

Pattern and Distribution of Human Brucellosis Diagnosed in the National Animal and Plant Health Laboratory of Eritrea

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Research Article

Keywords: zoonotic disease, bacteria, genus Brucella, profuse sweating, *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*

Posted Date: March 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-279310/v1>

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PATTERN AND DISTRIBUTION OF HUMAN BRUCELLOSIS DIAGNOSED IN THE NATIONAL ANIMAL AND PLANT HEALTH LABORATORY OF ERITREA

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ABSTRACT

Background: Currently, brucellosis is the most widespread zoonotic disease in the world. In Eritrea, the disease has been documented since the 1940s, with widespread infections throughout the country. That being said, the only facility that tested for brucellosis is the National Animal and Plant Health Laboratory of Eritrea (NAPHL). Therefore, this study will focus on the pattern and distribution of human brucellosis tested and diagnosed at NAPHL.

Method: This study is a retrospective cross-sectional look at all the Rose-Bengal test confirmed Brucellosis cases at the NAPHL from January, 2017 to December, 2019.

Results: A total of 848 cases were detected out of 2,669 patients tested. The male to female ratio was 2.78 in those who tested positive for brucellosis. In the years, 2018 and 2019, the age range was between 1 and 89 with a mean of 29 years. Patients came from 5 of the 6 zones of Eritrea. Across the study period, no tests were recorded from the Southern Red Sea zone. Overall, Maekel Zone had the most cases, followed by Debub Zone and Northern Red Sea Zone. Scatter plot imaging by village showed a dense scatter area around the Maekel zone. Cases mapping by sub-zone showed highest number of cases in the Sub-Zones of the Maekel Zone and those located closest to the zone (namely Ghindae, Segheneiti, Dubarwa and Dekemhare).

Conclusion: As human brucellosis is widespread in Eritrea, the government should take quick actions to increase accessibility to testing and treatment facilities, and implement an eradication program.

INTRODUCTION

Currently, brucellosis is the most widespread and the second most important zoonotic disease in the world [1-3]. About 500,000 new cases of brucellosis are reported annually [4]. The disease affects farm and marine animals, wildlife, and humans [5, 6]. It is caused by bacteria belonging to the genus *Brucella*. The species, *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* can cause severe disease in humans [6]. Infection can cause decreased milk production, abortion, infertility as well as weak offspring in animals [5, 7]. In humans, the disease is systemic and is characterized by acute or insidious onset of continued, intermittent, undulant or irregular fever of variable duration, headache, profuse sweating, chills, weakness, generalized aching, and joint pain [8].

Pappas et.al state that the epidemiology of human brucellosis has drastically changed over the past few years because of sanitary, socioeconomic, travel and political reasons [9]. Accurate data on human cases of brucellosis are lacking from many countries, especially developing countries, and the lack of active data leads to underestimation of the burden of the disease [10]. The true nature and extent of the disease in many African countries also remains largely unknown [11].

In Eritrea, the presence of *Brucella* spp. in livestock was well documented since the 1940s, with widespread infections throughout the country [12-17]. A slaughter-on-detection program of eradication in the dairy farms had been introduced in as early as the 1960s [18]. But brucellosis has not been brought under control in livestock, possibly due to continuous instability in The Horn of Africa. The situation may have been aggravated by absence of animal quarantine strategies, trans-boundary animal movement, a shortage of eradication and vaccination program and a lack of awareness of the disease in the general public.

In addition, there has been very limited access to diagnostic and treatment facilities for human brucellosis in Eritrea. In fact, the only facility that tests for the disease is located in Asmara; the National Animal and Plant Health Laboratory of Eritrea (NAPHL). Therefore, this study will focus on the pattern and distribution of human brucellosis tested and diagnosed at NAPHL, hoping to trigger more studies to follow that could focus on the eradication and control of the disease.

METHODS

Study Design

This study is a retrospective cross-sectional look at all the Rose-Bengal test confirmed Brucellosis cases at the NAPHL from January, 2017 to December, 2019.

Source Population

All samples of patients referred from all hospitals in Eritrea who had undergone Rose-Bengal test for confirming the diagnosis of Brucellosis in NAPHL have been assessed.

Data Collection and Analysis

Information regarding socio - demographic characters (gender, age, residence), date of test and result of brucellosis was extracted from the NAPHL database. The data was collected and cleaned using Microsoft Excel spreadsheet and all data analyses were completed using StataSE 15 (StataCorp LLC, College

Station, TX). Descriptive statistics on demographic characteristics of respondents were summarized using frequencies and percentages. Mapping of brucella cases were done by ArcGIS v 10.7.1.

Rose-Bengal Test Procedure

Samples were tested using the Rose Bengal plate test (RBPT) technique according to the manufacturer’s protocol. First the antigen and controls are equilibrated at room temperature and shaken to resuspend any bacterial sediment. After that 30 µl of plain serum is dispensed on an agglutination plate. It is then mixed with an equal volume of RBT antigen using a mixing stick. The tile is then rocked at room temperature for 4 minutes and any visible agglutination is taken as a positive result.

Then, positive sera undergo further testing. Eight drops, each of 30 µl, of saline are dispensed on the tile. The first one is mixed with an equal volume of the positive plain serum (1/2 serum dilution). Then 30 µl of this first dilution are transferred to the second drop with the help of a micropipette and mixed to obtain the 1/4 dilution. From this, the 1/8 to 1/128 dilutions are obtained by successive transfers and mixings. Finally, each drop is tested with an equal volume (30 µl) of the RBT reagent, so that the final dilutions ranges from 1/4 to 1/256 [19].

RESULTS

A total of 2,669 patients had undergone brucella testing from January 2017 to December 2019. Out of these patients, 848 were diagnosed with brucellosis. The male to female ratio was 1.92 in the patients who were tested and 2.78 in the cases positive for brucellosis. Due to lack of proper data recording guidelines, the age of patients tested for Brucellosis in 2017 were not available. But in the years, 2018 and 2019, age distribution of tested patients ranged from <1 to 89 years with a mean age of 33 years, while those who tested positive had an age range between 1 and 89 with a mean of 29 years. In both 2018 and 2019, the 19 – 40-year age group had the highest number of cases (62 and 110 respectively) followed by the 10 – 18-year age group (Table 1).

Age range (in completed years)	2018		2019		Total	
	Tested	Positive	Tested	Positive	Tested	Positive
0 – 10	56	16	149	63	205	79
10 – 18	109	48	215	106	324	154
19 – 40	186	62	368	110	554	172
40 – 60	130	36	297	76	427	112
60 – 80	49	7	128	34	177	41
> 80	2	0	10	3	12	3
Total	532	169	1167	392	1699	561

Table 1. Age distribution of patients

In Eritrea, brucellosis has been endemic across the study period. In 2017, the peak number of cases was observed in June, while in 2018, cases peaked in March. In 2019, the number of cases was highest in December with high number of cases being reported in January, April and July (Fig 1).

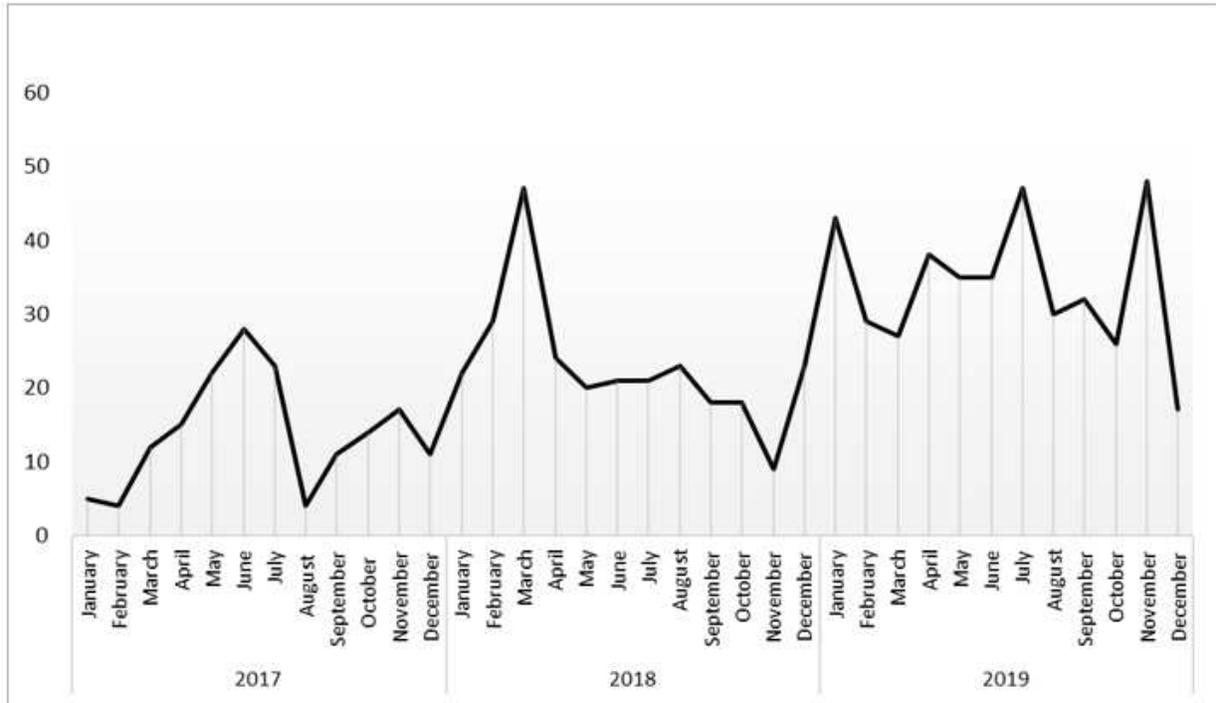


Fig 1. Total Cases of Brucellosis by year and month, 2017 – 2019

Patients were tested and came positive from 5 of the 6 zones of Eritrea. Across the study period, no tests were recorded from the Southern Red Sea zone. In 2017, most cases of brucellosis were found in Debub Zone (80), followed by Maekel Zone (28). In 2018 and 2019, Maekel Zone reported the most cases with 86 and 152 respectively. Overall, Maekel Zone had the most cases (266) followed by Debub Zone (235) and Northern Red Sea Zone (212). The results also show that the number of cases detected has increased across the study period (Table 2). We believe this could be due to the increasing awareness of the disease among health professionals in the country.

Zone	Number of Cases			
	2017	2018	2019	Total
Maekel	28	86	152	266
Debub	80	79	76	235
Northern Red Sea	22	58	132	212
Gash Barka	22	21	35	78
Anseba	13	28	11	52
Southern Red Sea	0	0	0	0
Total	165	272	406	843

Table 2. Distribution of cases by zone

Scatter plot imaging by village was done to show the distribution of the cases across the country. The results showed a dense scatter area around the Maekel zone. Few cases were located outside the villages closest to the Maekel region (Fig 2.). In addition, cases mapping by sub-zone showed highest number of cases in the Sub-Zones of the Maekel Zone and those located closest to the zone (namely Ghindae, Segheneiti, Dbarwa and Deqemhare) (Fig 3.)

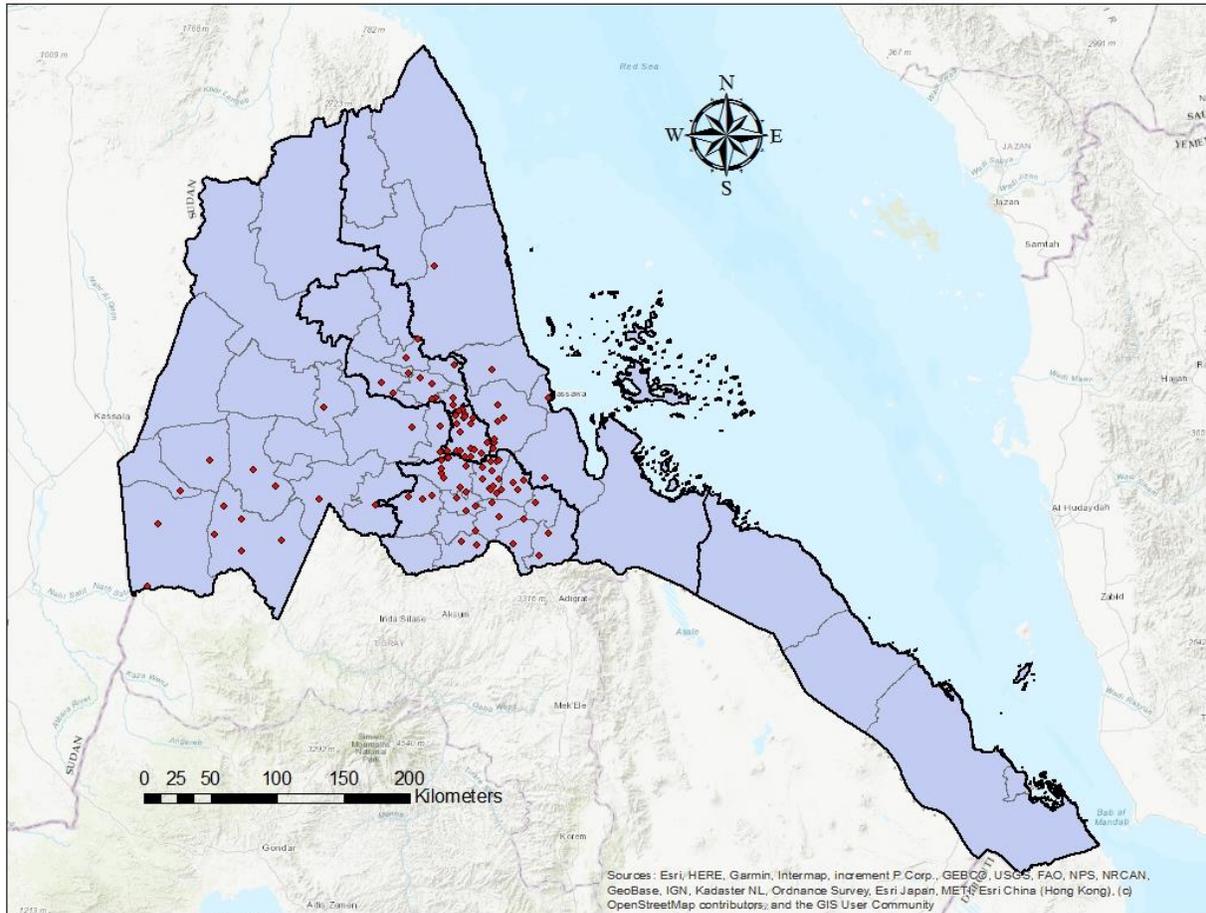


Fig 2. Scatter Map of Case Distribution by Village

DISCUSSION

This study showed that males with ages between 10 and 40 years were more likely to test positive for brucellosis. This can be attributed to the community roles in Eritrean agropastoral communities. Generally, adolescents and young adult males carry out herding, milking, farming and slaughtering duties, thus increasing their contact with animals and the risk of infection.

This study shows that Maekel zone has the highest incidence of brucellosis, followed by Debub and Northern Red-Sea zones. This is in contrast with the findings of Scacchia et al. who found more cases of brucellosis in Gash-Barka region than in Northern Red-Sea [20]. In Gash-Barka, boiling of milk before drinking is a common practice. As this is a human brucellosis study, this practice may be the reason for the relatively lower number of cases in that region. This study, similar to the study carried out by Scacchia et al. [20], also shows that there were no cases reported from the Southern Red Sea zone. The arid climate of this zone allows little agropastoral activity, thus the disease prevalence may be low. Moreover, this region doesn't have common market place with the other zones which have high disease incidence. In addition, the only major hospital in this zone is located in Assab, which is around 700km from Asmara. Therefore, cases, even if are present, are more likely to be treated clinically.

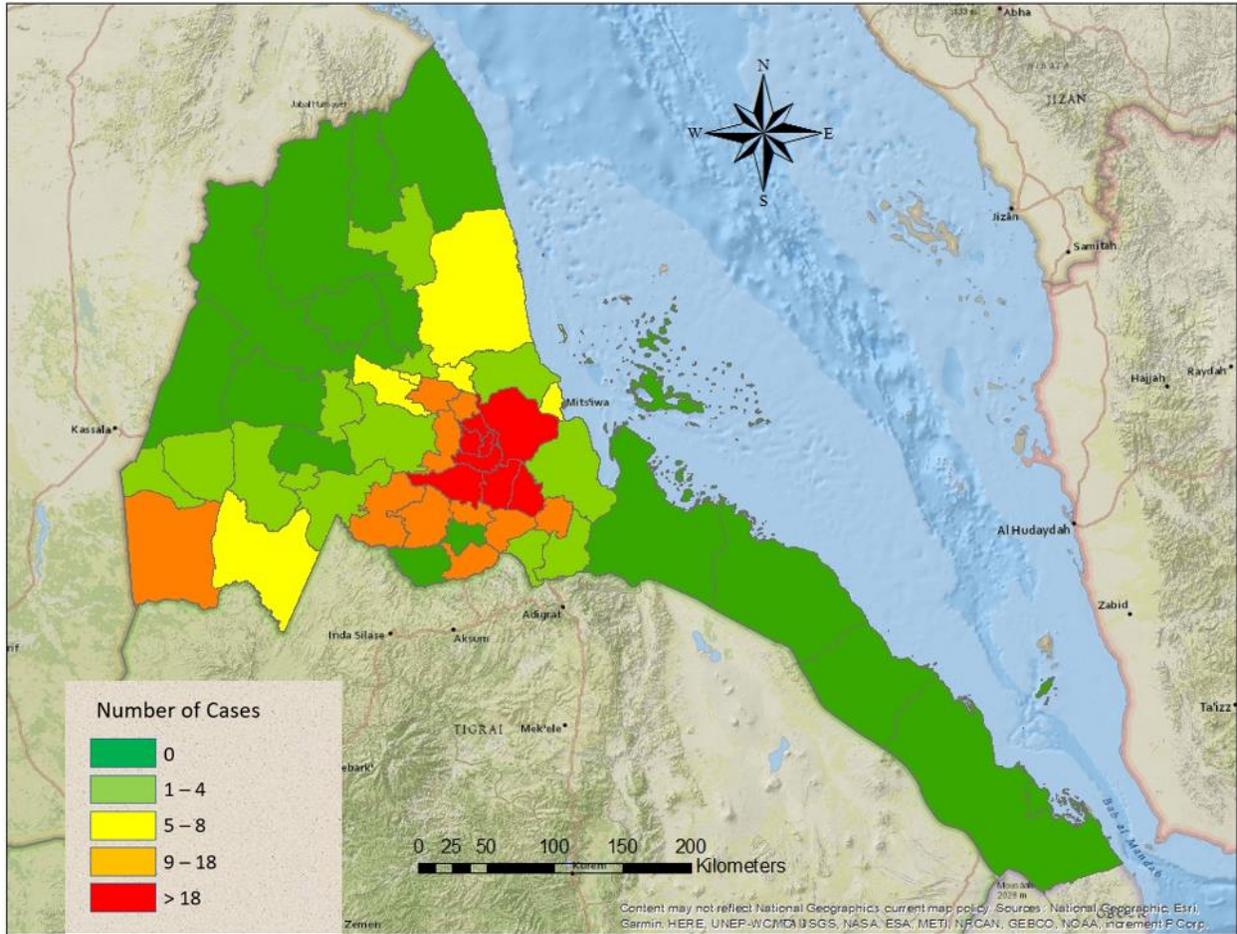


Fig 3. Case distribution by Sub-Zones.

Zone	Number of Cases					
	2017		2018		2019	
	NAPHL	HMIS	NAPHL	HMIS	NAPHL	HMIS*
Maekel	28	134	86	276	152	241
Debub	80	881	79	876	76	422
Northern Red Sea	22	34	58	86	132	129
Gash Barka	22	87	21	92	35	134
Anseba	13	52	28	192	11	165
Southern Red Sea	0	2	0	1	0	0
Total	165	1190	272	1523	406	1091

* HMIS data for 2019 is from January to September

Table 3. Confirmed cases at NAPHL vs. cases retrieved from HMIS of Eritrea

In the study time, consistent accessibility to RBT was an issue in the country. Table 3 compares the number of brucellosis cases reported to the Health Management Information System of Eritrea (HMIS) with the data acquired in the study [21]. The HMIS data shows cases diagnosed with brucellosis, but does not differentiate whether the patients were clinically diagnosed or RBPT positive. Brucellosis is

expressed in non-specific clinical manifestation, making the clinical diagnosis of the disease a challenge [22]. Although it has similar clinical manifestation with other infectious and non-infectious diseases [22], the clinical diagnosis is greatly dependent on a patient's medical and epidemiological history, clinical signs, which can result in misdiagnosis. But even keeping in mind the potential misdiagnosis of clinical brucellosis, the results of this study hugely underestimate the true extent of the disease in the country.

Two serological tests are recommended for the diagnosis of brucellosis, but only RBPT is widely used in most resource-poor countries [23], including Eritrea. Resource poor countries are recommended to use a simple modification of using 3 volumes of serum and one volume of antigen to increase RBPT sensitivity and minimize the discrepancies between RBPT and Complement Fixation Test (CFT) [22]. This study has adopted this technique with a simple modification of using 3 volumes of serum and one volume of antigen to increase RBPT sensitivity and minimize the discrepancies between RBPT and CFT.

The overall prevalence of human brucellosis in Eritrea is not known. Studies done in the early 2000s show that brucellosis is widespread in the country [24, 25]. The most recent study was carried out in 2013, and only the prevalence of the disease in cattle has been updated, showing a prevalence of 2.77% [20]. Nevertheless, brucellosis is reported to be widespread in neighboring countries, including Ethiopia, Sudan, Djibouti, Saudi Arabia and Yemen [26-35]. As movement of animals between Eritrea and these countries is unrestrained, infections are also expected to be widespread in Eritrea. Considering human brucellosis is largely affected by prevalence of the disease in animals [36], this study also shows the disease is widespread in animals in Eritrea.

Despite eradication efforts, brucellosis also affects several developed industrialized countries. In the United States, an estimated 839 new cases of brucellosis illness are reported annually with 55 hospitalization and 1 death [37]. Australia, which is not considered endemic for human brucellosis, has annually reported 34 cases per year between 1991 and 2012, [38]. From 1998 to 2010, a total of 8483 cases were notified in Italy. But further analysis depicted that the number of cases should have been much higher, ranging from 41,821 to 155,324 [39].

Cost effective measures for controlling brucellosis are known. Effective vaccines, *B.abortus* S19 and *B.melitensis* Rev.1, have been available since the 1990s [40, 41]. Nevertheless, the disease is prevalent all over the world. Controlling brucellosis requires considerable collaboration to raise awareness, provide proper testing and treatment facilities, train personnel and maintain an active surveillance programs [42]. Recently, a new approach named the 'One Health approach' is recommended to combat brucellosis. This approach sets up a platform where veterinary, medical, environmental and allied professionals can work together with stakeholders and the government, to design a suitable approach to combatting this infection [4, 42]. In Eritrea, interdisciplinary cooperation is on the rise. We believe the government can harness this newly developing platform and achieve control of the disease in the country.

Limitation of the Study

At the time of the study, all medical laboratories in Eritrea, including the national health laboratory, don't have the Brucella diagnostic kits. Since the study period, test kits have been distributed throughout the country, but the supply has been inconsistent. The only facility that has consistently had the test kits is NAPHL which is located in Asmara. Therefore, our data shows a larger portion of the sample to be from the areas surrounding Asmara and less or absent from the more distant areas.

Conclusion

This study shows that human brucellosis is widespread in five of the six zones of Eritrea. With comparison to HMIS data, it also shows that lack of access to proper testing facilities underestimates the true extent of the disease. We recommend that the government take quick actions to increase accessibility to testing and treatment facilities throughout the nation. We also suggest an eradication programs should be implemented with a One Health approach in mind.

DECLARATIONS

Ethical Approval and Consent to Participate

Ethical approval was sought out from the Research Ethics and Protocol Review Committee of the Ministry of Health of Eritrea in Asmara, Eritrea. Due to retrospective nature of the study, informed consent was waived by Health Research Protocol Review and Ethical Committee of the Ministry of Health of Eritrea. Confidentiality was assured before the study and hence data use was permitted for study purpose only. All methods were carried out in accordance with relevant guidelines and regulations.

Abbreviations

CFT: Compliment Fixation Test; HMIS: Health Management Information Services; NAPHL: National Animal and Plant Health Laboratory; RBPT: Rose Bengal Plate Test

Acknowledgments

We acknowledge all the members of ministry of health and the ministry of agriculture for their support in conducting this research.

Funding

Not applicable.

Availability of data and materials

The data of this study are available from the corresponding author on reasonable request.

Authors' contributions

ASG1 and ASG2 conceived and wrote the proposal. SBM, MWA and MW analyzed the samples and entered the data. ATT carried out the statistical analysis. ASG1 and ASG2 wrote the initial draft. The draft was edited by all the authors. All the authors read and approved the final draft.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Figures

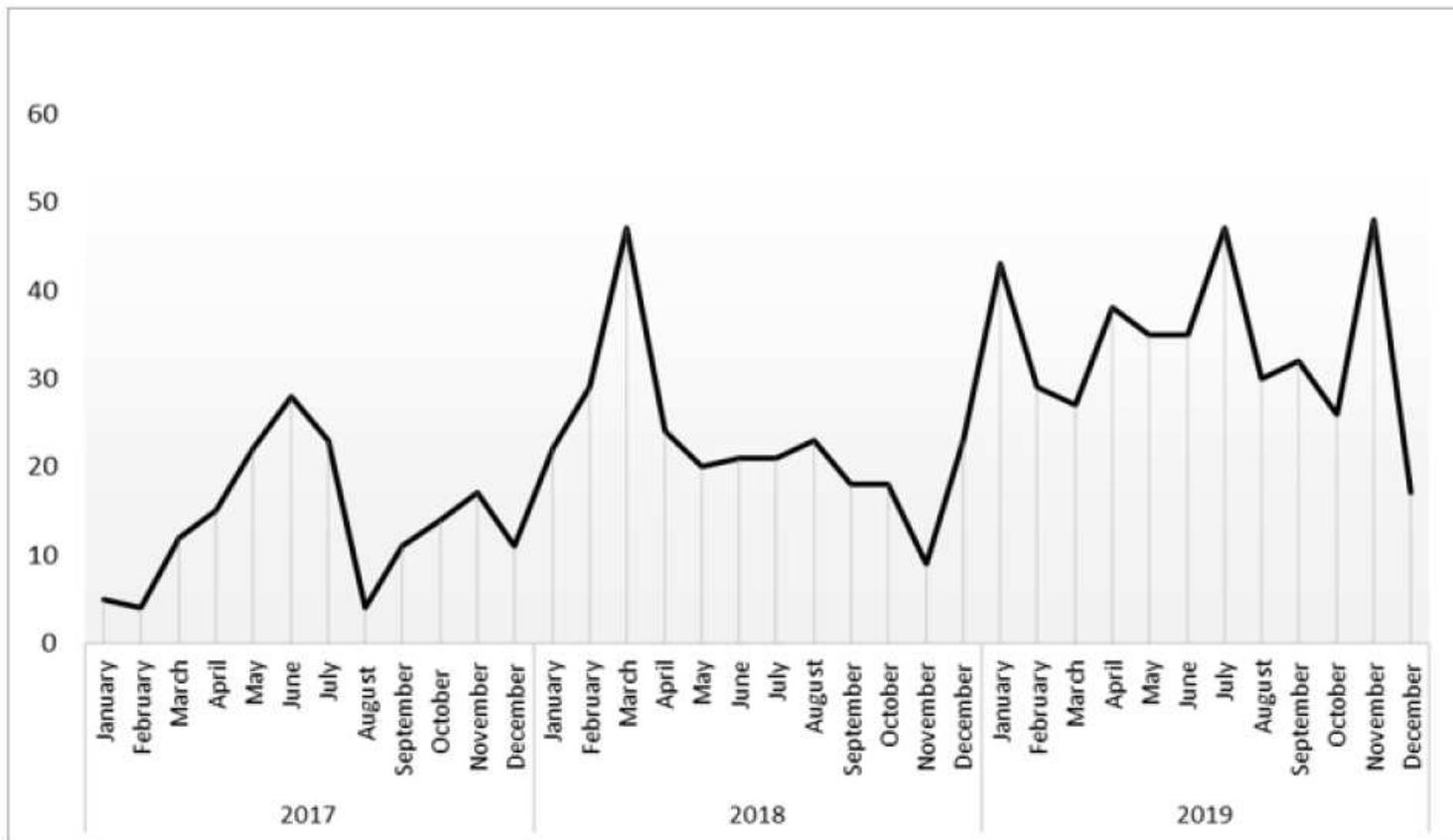


Figure 1

Total Cases of Brucellosis by year and month, 2017 – 2019

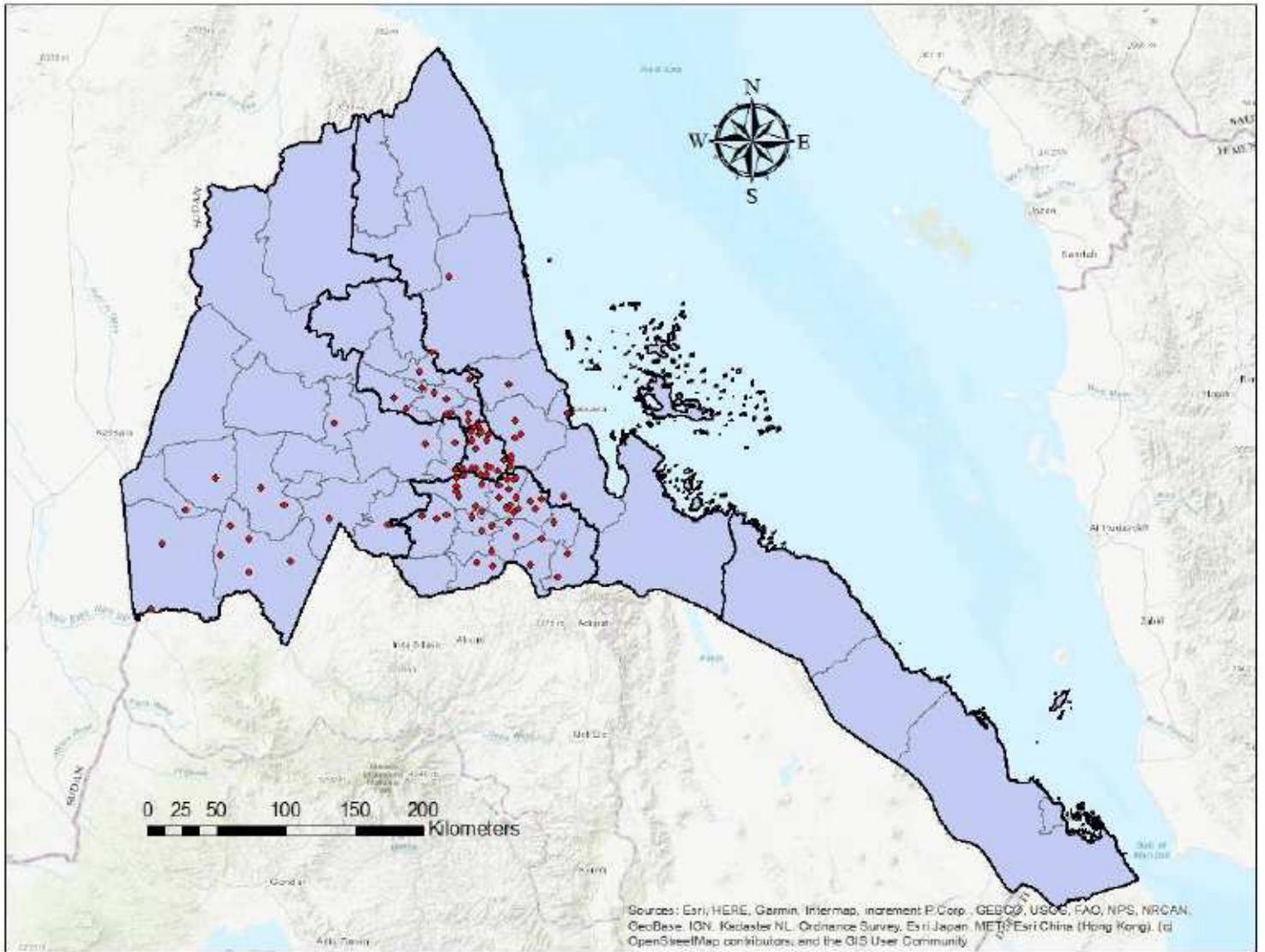


Figure 2

Scatter Map of Case Distribution by Village Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

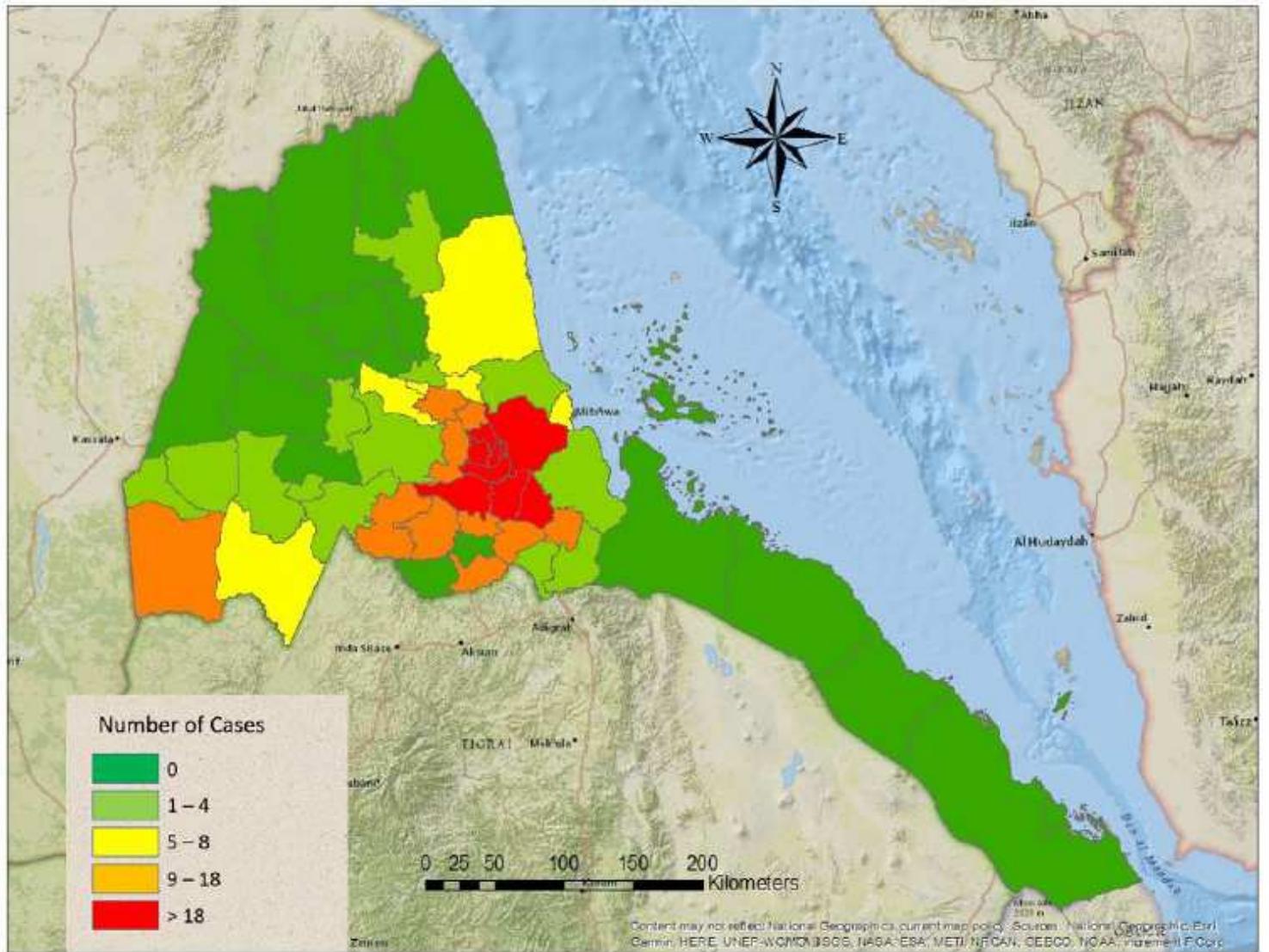


Figure 3

Case distribution by Sub-Zones. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.