infections (CDC, 2016) associated with *Rickettsia rickettsi* and *Rickettsia parkeri* in which *Amblyomma maculatum* and *Amblyomma americanum* were involved, in the USA. In this country, the estimated case fatality rate due to *Rickettia rickettsi* was 5%-10% (CDC, 2016). In Africa, according scientific publications, the prevalence of *Rickettsia africae* in *Amblyomma* ticks was 70% (Jensenius M *et al*., 2003) and in Cameroon, the country from which zebus were imported to Gabon, many human cases of infections due to Rickettsia africae associated with *A.variegatum* were reported with prevalence of 32% in both regions of Iko and Buea, therefore there is a risk to introduce this pathogen in Gabon or for a human to be infected after a bite of *Amblyomma variegatum*, because a tourist who had spent holidays in Gabon was infected with *Rickettsia africae* (Raoult D *et al*., 2001).

Hyalomma truncatum has a two-host life cycle. Great herbivores are his preferred hosts but this arthropod can also feed; on dogs. Immature stages infest hares and rodents and can also attach to human body. This acarian is widely distributed in the south of Sahara (Walker A *et al*., 2003). It was demonstrated in laboratory conditions that its best biotopes are those with a temperature around 26°C, with humidity of 92 to 96% (LoganT *et al*., 1989) and where he could

complete his cycle of life in 108 days. With annual averages of temperature, of precipitations and hygrometry that are 25°C, 1500mm and 84% respectively; with these information, Gabon seems to be considered as one of its favorable environments; but the biotopes of this arthropod are the arid and semi-arid regions where hygrometry varies remarkably: 25-30% during dry season and 70-80% during rainy season with variation of temperature between 21°C to 30°C (Nicholson S, 2018); in addition the hydrophilic forests like those of the area of Libreville that have annual precipitations around 2800mm are environments that are uncomfortable for this acarian (Aeschlimann,1967); therefore *Hyalomma truncatum* can't support Gabon climatic conditions ; this is not surprising, because the last tick distribution studies in Gabon has confirmed the absence of this acarian in this country (Pourrut X *et al*., 2011). In some cases, the notification of the presence of *Hyalomma truncatum* in certain wet areas is due to the confusion in identification between this specie of tick and *Hyalomma niditum* (Walker A *et al*., 2003); this can lead to the wrong results in the study of the distribution of these acarian. This arthropod transmits Crimea-Congo hemorrhagic fever to humans, with following symptoms: fever, myalgia, dizziness, backache, headache, sore eyes, neck pain, petechial rash and other hemorrhagic phenomena. The human mortality rate due to this disease is 10%-40% (WHO, 2013). To date, it has never been reported in Gabon that any virus of Crimea-Congo hemorrhagic fever has been detected in ticks but in 2016 a survey conducted with bats has shown that the prevalence of this pathogen to these animals was 10% (Müller M *et al*., 2016), this has confirmed that bats are reservoirs of CCHFV in Gabon and competent anthropophilic ticks like *Hyalomma sp* could transmit this arbovirus to humans from bats. *Hyalomma truncatum* can be infected with the Rift valley fever virus and also with *Rickettsia africae* although no human transmissions have ever been reported in both cases. This arthropod like *Hyalomma marginatum marginatum* is a vector (Fernández-Soto P *et al*., 2009) of *R. aeschlimannii* and transmits this rickettsia to humans with the following symptoms: fever of 39.5° C, a generalized maculopapular skin rash; these clinical signs are similar with those caused by R. africae. Doxycline remains the best treatment against rickettsial infection (Raoult D *et al*., 2001). The surveillance of these arthropods has to be conducted frequently in countries like Soudan and Ethiopia that have common and comfortable biotopes for this species of ticks. *H. truncatum* can also harbor the Semliki Forest virus, a pathogen for which mosquitoes are the main vectors. The disease caused by this virus is benign and the symptoms include fever, myalgia, arthritis and bellyache (Raoult D *et al*., 2002), but this pathogen is fatal for immunodepressive persons. This survey, has reported for the first time the introduction of *Hyalomma truncatum* to Gabon with the imports of cattle.

Like in the case of *Hyalomma truncatum*, it was the first time that the presence of *Hyalomma impeltatum*; has been reported in Gabon through this survey. This arthropod has a three-host life cycle. Often, adults infest cattle and camels, whereas immature stages feed; both on rodents and birds. The habitats of this tick species are the arid and semi- arid areas with annual precipitations of 50mm to 600mm.The distribution of this arthropod covers Sahel, North Africa and some middle Eastern countries such as Iran, Turkey and Israel (Walker A *et al*., 2003). The south of Cote d'Ivoire and Gabon where the annual amount of rainfalls is higher than 1000mm are uncomfortable biotopes (Aeschlimann, 1967) for the achievement of the life cycle of this tick. Like *Hyalomma truncatum*, this arthropod is also a vector of Crimea- Congo hemorrhagic fever virus (Camicas J *et al*., 1990) [31]. A bacterium called *Ehrlichia chaffensis* were detected in this arthropod in Nigeria (CDC, 2019). To date, no case of human infected with this pathogen associated with this acarian has been reported. Signs and symptoms observed in patients suffering about this ehrlichiosis are: fever, chills, headache, muscle aches, nausea, vomiting, diarrhea, loss of appetite, confusion, rash, damage to the brain or nervous system, respiratory failure and uncontrolled bleeding. Among antibiotics, doxycline is designated as the best drug in the treatment of Ehrlichiosis. In 2017, in USA 1,642 cases due to *Ehrlichia chaffensis* were reported (CDC, 2019) and the global case fatal rate was 1%. But for children under 5 years old and people over70 years it was higher and was respectively 14% and 53%. The environment of Gabon does not offer a favorable habitat for this arthropod because the humidity and the precipitations are high. Because of climatic conditions of Gabon the risk of transmission of *Ehrlichia chaffeensis* and CCHFV by *H. impeltatum* seems to be weak but wildlife could maintain these pathogen after dontacts with this arthropod thus that might allow *A. variegatum* to inoculate *E. chaffeensis* to humans like *Amblyomma americanum* does it in the USA (CDC, 2019).

In this survey, in accordance with the health problem analysis framework, the introduction of anthropophilic ticks related to the cattle imports to Gabon has been designated as health problem because these imports have introduced Hyalomma truncatum and Hyalomma impeltatum; two anthropophilic tick species of the dry regions; Gabon was unsuitable living place for these arthropods threating public health. In addition, 86% of interviewed persons and AGASA have confirmed that cattle crossing the frontier in Bitam have never undergone any veterinary examinations and treated with acaricides because of the lack of a veterinary control service. This factor was designated as health determinant (Brown L, 2017) and was closely associated with the introduction of the ticks. The lack of the governmental decision to create a veterinary service at the border in Bitam was another limiting factor to perform animal examinations, this last element was

defined as contributing factor linked to the health determinant and has acted back at the last level in the chain of causation (Turnock, 2009). In this survey determinants and contributing factors were considered as risk factors (Turnock, 2009) and were associated with the introduction of foreign ticks to Gabon with the cattle imports. Thus a governmental decision to create a veterinary control service in Bitam, at the frontier between Gabon and Cameroon, could stop or reduce the introduction of anthropophilic ticks to Gabon. In some developed continents, despite of veterinary border controls, vectors of diseases can cross frontiers. It was what happened in Europe when mosquitoes belonging to the *Aedes* genus had accidentally crossed borders in goods such as the tires and lucky bamboo [37]. The lack of border veterinary control service were not associated with these imports; therefore, goods else than domestic animals can introduce vectors of diseases in countries or continents, despite of the presence of border veterinary control service.

In order to struggle against ticks, the acaricide called Topline (fiprolin) pour-on at 1% could be used to treat cattle before passing the border and entering to Gabon because this drug has shown it effectiveness against ticks belonging to the *Amblyomma* and *Hyalomma* genus in Cameroon and in Mauritania (Dia M *et al*., 2018), to date no resistance has never been observed. Likewise, indigenous drugs such as: *Vernonia amygdalina (Asteraceae*), *Zanthoxylum zantholoïdes (Rutaceae), Khaya senegalensis (Meliaeae), Securidaca longepedunculata (Polygalaceae*) and *Sclerocarya birrea (Anacardiaceae)* (Azokou A *et al*., 2016); have also demonstrated their effectiveness to kill ticks of cattle in Côte d'Ivoire but the afterglow of these products and their using dose are not known.

CONCLUSION

The zebus Bororo and Fulbe imported from Cameroon have introduced three anthropophilic tick species *Amblyomma variegatum*, *Hyalomma truncatum* and *Hyalomma impeltatum* to Gabon. The first specie was the most abundant among all arthropods and can transmit *Rickettsia africae* to humans whereas the two last were vectors of the virus of Crimea-Congo hemorrhagic fever and are able to inoculate it to men. Among these three tick species, only *Amblyomma variegatum* is able to support the climatic and environmental conditions of Gabon, this arthropod can in live in this country. The lack of a veterinary control service at the border in Bitam was the factor responsible in the introduction of anthropophilic ticks to Gabon. A governmental decision to establish a veterinary control service at the border in Bitam will be helpful to struggle against the introduction of anthropophilic ticks to Gabon.

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