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Contribution to the study of the biological diagnosis of Q fever in humans in the prefectures of Kindia, Kissidougou, Guéckédou and N'Zérékoré (Republic of Guinea)

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Abstract

Q fever is a zooanthroponosis found almost worldwide, with the exception of New Zealand and Antarctica. It is a cosmopolitan disease caused by Coxiella burnetii whose main modes of contamination in humans are the digestive and airways. His clinic includes an incubation period of about 20 days. The onset is marked by a flu-like syndrome with fever, chills, sweats, asthenia, anorexia, headache, myalgia which lasts 1 to 3 weeks. Its prevalence varies greatly from one country to another, due to epidemiological disparities, the frequency of breeding and the knowledge of the disease by doctors. This study contributes to improving the biological diagnosis of Q fever in the health structures of certain prefectures of Guinea (Kindia, Kissidougou, Guéckédou and N'Zérekoré) during the period from October 2017 to October 2020. C. burnetii DNA was detected in febrile patients seen in hospitals in the investigation areas. ELISA and real-time PCR methods were used for data analysis. This disease occupies a share of the febrile diseases that prevail in Guinea through the areas of the present study.

Keywords: Q fever, zooanthroponosis, asymptomatic, biological diagnosis and humans

Introduction

Q fever is a zooanthroponosis, of which humans are only accidental hosts. The reservoir is vast and includes domestic mammals (cattle, sheep, goats, pigs, camels, dogs, cats, rabbits) and wild mammals (rodents, rabbits, hares, marsupials, foxes, bears, deer), but also birds domestic or wild (chickens, turkeys, geese, ducks, pigeons) (M. Maurin and D. Raoult, 1999) [16]. Its causative agent Coxiella burnetii (Cb) is also found in many species of ticks. Infected mammals excrete Cb in urine, feces, milk and parturition products (Epelboin L., 2017) [12]. Coxiella burnetii is the agent of a ubiquitous zooanthroponosis (Q fever) described all over the world with the exception of New Zealand, as is repeated from one article to another (Epelboin, Nacher *et al*., 2016).

The starting point of epidemics in humans comes most of the time from contamination of animal secretions. The bacteria will mainly be found in urine, stool, genital secretions, milk, etc. Thus, contact with vaginal secretions, the placenta and whelping products of an infected animal exposes humans to risks of contamination. In addition, many animals can prove to be healthy carriers, which further increases the risk (Marie C., 2014).

Q fever is an important public health issue. The clinical manifestations of this disease, when they develop, are very varied, not very specific and often self-limiting, which makes its diagnosis difficult and tends to underestimate the number of cases of Q fever in the population. (Dahlgren *et al*., 2015).

The epidemiological situation of Q fever in Cayenne is not observed anywhere else in Guyana or even in South America or the rest of the world (LOÏC EPELBOIN, 2017) [12]. The declaration of any case would require an active search for other cases in the entourage (family, neighborhood) and their management. Thus, during the Q fever epidemic in a military environment on the Tiger Mountain, the active search for cases around the index case made it possible to diagnose 10 others in the entourage, 50% of them retrospectively. (Vincent PS *et al*., 2016) [18].

In the Netherlands, in 2007, an upsurge in cases of pneumonia was noticed by some general practitioners working in rural areas. After investigation, these cases of pneumonia were found to be clinical signs of acute Q fever. The country then went through a major Q fever epidemic from 2007 to 2010, which was stemmed by the systematic slaughter of pregnant goats and lambs (EFSA Journal, 2010). A geographical link had been established between dairy goat farms and the emergence of new cases of Q fever, particularly during waves of spontaneous abortions in goats. Better knowledge of Q fever by general practitioners during this epidemic reduced the frequency of hospitalizations in affected patients (E. Bosnjak1 *et al*., 2009; Alexandra Aubin., 2020) [2].

Regarding the Indian Ocean zone, Q fever has been studied in Mayotte, Madagascar and the Comoros archipelago. In Mayotte, an overseas department near Reunion Island, only one case of Q fever-related endocarditis has been reported to date. A case of co-infection between Q fever and Plasmodium falciparum has been described in a patient returning from the Comoros archipelago. In Madagascar, sera from zebus were analyzed and an animal seropositivity of 2.00% was demonstrated. The human samples tested, on the other hand, were all negative for Cb. Concerning the Seychelles and Mauritius, no data is available on the epidemiology of Q fever (M Georgiev *et al*., 2013) [14].

The Q fever situation in Africa is endemic and the infection is widespread across the continent. Indeed, various seroprevalence studies have revealed very high seropositivity rates in many countries from South Africa to the Maghreb: 18.3% in Morocco, 26% in Tunisia, 37% in Zimbabwe, 44% in Nigeria, from 10 to 37% in North-East Africa, 26% in Namibia, 15% in Algeria with peaks of 30% in certain villages, 24.5% in Senegal. (Kelly P.J., *et al*. 1993; J. Njeru *et al*., 2016) [10, 9].

The overall impact of Q fever on public health is vastly underestimated. In a recent study, Cb was found as the etiological agent in 5% of 109 cases of severe pneumonia in Tanzania in 2011. In the same country, a survey carried out in a cohort of severe febrile patients revealed 26.2% of zoonoses, including 30% Q fever in 2013 (J. Njeru *et al*., 2016) [9]. In Tunisia and Algeria, Cb represents 1 to 3% of infective endocarditis. In Burkina Faso, Q fever was responsible for 5% of acute febrile illnesses in 2014.

In Cameroon, 9% of community-acquired acute pneumonias in people aged over 15 tested positive for Cb in 2014. (Mohammed H. *et al*, 2017) [15]. These studies are perhaps the tip of the iceberg, indicating a widespread circulation of the bacterium in rural Africa, especially since in most African countries, seroprevalence rates are high in domestic ruminants. Cattle surveys have shown rates ranging from 4% in Senegal to 55% in Nigeria (Eldin, Melenotte *et al*., 2017) [6].

In Guinea, the study carried out by Siba Kalivogui *et al* carried out in 1986, indicates a low prevalence of Coexilla burnetii and that carried out by Abdoulaye Ahmat Nassour *et al* in 2020 in the Prefecture of Kindia, carried out at the Guineo-Russian laboratory of the IRBAG showed the existence of Coxiella burnetii in humans (6.5%), in reservoir animals (10%) and vector ticks (42.12%). Finally, the study conducted by Fatoumata Dramé *et al* in 2021 on the circulation of Coxiella burnetii in Guinée

Materials and Methods

Presentation of the study area

The study area for this research work was the prefecture of Kindia in lower Guinea and three prefectures of Guinée Forestière (Kissidougou, Guéckédou and N'Zérékoré) which are important agro-pastoral areas of Guinea.

The prefecture of Kindia has an area of 9115 Km2. It has a population of 438,315 inhabitants, including 226,300 women. The main activities are trade, agriculture and livestock. (Abdoulaye Ahmat N., 2020) [1]. The density is 52 inhabitants per km2 unequally distributed between ten (10) decentralized communities and one (01) urban municipality which includes thirty-three (33) districts. With a growth rate of 34%. (General Census of Population and Housing 2014). Its climate is of the humid tropical type, characterized by the alternation of two seasons of variable duration, a dry season from November to April and a rainy season with abundant rainfall from May to October with an average rainfall of 2500 mm of water per year. With temperatures varying from 25°C to 39°C (Fig.1) (Abdoulaye Ahmat N., 2020) [1].

The prefectures of Kissidougou, Guéckédou and N'Zérékoré are the three largest prefectures in the Forest region. This region is located in the south-east of Guinea and covers an area of 49,500 km², or 20% of the national territory. Its population is 1.1 million. Its density is estimated at 22 inhabitants per km². Its relief is on a plateau. It has seven (7) prefectures: Beyla, Guéckédou, Kissidougou, Lola, Macenta, N'Zérékoré and Yomou (Barry M.S *et al*., 2019) [3]. Its climate is of the humid subequatorial type characterized by the alternation of two seasons, a rainy season from March to November and a dry season from December to February. In August and September, the monthly rainfall can reach 300 to 400 mm³ with relatively high intensities (Fatoumata Dramé., 2021) [7]. Average temperatures are lowered by altitude and vary between 17°C and 22°C (Ministry of Planning and International Cooperation, 2016).

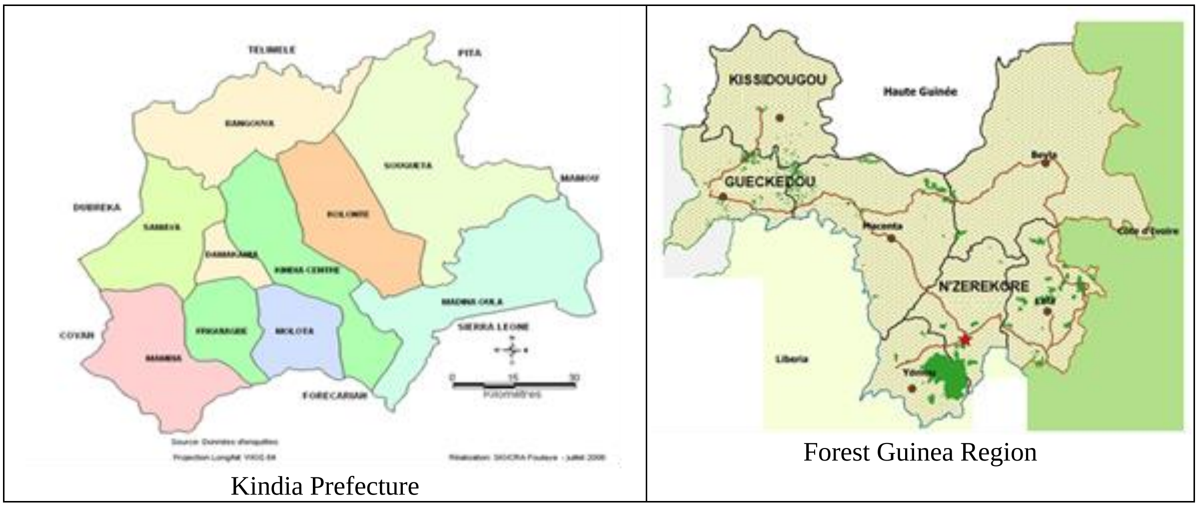
Sampling and analysis materials

Sampling and analysis materials include: 1.5ml eppendorf tubes; automatic pipette from 100 to 1000µl; icehouse with cold accumulator; microtube rack; microtubes; Centrifuge; vortex mixer; refrigerator (-20 to -80°C); thermostat; bleach tray (with disinfectant solution); booster; autoclave; reagent homogenizer (microspin); bio analyzer computer with software; Extraction solutions; vacuum; mixed (PC-2); Taq-polymerase; revertase (RT-PCR); RT-Gmix; positive control (k+); negative control (K-); OKV analysis; tabletop centrifuge; 1.5-2.2ml polypropulain tube; spectrophotometer for plate reading; 5 cc syringe; marker and work protection or safety equipment. The relevant population of febrile patients consulted in the hospitals of Kindia, Kissidougou, Guéckédou and N'Zérékoré during the period from October 2017 to November 2020.

Methodology

This is a prospective descriptive and analytical study which consists of to detect the DNA of the causative agent of Q fever, as well as to evaluate the prevalence and determine the seroprevalence of Q fever in febrile patients seen in consultation in Kindia, Kissidougou, Guéckédou and N'Zérékoré hospitals during the period from October 2017 to November 2020. We took random samples in the study area, i.e. 693 in Kindia, 39 in Kissidougou, 25 in Guéckédou and 60 in N'Zérékoré These samples are used for the detection of C. burnetii

DNA and class G immunoglobulins directed against C. burnetii infection in the infected person. The human blood samples that were the subject of the study were collected in sterile 5ml hemolysis tubes, using the blood collection system according to the standard method from the elbow crease vein.



Kindia Prefecture

Forest Guinea Region

Fig 1: Study area

Immuno-enzymatic analysis

The indirect ELISA technique is used for the detection of class G immunoglobulins (IgG) in the blood collected and the direct ELISA technique is used for the search for the C. burnetii antigen in the same blood (Lai C-H *et al*., 2013). The detection of C. burnetii DNA is carried out by the PCR method according to the recommendations of the manufacturer of the “Ampli Sens Coxiella FLR” reagent kit (Interlab Service, Russia). This kit makes it possible to perform the polymerase chain reaction of C. burnetii and to amplify the DNA of pathogenic genotypes, this is real-time PCR (Hubert Bassene *et al*., 2014).2.5 Statistical analysis In the statistical treatment of the results, the proportion of positive cases in each sample was calculated by formula 1 (Mohammed H. *et al*, 2017).

$$\hat{p} = \frac{X_n}{n} \qquad (1)$$

Where: X_n is the number of positive cases in the sample and is the sample size. For a large sample of size n($n \geq 30$), one can apply to$\hat{p}$, the central limit theorem and conclude that follows a normal distribution $\hat{p}$ mathematical expectation and standard deviation (formula 2 and 3).

$E(\hat{p}) = p$ and standard deviation $\sigma = \sqrt{\frac{p(1-p)}{n}}$ (2, 3)

We therefore determined, for each estimated proportion, a 95% confidence interval to ensure that the true proportion lies within this interval, according to formula 4.

$$IC = \left[\hat{p} - 1{,}96\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}\,;\hat{p} + 1{,}96\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}\right] \qquad (4)$$

Results and Discussions

Results

Between October 2017 and October 2020 a total of 817 samples were taken from humans in the different investigation areas of this study. The distribution of positive cases by prefecture is given in Table 1.

Table 1: Distribution of cases of detection of DNA of C. burnetiid

Prefecture	**Sampling**	**Positive cases**	**Prevalence (%)**
Kindia	693	45	5,51
Kissidougou	39	0	0
Gueckedou	25	0	0
N’Zérékoré	60	2	0,24
Total	817	47	5,75

The results of this table showed that the Kindia area was the most investigated, but also the most affected: 45 positive detections for C. burnetii corresponding to a prevalence of 5.51%. In Forest Guinea, N'Zérékoré presented 2 cases of positive detections for C. burnetii, i.e. a prevalence of 0.24%. In sum, for all the areas investigated by this study, 47 positive cases for C. burnetii were obtained, resulting in a total prevalence of 5.75%. The distributions of positive cases by prefecture and by gender are given in Table 2.

Table 2: Detection of C. Burnetii DNA by gender

Prefecture	Gender	Sampling	Positive cases	Prevalence (%)
Kindia	F	293	12	1,47
	M	400	33	4,04
Kissidougou	F	20	0	0
	M	19	0	0
Guéckédou	F	10	0	0
	M	15	0	0
N’Zérékoré	F	31	1	0,12
	M	29	1	0,12
Total		817	47	5,75

Table 2 shows that the DNA detection of C. burnetii by sex has a positivity of 33 cases in males with a prevalence of 4.04% and 12 positive cases in females with a prevalence of 1, 47% in the Kindia area. In Forest Guinea, it is only in the N'Zérékoré area that 2 cases of C. burnetii DNA were detected, with one (1) male and one (1) female for a prevalence of 0.24% in total. For all the areas investigated, there were 47 detections of C. burnetii DNA including 13 female cases corresponding to 1.59% and 34 male cases corresponding to 4.16% and all corresponding to a total prevalence of 5.75%. The distribution of confirmed cases carrying C. burnetii DNA according to the age group of febrile patients is shown in Table 3.

Table 3: Distribution of confirmed cases according to age groups

Age groups (years)	Sampling	Positive cases	Frequency (%)
1-10	89	1	0,12
15 - 25	96	4	0,49
26 - 35	154	15	1,84
36 - 45	168	12	1,47
46 - 55	104	8	0,98
≥ 55	206	7	0,86
Total	817	47	5,76

Table 3 showed that the 26 - 35 age group was the most carrier of C. burnetii DNA with a proportion of 1.84%. It was followed by that of 36 - 45 years for a proportion of 1.47%. The least carrier age group was that of 1 - 10 years with a proportion of 0.12%. The distribution of the prevalence of C. burnetii in febrile patients according to socio-professional categories is given in Table 4.

Table 4: Distribution of the prevalence of C. burnetii according to socio-professional categories

Professions	Sampling	Positive cases	Prevalence (%)
Agriculteurs	101	9	1,10
Eleveurs	102	10	1,22
Bouchers	100	17	2,08
Vétérinaires	95	2	0,24
Etudiants	122	3	0,36
Ménagères	122	1	0,12
Commerçants	85	3	0,36
Sans Profession	90	2	0,24
Total	817	47	5,72

Table 4 shows that butchers were the most carriers of C. burnetii DNA with 17 positive cases, ie a prevalence of 2.08%. They were followed by breeders who presented 10 DNA-positive cases of C. burnetii with a prevalence of 1.22%. Housewives constituted the occupational group with the least C. burnetii DNA with one (1) positive case for a prevalence of 0.12%.

Discussions

The analysis of 817 human blood samples produced the following results for all the areas investigated: 47 detections of C. burnetii DNA resulting in a total prevalence of 5.75% including 13 cases in female sex corresponding to 1.59% and 34 cases in the male sex corresponding to 4.16%. The study conducted by Abdoulaye Ahmat Nassour in 2020 on the circulation of the causative agent of Q fever in the prefecture of Kindia has found a prevalence of 6.5% in the area. This prevalence is higher than the present one, which is

5.75% for all the areas investigated. Found in humans a prevalence of 1.61%. This prevalence is lower than the present, which is 5.75% for all the areas investigated. Another study carried out in febrile humans in Bamako and Mopti by Bonfoh B *et al*. (2003) showed a seroprevalence of 36% in Bamako and 61.9% in Mopti; these results are significantly higher than those found in Guinea in 2020 (6.5% in the prefecture of Kindia), in 2021 (1.61% in Guinée Forestière) and that of this present which is 5.75%.

Kisito Nagalo, who worked from 1996 to 1997 in Burkina Faso (Fatoumata Dramé *et al*., 2021) [7], reported a prevalence of 17.50% for the 30-44 age group among the 183 hospitalized febrile patients; men made up 55.7% and women 44.3% of the population. For the profession variable, farmers are much more represented with a prevalence of 20.2% of the sample. This prevalence of 17.50 is clearly higher than those found in Guinea, including the present one, which is 5.75%.

The highest seroprevalence was found in 1983 in the Alps, where 30% of the inhabitants of a village were seropositive for IgG due to C. burnetii DNA (Magisson-Ricci, F., 2003).

Tissot-Dupont, H *et al*. (2007) reported a prevalence of 9.8% in their studies conducted between August 2002 and July 2003, involving 1946 sera tested, from 1089 people. Overall, the latter presented a diagnosis of acute Q fever in 101 people (9.3%), while chronic Q fever was diagnosed in 5 patients (0.5%). Depending on the host, acute Q fever was found in 11 pregnant women (2.6%), 5 valvulopathies (5.5%) and 85 people with no known risk factor (14.7%) (Fatoumata Dramé, 2021) [7].

Conclusion

The examination of a total of 817 human blood samples, including 693 samples in Kindia and 124 samples in three prefectures of Forest Guinea is based on the detection of Coxiella burnetii DNA and IgG, carried out during the period From October 2017 to October 2020, the population concerned by this study is made up of febrile patients residing and consulted in the hospitals of the chosen areas. The Kindia area was the most investigated, but also the most affected: 45 positive detections for C. burnetii for a prevalence of 5.51%. In Forest Guinea, the N'Zérékoré area presented 2 cases of positive DNA detections of C. burnetii corresponding to a prevalence of 0.24%. For all the areas investigated, 47 detections of C. burnetii DNA in humans were found, including 13 cases in the female sex corresponding to 1.59% and 34 cases in the male sex corresponding to 4.16% and all correspond to a total prevalence of 5.75%.

The age group 26-35 years was the most carrier of C. burnetii DNA with a proportion of 1.84%. Butchers were the most affected by C. burnetii with 17 positive cases for a prevalence of 2.08%. The detection of C. burnetii DNA in febrile patients subjected to the present study confirms the circulation of C. burnetii in the investigated areas of (Kindia and Guinée Forestière).

This demonstrates that Q fever has a significant role in the development of febrile illnesses that are rampant in these two agro-pastoral areas of Guinea. The fight against this zoonotic pathology is a necessity in order to reduce its prevalence. This sero-epidemiological research on Q fever is continuing in cattle and ticks, which are risk factors for zoonotic transmission in rural communities. These results will be the subject of other scientific articles.

References

1. Abdoulaye Ahmat. N. Etude de la circulation de l’agent causal de la fièvre Q dans la Préfecture de Kindia. Mémoire de Master, 2020, 85.
2. Alexandra Aubin. Données cliniques autour de la fièvre Q aiguë à La Réunion; étude de cohorte rétrospective. Thèse de doctorat de L’Université de la Réunion, U.F.R Sante, 2020, 62.
3. Barry MS, Diallo AS, Barry OD, Camara FS, Dore Set Balde AM. Contribution aux études ethnobotaniques sur des plantes médicales employée dans le traitement du paludisme en médecine traditionnelle Guinéenne, 2019.
4. Bosnjak1 E, AMSW Hvass, S Villumsen H Nielsen. Emerging evidence for Q fever in humans in Denmark: role of contact with dairy cattle, Journal Compilation, European Society of Clinical Microbiology and Infectious Diseases, CMI,2009:16:1285-1288.
5. EFSA Panel on Animal Health and Welfare (AHAW), EFSA Panel on Biological Hazards (BIOHAZ), Chapter 4 on Food Safety, European Food Safety Authority (EFSA), Parma, Italy, Journal,2010:8(5):1595, doi:10.2903/j.efsa.2010.1595
6. Eldin C, C Melenotte, O Mediannikov, E Ghigo, M Million, S Edouard, *et al*. From Q Fever to Coxiella burnetii Infection; à Paradigm Change. Clin Microbiol Rev,2017:30(1):115-190.
7. Fatoumata Drame. Mise en évidences de la circulation de *Coxiella burnetii* en Guinée., 2021.
8. Emmanouil Angelakis, Oleg Mediannikov, Cristina Socolovschi, Nadjet Mouffok, Hubert Bassene, Adama Tall, *et al*. Didier Raoult, Coxiella burnetii-positive PCR in febrile patients in rural and urban Africa Emmanouil Angelakis, Oleg Mediannikov, Cristina Socolovschi, Nadjet Mouffok, International Journal of Infectious Diseases,2014:28:107-110.
9. J Njeru, K Henning, MW Pletz, R Heller, H Neubauer. Q fever is an old and neglected zoonotic disease in Kenya: a systematic review, BMC Public Health,2016:16(297):1-8.
10. Kelly PJ, Metthewman LA, Mason PR, Raoult DQ. Fever in Zimbabwe: A Review of the Disease and the Results of a Serosurvey of Humans, Cattle, Goats and Dogs. SA Med. J.,1993:83:21- 25.

11. Lai C-H, Chang L-L, Lin J-N, *et al*. High Seroprevalence of Mycoplasma pneumoniae IgM in Acute Q Fever by Enzyme-Linked Immunosorbent Assay (ELISA). PLoS ONE, 2013, 8(10).
12. Loïc EPELBOIN, Émergence de zoonoses en Amazonie; épidémiologie comparée de la leptospirose et de la fièvre Q en Guyane française, 2017, 271.
13. Ministère du Plan et de la Copperation Internationnal. Prospective et du plan et de propective, situation economique et social de la Guinée, »,2016:19:2012-2014.
14. M Georgiev, A Afonso, H Neubauer, Howard Needham, R Thiéry, A Rodolakis. *Q fever in humans and farm animals in four European countries, 1982 to 2010,* Review articles, 2013, 1-13.
15. Mohammed H. Benaissa, Samir Ansel, Abdallah Mohamed-Cherif, Karima Benfodil, Djamel Khelef, Curts R. Youngs, *et al*. Seroprevalence and risk factors for Coxiella burnetii, the causative agent of Q fever in the dromedary camel (Camelus dromedarius) population in Algeria, Journal of Veterinary Research, 2017, 1-7.
16. M. Maurin and d. Raoult. Q Fever, Clinical Microbiology Reviews, American Society for Microbiology, All Rights Reserved.1999,12(4):518-553.
17. Sky Vanderburg, Matthew P Rubach, Jo EB Halliday, Sarah Cleaveland, Elizabeth A Reddy, John A Crump Epidemiology of Coxiella burnetii Infection in Africa: A OneHealth Systematic Review, PLOS Neglected Tropical Diseases,2014:8:1-8.
18. Vincent Pommier De Santi, Jean-Lou Marié, Sébastien Briolant, Aba Mahamat, *et al*. Spécificités épidémiologiques de la fièvre q en Guyane, 2016, 7.