Effect of population characteristics and seasonal variation on anthrax epidemiology

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Abstract

Introduction
Anthrax is a disease caused by the spores of *Bacillus anthracis* and can have a high fatality rate. It is a zoonosis and mostly affecting animals. In this study I want to find out risk factors on population scale for anthrax cases and deaths in humans and animals, and look at the relation of anthrax with weather patterns.

Methods
I searched for anthrax outbreaks in different countries, mainly yearly reports. I looked at human cases, human deaths, livestock deaths and wildlife deaths. Different risk factors were considered: country size, population characteristics, Human Development Index (HDI), total cattle number, cattle per human ratio, mean annual temperature, mean temperature of the warmest 1 and 3 months, annual precipitation and minimum and maximum precipitation in 1 month and 3 months. Linear regression was used. Statistics were repeated without China because it was often the single outlier in the figures. Statistics were also repeated with the countries aggregated in continents because of the modifiable area unit problem.

Results
Data was found for 28 countries resulting in 36 data points. There was a significant relation between human cases and cattle number, human deaths, country size and population size. There were also significant relations between wildlife deaths and population size, country size and mean temperature of the warmest month. Without China relations between human cases and maximum precipitation in 1 and 3 months, and between livestock deaths and country size were significant. For continents a significant relation between human cases and cattle ratio, cattle deaths and HDI.

Conclusion
This study mainly shows that high cattle numbers and cattle deaths due to anthrax are risk factors for human cases. Also seasonal precipitation is a risk factor. Bigger country size and population size may be indirect risk factors as these usually accompany higher cattle numbers.

Summary

In my study about anthrax I found three interesting things. It became clear that cattle are a source of anthrax to humans. It also became clear that seasonal rain has an influence on the amount of human anthrax cases. And finally it became clear that anthrax is more present in less developed countries.

An interesting fact about anthrax is that it can survive for a very long time outside the body. Probably around hundred years. And because anthrax can also be very deadly, I wanted to find out how we can predict where anthrax will occur in the future, so that we can take extra precautionary measures in those situations. To do this I collected outbreak data for different countries, so amount of cases of anthrax in humans and animals. After this I started looking if I could find relations between different factors, like cattle numbers, temperature, rain, population size and population density and development of a country.

I got different results but the most important ones are the 3 factors mentioned in the beginning of this summary, so cattle, seasonal rain and development of the country. The cattle factor shows that the more cattle in a country, the more human cases there will be in the country. This is probably because anthrax is most often found in animals, especially livestock. Humans get easily infected from this livestock, like during slaughtering of this dead animal or after eating the meat from this animal. So it is a good idea to tell people to call the veterinary health system if they find a dead animal and not investigate themselves. Next, the seasonal rain factor is difficult to find an explanation for. If there is a lot of rain in a short period of time, up to 3 months, there will be more human anthrax cases in the year. It is not clear how anthrax itself would profit from the rain. Maybe it is not the anthrax itself, but the behavior of humans and animals that changes with the rain. And this behavior change could result in more contact to anthrax. And finally, the development factor shows that there are more human anthrax cases in poorer countries. This could be a coincidence, but there are also some possible explanations for this. Like written before, especially in poorer countries an animal who died will be slaughtered and eaten. This is a big source of infection to humans. Also, bad human and veterinary health systems play a role.

So, to conclude, people should be careful around livestock, especially in poorer countries. They should call veterinarians to handle dead animals in order to prevent anthrax infections in humans.

Introduction

Bacillus anthracis or anthrax is a bacterium which causes fear in many people because it is a deadly disease and it is associated with anthrax letters. It is a zoonosis meaning it is transferable from animals to humans. This bacterium is usually found in the soil in its spore from, waiting for certain animals to come into contact with them. After these spores get access to the body these spores transform into live bacteria (1,2,3). They will then produce toxins which can ultimately lead to death.

Background

If a human gets infected, this will most probably be a result of coming into contact with the spores. It is believed that live bacteria do not cause infection, but this is not yet clear (4,5). In humans there are four different ways of manifestation after coming into contact with *Bacillus anthracis*, either spores or possibly live bacteria. All of these forms can be deadly. Skin anthrax will occur after the anthrax comes into contact with a little cut in the skin, inhalation anthrax after the anthrax is inhaled and ingestion anthrax after the anthrax is ingested. A fourth option is injection anthrax, this is a relatively new form and can be seen as a more deep form of skin anthrax. Skin anthrax is the most common type presenting about 95% of the anthrax cases, and usually presents itself in people who handle animals which died from anthrax or animal products from infected animals. Contamination can happen while simply touching the dead animals, during slaughtering, and handling animal products like skin, bones, meat and hair (1,6,7). A not painful lesion will occur at the site of entrance, with a black necrotic center, explaining the name anthrax which is Greek for coal. If left untreated, around 10-40% of people will die of this form, if treated with antibiotics less than 1% will die. Inhalation anthrax is far less common but is the most deadly manifestation, around 90% can die. This is in part because the first stage of this infection is asymptomatic. When symptoms occur and healthcare is visited, the bacteria are usually already too widespread to be effectively treated. The first symptoms can include fever, sweating, nausea, vomiting and coughing. In a later stage dyspnea, cyanosis and death can occur. This form can occur in any situation where spores are present in the air, for example during handling of the same animal products like skin, bones, meat and hair or at a site with dead animals on the ground. Ingestion anthrax is also not very common in humans and usually occurs after eating meat from an infected animal or after drinking contaminated water (1). The lesions can occur in any part of the gastrointestinal tract and usually results in local hemorrhage. Symptoms include fever, nausea, vomiting, diarrhea and abdominal pain. If death occurs this is usually 2 to 5 days after the first symptoms. For all these 3 forms the history of the patient regarding anthrax exposure plays an important role in diagnosis as symptoms beside the black lesion in skin anthrax are not very specific. The incubation time will differ with the way of coming into contact with the pathogen but is usually a few days, between 2-10. Humans who are most often affected are those who work with animals, for example in a study in Turkey where 87% of anthrax infected people were farmers, butchers or their housewives (8) or another study in Turkey (9) where 57 out of 58 infected persons were involved in agriculture. Transmission from human to human is extremely rare, and if this happens the source was skin anthrax (1). Transmission from alive animals to humans or other animals is also extremely rare.

The fourth type of anthrax, injectional anthrax, was first seen in the year 2000 in one person in Norway (10,11). He was infected after injecting contaminated heroine. Then in the years 2009-2013 more cases appeared in Europe. Out of 70 laboratory confirmed cases in these years 26 died resulting in a fatality rate of 37%. This type of anthrax usually results in deep tissue infection at the site of injection and is more severe than skin anthrax. The main symptom in these outbreaks was swelling at the local site, always missing the black lesion. This difference in presentation makes diagnosis more difficult which could in part explain the higher fatality rate compared to skin anthrax (10,11).

Treatment of anthrax mainly consists of antibiotics to save the patient (1,6,8,9). Penicillin works as these bacteria are very rarely resistant, but many other antibiotics also work. This makes it that even in developing countries this disease can be treated. It is very important to give the antibiotics as fast as possible. If the antibiotics are administered after a certain point in the disease, the bacteria could all be killed but the present toxins could still cause death. Therefore, if the antibiotics are administered timely there will not be as many toxins. Especially in inhalation anthrax it could already be too late for administration at the first time of symptom presentation, leading to the high fatality rate in this form of anthrax. Besides antibiotics it may also be necessary to keep the patient on the intensive care for supportive care (1). Surgical intervention may be needed to cut out infected areas, especially in skin anthrax and injection anthrax (8-11).

Live anthrax bacteria cannot survive outside their hosts for a long time because they cannot survive in such environments. To be able to survive the bacteria will transform itself into spores (1,2,4). These spores are capable of surviving many different impacts, like high and low temperatures, anaerobic environments, many disinfectants, UV and ionizing radiation and a high pH range. These spores also do not need nutrients as they are metabolically inactive (1,5). This means these spores can survive for many years, even decades in many environments on earth. The spores wait for the environmental conditions to be suitable again for the living state, after which they transform back into live bacteria. This can be after they somehow ended up in a host and maybe also after ending up in an environment outside a host with a suitable condition for the living state, but there is no consensus about this last statement. The live bacteria will multiply and when the conditions are unfavorable again, they will all transform back into spores. These will then wait again for favorable conditions to go through the same loop (1,5). Reinfections of anthrax at the same location are reported after 60 years (12) or 75 years (2), showing how long the spores can survive.

After the spores arrived inside the host, they will transform into live bacteria (5,6). They do this because of specific signals they get in the body. The spores itself are not harmful and cannot multiply by their self. The spore will lose its great resistance properties while transforming, however these live bacteria will have a surrounding capsule which makes it harder for the immune system to attack them and this is one of the two well known virulence factors of this pathogen. More specifically this capsule consists of poly-γ-D-glutamic acid and it prevents phagocytosis and the complement system from affecting the bacteria (13). The bacteria will also produce toxins, namely protective antigen (PA), lethal factor (LF) and edema factor (EF), which are the second virulence factors. These toxins will ultimately lead to the death of the host, it is the LF and EF that have harmful effects on the body. PA only facilitates the uptake of the LF and EF into cells. PA will bind to LF or EF and this complex will bind to receptors on the cell surface. These receptors are tumor endothelial marker 8 (TEM8), also named ANTXR1 and capillary morphogenesis gene 2 (CMG2), also named ANTXR2 (4,14), where CMG2 seems the most important in anthrax pathogenesis (14,15). After binding, this receptor-toxin complex will enter the cell through endocytosis in a lysosome (4). Because of the low pH in this lysosome the PA will undergo a conformation change to form a pore in the lysosome. This allows the LF or EF to enter the cytoplasm. Another possibility is that the toxin complex will first end up in an intraluminal vesicle. Here the complex can stay for a longer period of time to fuse again with a lysosome and to be released in the cytoplasm (4). LF will mainly induce apoptosis of the cells and EF will mainly lead to edema, as the names also imply. How they exactly do this is not fully understood. It seems also that these 2 toxins have a favorite tissue to affect. LF mainly affects the cardiomyocytes and smooth muscle cells and EF mainly effects the hepatocytes, but in this disease almost all organs will be affected. An infected person will eventually die due to presence of large amounts of bacteria and toxins which will affect many organs and respiratory dysfunction, brain hemorrhage, hypotension and/or heart failure will often be the direct cause of death of a systemic anthrax infection (14). This is thought to be mostly a direct effect of the toxins on the cardiovascular system and not the result of cytokines.

In a mice study published in 2016, researchers found that in skin anthrax, the two anthrax toxins already spread throughout the mice bodies before the bacteria are detectible at either the site of infection or in the bloodstream (16). Before it was thought this was not the case and the anthrax was only present at the local site of inoculation in the beginning stage. This shows that there is still a lot to be discovered about this disease, despite this bacterium has already been discovered in 1875 by Robert Koch (1).

There are some suggestions that anthrax causes more outbreaks in particular environmental conditions, like a temperature above 15 degrees Celsius, a ground pH >6, enough precipitation and availability of organic contents, there seems to be especially a link with calcium contents. But the exact role of these environmental influences are not very well clarified nor understood (1,6,8).

When an infected animal dies due to anthrax, it will also spread the bacteria (1,6). The anthrax toxins will cause damage to the blood vessels leading to hemorrhage, this means that when the animal is lying on the ground, contaminated blood will come out of the orifices. This outside environment is not sufficient for the bacteria to survive, so they will transform into spores again. The carcass will stay infective and these spores will also contaminate the soil. These spores will wait for a new animal to come into contact with these spores. This can be by animals grazing which then come into contact with the contaminated soil or the dead animal. This makes it clear why anthrax is a disease which is much more common in animals than in humans and it is especially prevalent in grazing herbivores, with cattle being affected often. For example in Ukraine over a period of 100 years, 72% of infected livestock were cattle and 15% were sheep and goats, 8% swine and 4% horses (17). Carnivores also seem to be less sensitive to anthrax. Chickens, pigs, dogs and cats are also less sensitive. But any animal, resistant or not, can spread the spores as a mechanical vector, like birds and flies (1,6,3,18). Buried carcasses can also be a source of infection in at least two ways. First, earth worms can bring the spores to the surface. Second, in digging activities people or animals can encounter such carcasses (19), or these carcasses can reach the surface because of environmental impacts, like erosion or melting ice (2). Dead animals should therefore preferably be burned and not buried (1). Decontamination of infected sites or cleaning products by killing the spores is possible with for example hydrogen peroxide, formaldehyde or dry heat (1).

There is an anthrax vaccine available for animals. The first version of this vaccine has been discovered a long time ago, it was developed by Louis Pasteur in 1881 (20). The current vaccine is based on a different strain (6). This vaccine should be given annually and can be used to stop current outbreaks or prevent future outbreaks (1), but this demands that the vaccination should continue annually. If the vaccination is stopped, there is a risk of new outbreaks. The vaccine is not frequently used however (21,22). In a study in Azerbaijan it was found that the anthrax incidence in livestock increased after the collapse of the Soviet Union when the public and animal health system collapsed. A few years later when animal vaccines were introduced the livestock incidence decreased dramatically (23). This also decreased the number of human cases. The same pattern was found in Georgia, where in 2007 the annual livestock vaccination was not mandatory anymore (24). After this the number of human cases increased significantly. This shows that changing policy for animal health can also have influence on human health. This emphasizes the importance of the One Health concept. This does not mean however that this pattern is always seen. In Israel where the vaccine policy of yearly vaccination in deer was stopped after it was started 10 years earlier after an outbreak (25), there were no new cases of anthrax found since then.

In a study in the Etosha National Park in Namibia which is known for its frequent anthrax outbreaks (26), researchers looked at antibodies against anthrax in wild animals, namely zebra, springbok and elephants and also tried to retest these within 2-3 years. Zebras were most often found positive, 62% of the tested zebras had antibodies against anthrax. Moreover both negative and positive seroconversion were seen in retested zebras. This shows that anthrax is not always a lethal disease and suggests that an immunity against anthrax is build, however not long lasting with a mean time to negative seroconversion of 6 months. On the other hand these subsequent infections can be seen as a natural way of boosting, resulting in a memory response. In a study in guinea pigs (27) a low dose of anthrax did not result in detectable antibodies, but after a second dose in a later point in time there was a quick immune response showing that immunological memory was created earlier. This could indicate that the negative seroconversion of the zebras does not mean that the immunity against anthrax is lost.

Aim of study

With this study I want to find out more about the behavior of anthrax, how, where and why it is causing infections and outbreaks. Therefore I want to find risk factors for anthrax on human population level to understand why the disease is present in one place and not in another. I also want to look at the relation of anthrax to different weather patterns.

Research questions:

- What is the relation of climate/weather with human or animal anthrax incidence?
- What are risk factors on human population scale for anthrax?

Methods

First, I collected data on anthrax outbreaks. For outbreak data I searched for human cases and deaths and livestock and wildlife deaths. To do this I searched for a big database with anthrax data on country or world level. Unfortunately, I could only find this for Europe, covering only human cases and deaths. I then decided to search for individual outbreak reports with data for at least a whole year. When I found outbreak reports in which it was not clear if this covered at least a whole year I searched on Google for other outbreaks in that country in that year. If I did not find anything I assumed that there were no other outbreaks in that year and included the data in my dataset. This way I can fairly compare different countries with each other. I searched for data using Google search, with the search terms: anthrax, anthrax cases, anthrax deaths, anthrax outbreak(s), anthrax in animals and anthrax animal outbreak. I searched the normal results and the picture results. I also searched in PubMed with the term: "Anthrax"[Mesh] and when I found a study with outbreak data I searched in the references, “similar articles” and “cited by” for more data. I focused on outbreaks in the 21st century but did not exclude older outbreak data if it was included in studies. These searches were done between the 1st and 29th of April 2020. This resulted in data for 28 countries, Appendix 14. Ghana had double data for cattle deaths for multiple years, the cattle deaths 2003-2012 were not used.

To find out which factors are risk factors for anthrax I collected data on possible risk factors on country level. These are country size, total population size and urban and rural population size, percentage of people living in rural areas, population density, Human Development Index (HDI) which combines the 3 factors human health, knowledge and income, total cattle number, cattle per human ratio, mean annual temperature, mean temperature of the warmest three months and mean temperature of the warmest month, annual precipitation, minimum and maximum precipitation in one month and minimum and maximum precipitation in 3 months. This was done for countries for which outbreak data was found.

I did not search for livestock numbers in general but cattle data specifically. I did this because cattle are among the most affected livestock by anthrax and because there are livestock which become rarely infected. So to avoid getting livestock numbers which contain very few infectable animals I chose to use cattle numbers.

The weather data was found on the website of the Climatic Research Unit (CRU), part of the University of East Anglia (28). The HDI data was found on the United Nations Development

Programme website (29). The data about total and urban and rural human population and cattle population were found on the Our World in Data website (30). Population density and cattle ratio were manually calculated. Rural percentage was calculated by dividing people living in rural areas by total population size.

Statistics were done using the statistical program R, version 3.6.1. Linear regression was used to look at the relationship between the four response/dependent variables, human cases and deaths and cattle and wildlife deaths, with all the explanatory/independent variables, and also the response/dependent variables with each other. A p value below 0.05 was regarded as significant.

While looking at the results it became clear that China was a frequent outlier in the graphs. For this reason, I decided to do the same tests again, this time without China in the dataset. Data for China only contained human cases and cattle deaths, so only new tests with those 2 factors were done.

Also, I put the countries together in continents to get somewhat more comparable size and human and animal populations. This way we can look at the different relations on a different scale, which can give different results. This is sometimes called the modifiable area unit problem. Now differences between individual countries are evened out and this allows for more statistical power to see relations between factors. I made continents for Europe, Africa, Asia and kept the individual countries Russia and China because of their size and population. I only summed the data for the countries which were in this dataset, to create the continents. I did not look at weather data (temperature and precipitation) because of the large size of the continents.

Results

Data was found for the following countries, immediately divided into continents for good overview: Africa included Kenya (31,32), Zambia (33,34), Uganda (35), Tanzania (36,37), Namibia (3,38-40), Ghana (41), Lesotho (42), Zimbabwe (43), Niger (44). Asia included Kazakhstan (45,46), Georgia (24), Azerbaijan (23), Turkey (8,9,47), Bangladesh (48), Indonesia (49), and Pakistan (50). Europe included Ukraine (17), Hungary (51), United Kingdom (10,51), The Netherlands (51), Spain (51), Romania (51), Greece (51), Sweden (51), Poland (51). The 3 countries Canada (52), China (53,54) and Russia (2) were not put into continents.

I found data for individual years and combined years. Data from combined years was calculated back to single years by dividing the cases and deaths by the amount of years. The data can be found in the appendix, Table 4 and 5.

Results for all the countries can be found in Table 1. There is a significant positive relation between human cases and number of cattle with a p value of <0,001. A significant positive relation between human cases and deaths with a p value of 0,009. A significant positive relation between human cases and total population with a p value of <0,001. And a significant positive relation between human cases and country size with a p value of 0,007. Furthermore there is a significant negative relation between wildlife deaths and the mean temperature of the warmest month with a p value of 0,044. There is a significant positive relation between wildlife deaths and total population with a p value of 0,001 and a significant positive relation between wildlife deaths and country size with a p value of <0,001. Figures can be found in the appendix, Figure 1 to 7.

Removing China from the dataset gave different results and can be seen in Table 2. Since China did not have data for human deaths or wildlife deaths these 2 factors are removed from the Table 2 to show only relations affected by removing China. Now the relation between human cases and number of cattle, country size and total population are not significant anymore. There was a new significant positive relation between human cases and the month with maximum precipitation with a p value of 0,006. There was also a new significant positive relation between human cases and the maximum precipitation in 3 months with a p value of 0,001 and finally there was also a new significant positive relation between cattle deaths and the country size with a p value of 0,013. Figures can be found in the appendix, Figure 8 to 10.

For the continent data completely different results were received, Table 3. A significant positive relation between human cases and cattle ratio with a p value of 0,039. A significant positive relation between human cases and cattle deaths with a p value of 0,012 and a significant positive relation between human cases and HDI with a p value of 0,44. Figures can be found in the appendix, Figure 11 to 13.

Table 1. P-values for possible risk factors for anthrax in humans, livestock and wildlife. All countries. ns= not significant

	Human cases (anthrax)	Human deaths (anthrax)	Livestock deaths (anthrax)	Wildlife deaths (anthrax)
Human cases (anthrax)	x	x	x	x
Human deaths (anthrax)	0,009	x	x	x
Livestock deaths (anthrax)	ns	ns	x	x
Wildlife deaths (anthrax)	ns	ns	ns	x
HDI	ns	ns	ns	ns
Total population	<0,001	ns	ns	0,001
Urban population	ns	ns	ns	ns
Rural population	ns	ns	ns	ns
Rural %	ns	ns	ns	ns
Population density	ns	ns	ns	ns
Country size	0,007	ns	ns	<0,001
Cattle number	<0,001	ns	ns	ns
Cattle ratio	ns	ns	ns	ns
Mean annual temperature	ns	ns	ns	ns
Mean temp warmest month	ns	ns	ns	0,044
Mean temp warmest 3 months	ns	ns	ns	ns
Annual precipitation	ns	ns	ns	ns
Least precipitation (month)	ns	ns	ns	ns
Maximum precipitation (month)	ns	ns	ns	ns
Least precipitation (3 months)	ns	ns	ns	ns
Maximum precipitation (3 months)	ns	ns	ns	ns

Table 2. P-values for possible risk factors for anthrax in humans, livestock and wildlife. Excluding China. Only showing factors with possible new relations. ns= not significant

	Human cases (anthrax)	Livestock deaths (anthrax)
Human cases (anthrax)	x	x
Livestock deaths (anthrax)	ns	x
HDI	ns	ns
Total population	ns	ns
Urban population	ns	ns
Rural population	ns	ns
Rural %	ns	ns
Population density	ns	ns
Country size	ns	0,013
Cattle number	ns	ns
Cattle ratio	ns	ns
Mean annual temperature	ns	ns
Mean temp warmest month	ns	ns
Mean temp warmest 3 months	ns	ns
Annual precipitation	ns	ns
Least precipitation (month)	ns	ns
Maximum precipitation (month)	0,006	ns
Least precipitation (3 months)	ns	ns
Maximum precipitation (3 months)	0,001	ns

Table 3. P-values for possible risk factors for anthrax in humans, livestock and wildlife. Continents. ns= not significant

	Human cases (anthrax)	Human deaths (anthrax)	Livestock deaths (anthrax)	Wildlife deaths (anthrax)
Human cases (anthrax)	x	x	x	x
Human deaths (anthrax)	ns	x	x	x
Livestock deaths (anthrax)	0,012	ns	x	x
Wildlife deaths (anthrax)	ns	ns	ns	x
HDI	0,044	ns	ns	ns
Total population	ns	ns	ns	ns
Urban population	ns	ns	ns	ns
Rural population	ns	ns	ns	ns
Rural %	ns	ns	ns	ns
Population density	ns	ns	ns	ns
Country size	ns	ns	ns	ns
Cattle number	ns	ns	ns	ns
Cattle ratio	0,039	ns	ns	ns

Discussion

When we look at the results we see a few significant risk factors for anthrax. Firstly human anthrax cases is significantly related to number of cattle meaning that there are more human anthrax cases if there are more cattle present in the country. If we look at the anthrax cycle we can maybe find an explanation for this. If there are more cattle in an area, there could be a higher chance that some of this cattle come into contact with anthrax spores. This cattle has a high chance of dying and because this is livestock, subsequent contact with a human is very likely. Transmission can occur with just touching this dead animal, but also especially in poorer countries this animal will be slaughtered for the meat to sell (22,32,33,48). The person eating this meat also has a risk of getting infected. So one infected cattle could infect multiple people. Keeping this in mind it is strange that anthrax cattle deaths is not significantly related to number of cattle on one hand or human cases on the other hand. One explanation for this could be that there was very few data in the dataset regarding cattle deaths. There were only 14 out of 36 data points with data for cattle deaths so there could have been too little statistical power to show this relation. In addition, after China was removed from the dataset, cattle number was not significantly related to human cases anymore. This could mean that there is indeed no real relation between these human cases and cattle number which could in turn also explain the lack of the relation between human cases and cattle deaths. However cattle deaths is significantly related to human cases when testing with continents. This will be discussed later.

There was also no significant relation between human anthrax cases and wildlife anthrax deaths. There could be several reasons for this. An anthrax related reason could be that humans are not so likely to come into contact with wildlife so there will be only few possibilities of transmission. In addition, wildlife is not as likely to be slaughtered compared to livestock. Another explanation can be that there was not enough data for good statistical power. Only 10 out of 36 data points contained data about wildlife deaths.

Human anthrax cases are significantly related to human anthrax deaths meaning if there are more cases there are also more deaths. This does not need much explanation. If there are more anthrax infected people then it is very likely that more people will die. This does depend however on the form of anthrax. If skin anthrax is the cause then it might not necessarily mean that there will be more deaths with more cases. An example of this is skin anthrax in Azerbaijan (23) over 27 years with 498 cases and zero deaths, or skin anthrax in Bangladesh (48) over 2 years with 273 cases with also zero deaths, probably due too good and timely treatment. It would have been interesting to split up the 3 anthrax types in the current study and see how they are related to the risk factors but unfortunately the data did not allow this. Too few reports made distinction between the types of anthrax.

Human population and country size are both significantly related to human cases. I think these 2 factors may maybe not be seen independently from each other because in a bigger country usually more people are living. It is unlikely that this relation with human population will be a causative relation because anthrax hardly spreads between humans (1). Rather there will maybe be a higher chance to find anthrax cases if there are more people in a country, maybe because more people usually means more livestock and these can spread the anthrax. We already saw a significant relation between human cases and cattle number. This same explanation can explain the relation for country size. In a bigger country there will usually be more people and more livestock, if there is enough land available for grazing. After China was removed from the dataset these 2 relations were not significant anymore and this also became clear in the graphs, appendix Figure 3 top datapoint and Figure 4 most right datapoint. The China datapoint was found far away from the other datapoints and was the only reason these relations were significant. So it is hard to say if these 2 factors are indeed risk factors for human anthrax cases. They could be indirect risk factors as they usually go along with more cattle.

Furthermore, there is a significant relation between wildlife deaths and both human population and country size, suggesting there are more wildlife deaths in a bigger country and if there is a bigger population. This last relation seems hard to explain, since humans do not infect wildlife with anthrax. It seems thus very unlikely that this is a true relationship. In the graph, appendix Figure 6, top datapoint, it becomes clear that there is again a single datapoint responsible for this, Russia with its single outbreak. The relation with country size has some possible explanations. If the country is bigger there will be more wildlife and if the country is bigger there is a higher chance that there is some ground which is infected with anthrax spores, which could kill wildlife. So just statistically there is a higher chance to find more wildlife deaths in a bigger country and this is not a risk factor in itself. This relation is probably also the result of the Russia datapoint, appendix Figure 7, top datapoint, so it is unclear what the value of these 2 relations are. It seems they are a result of the small dataset.

There is a significant negative relation between wildlife deaths and the mean temperature of the warmest month which means there are fewer wildlife deaths if the temperature is higher in the warmest month. If I look at the graph, appendix Figure 5, top datapoint, it becomes clear that there is only a single datapoint resulting in this relation. This is Russia, with one large outbreak in wildlife which was in a colder area with melting ice, probably resulting in an infected carcass coming to the surface resulting in infection (2). Other studies show that outbreaks can happen in both warmer and colder periods. In the next paragraph I will go deeper into this.

After removing China from the dataset there were new significant relations between human cases and both the maximum precipitation in one and three months. This shows there are more cases in a country if there is more rain. The relations with annual precipitation and minimum precipitation in one and three months are not significant. This suggests that there are more anthrax cases if there is a seasonal rain period. On the basis of this result it is not possible to see when these cases occur, before the period of rain, during or after. In a study in Texas it was found that the anthrax epidemics in the summer occur after there have been heavy rains in the spring and only sporadic cases occur after drought in winter or spring (55). This suggest that rain is important for anthrax outbreaks, but that these anthrax outbreaks do not necessarily only have to occur in the rainy months. This is also seen in other studies in Africa, where the countries have seasonal rains and dry periods. In Kenya during a period of 18 years, 31% of the anthrax outbreaks in wildlife occurred during dry hot months, 36% in dry cold and 37% during rainy months (31). In Zimbabwe during a 11 year period, 40% of the cattle outbreaks occurred during hot dry months, 31% in rainy months, 15% in cold dry months and 14% in post rain months (43). In Tanzania over 11 years most anthrax cases in humans, livestock and cattle occurred in the dry months (36). In Ghana most outbreaks occurred in dry months during the onset of the wet season (22). In a study in Zambia most anthrax cases also occurred in dry months (33). The investigators suggested that this was mainly because the people took their livestock to floodplains in these dry months, where more anthrax spores were present and also leading to a higher density of livestock and people favoring outbreaks. In contrast, in Namibia the peak in wildlife cases occurs in the end of the rainfall season (3), as in Lesotho where 79% of the outbreaks over 11 years in livestock occurred in the rainy hot season (42). This shows that most outbreaks do seem to appear in hot and dry months but there is no consistency in the different countries in Africa. Taken all together, my result and the results from other studies suggest that climate and seasons plays a role in the anthrax incidence but a large part of the reason could be a different behavior of humans and animals during different seasons and not the ability of anthrax itself to cause more cases in a particular season. How anthrax itself would profit from rain or drought is not yet clear. Possible earlier suggested reasons (1,31,36,43,55) are that hot dry months stress animals which leads to a weakened immune system making the animals more susceptible to anthrax and other diseases. That during hot dry months animals are more likely to feed on short grass in absence of other vegetation and anthrax spores are much more present in this grass so close to the ground. Animals will be closer to each other as there are fewer sites to feed. Available water may collect and concentrate spores. However, there is not much evidence for all these reasons. One study even demonstrated that soil exposure for animals was higher in wet and not in dry months (3).

After combining the countries into continents and repeating the statistical test I got three new significant relations. Human cases and cattle ratio were significantly positively related. So there are more human cases if there are more cattle per person. This resembles the significant relation between human cases and cattle number in the individual country dataset statistics. Taking these 2 relations together gives a solid prove that there is indeed a higher risk of human anthrax cases if there are more cattle in a place, for the same reasons explained earlier that more cattle can mean a higher chance some of this cattle comes into contact with anthrax spores. It is likely that this also applies to other livestock. In addition there was a new significant relation between human cases and cattle deaths, showing there are more human cases if there are more cattle deaths. This is very good explainable following the anthrax cycle. Humans are very likely to come into contact with dead cattle. The anthrax infection can then easily be transferred to humans. The reason that these 2 relations were positive for continents but not for individual countries could be that there are some differences between countries within a continent making the datapoints too scattered to show a significant relation. When the countries are added together this scattered data has no influence anymore. This thus means that a country within one continent could still show a different view, with more cattle but with fewer human anthrax cases. This problem is known as the modifiable area unit problem. This same relation was also found in other studies. In one study human anthrax cases was significantly related to livestock cases (33). In another study human cases were related to both livestock and wildlife cases (36).

And finally there is significant negative relation between human cases and HDI showing there are fewer human cases in continents with a higher HDI, or more human cases in continents with a lower HDI. In other words, human anthrax is more present in lower developed parts of the world. This is also noted by others (1,2). For the same reason explained before, this relation could possibly not be shown between individual countries. So there can be countries with a higher HDI than others but still having more human anthrax cases. Some of the reasons for this relation can be for example the practice of slaughtering and eating sick animals in poorer countries, a less established veterinary health care system leading to more sick animals and thus more spreading of the disease, less knowledge of disease in the public leading to more risky behavior, no or late diagnosis due to bad/few human health facilities or bad hygiene leading to more infections.

There were no significant relations between human cases or deaths and urban or rural populations or rural percentage, while there are statements in some studies (1,2) that there is more anthrax in rural populations. Rural populations have a lower HDI compared to urban populations in the countries with a lower HDI, giving more risk of anthrax for the reasons given in the HDI discussion in the previous paragraph. Also there are more livestock in rural areas which can transfer the infection to humans. Maybe there was no significant relation found because a bigger rural population itself does not have to mean that there will be more anthrax cases or deaths. In theory a small rural area located near anthrax spores contaminated ground could be sufficient for several anthrax outbreaks. It is already shown that most cases appear in agricultural conditions (8,9).

It is impossible to find all death wildlife within a country and if dead wildlife is found there may or may not be testing done for the cause of death because there is not always enough testing capacity, especially in African countries (2,36). This means that from a large part of the death wildlife the cause will never be known. And if testing is done and anthrax is found as the cause of death than it is the question if this knowledge is reported to officials in the country. One study also suggested that sometimes scavengers remove wildlife deaths making it impossible to register these deaths (31). I found statements in different studies that there is a big underreporting of anthrax (18), even in human anthrax. In a study from Tanzania on human anthrax (36), 96 cases were reported in the electronic surveillance system for an entire region, but 134 cases were found in health facility's records in the hotspot district alone. For another region these numbers were 38 and 109 cases.

There are some weak points in this study. Firstly the dataset was rather small. This almost certainly had implications for the statistical tests done. On one hand I saw that a single datapoint/country could make a relation significant, while looking at the corresponding graph clearly showed this was probably not a true relation. On the other hand there could have been some real relations which I was not able to show with my small dataset. Another weak point is that the dataset was not complete. I am sure there is more data available on the internet which I was not able to find. Even more important and as indicated earlier, a lot of anthrax is either never diagnosed or not reported, especially in animals. This makes the available data biased. Despite this I think my dataset is a good representation of the available anthrax data online since I searched in multiple different ways.

Sometimes it was unclear whether an outbreak report was for a whole year or just covering a single outbreak. I then searched on Google to look for other outbreaks in that year and then included this data anyway and since a report on a single outbreak can also mean that there were no other outbreaks. I am sure this led to false data in my dataset because I did not find some outbreaks.

Also, I did not consider whether treatment for anthrax was applied or not. In some cases this information was also not written down in the outbreak reports so it was difficult to include this information. This certainly has influence on the amount of deaths for especially humans but also cattle. This could have been the reason that there were so little significant relations for human deaths.

The most important suggestion which can be concluded from this study is that there should be one universal database for anthrax in humans and animals. The available data now is scattered over the internet making research difficult and unnecessarily time consuming. One other study also assembled a big database for their study in a way somewhat similar to my methods (21). This study even stated that they are willing to share their dataset, but only after approval from country ministries for health or agriculture. It is a waste of time if every research into anthrax epidemiology is creating a dataset from zero. It seems that the Food and Agriculture organization of the United Nations (FAO) is collecting anthrax data to create a dataset like this (2), so it could be that such a dataset already exists but is not available to the public. If this is the case I think this is a bad case. Data should be available to everyone. I also found a link on the WHO website to a website called World Anthrax Data Site which should contain worldwide anthrax occurrence data, but this website is unavailable (56). E-mailing WHO about this issue did not result in a response.

Conclusion

This study gave several different insights into anthrax and its risk factors on population scale. To go back to the research questions, the first question was about the relation to weather patterns. This study showed that there will be more human anthrax cases if there is a seasonal rain period with a lot of rain in a relatively short period of time. The reason could lie in altered human and animal behavior or the anthrax spores could benefit from a lot of rain, but this needs further studies. The next research question considered risk factors on human population scale. There are more human cases if there are more cattle present, showing cattle are a big source of infection for humans, which was known before. Furthermore there are more human anthrax deaths if there are more human cases, but other studies show this can be dependent on the type of anthrax and how the treatment regime was. Country size and population size may be indirect risk factors since they go along with higher cattle numbers. The analysis with continents shows there are more human cases when there are more cattle per person and more human cases if there are more cattle deaths, confirming that cattle are a true source of infection to humans. And finally there are more human cases in a lower HDI setting. This probably has several reasons, like higher cattle numbers, lower health status and knowledge and higher tendency to slaughter sick animals. All in all it became clear that if anthrax spores are present, a high risk of human cases is present in situations related to livestock.

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I would like to thank Patrik Dinnétz who was the supervisor of this thesis. He helped me a lot in the beginning phase of this thesis with the data collection and the statistics. It was his idea to redo the statistics without China in the dataset and to make continents. This was absolutely a great improvement of this thesis.

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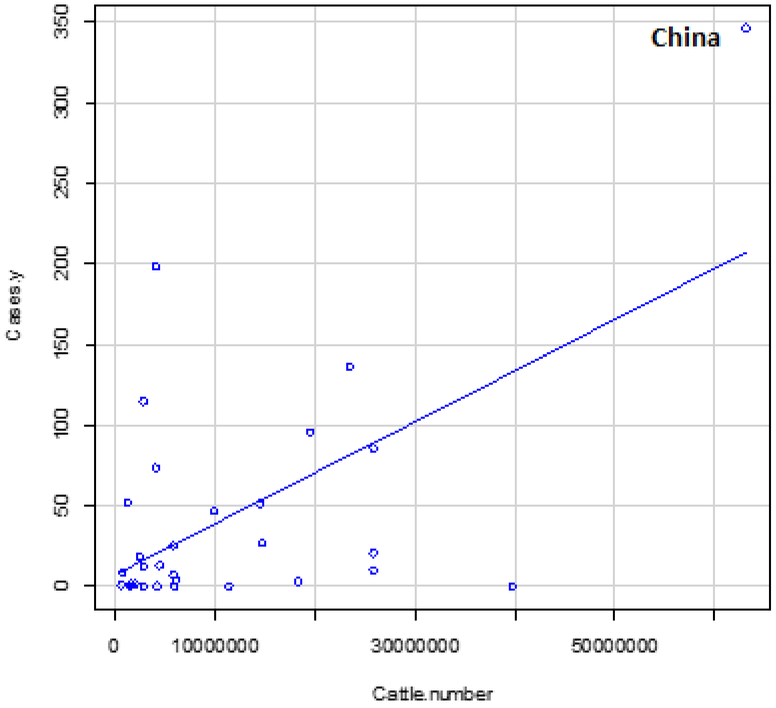
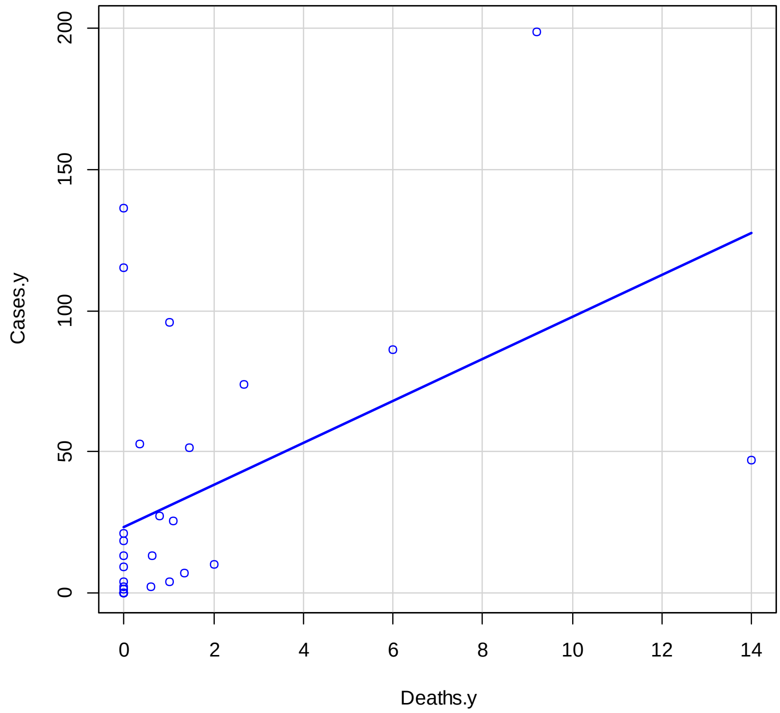
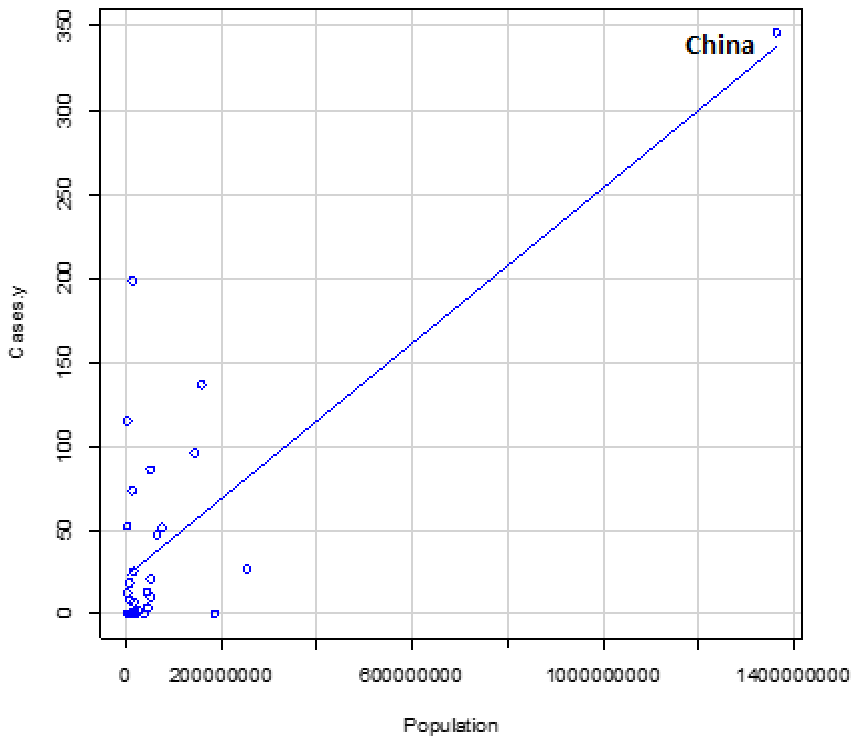
Appendix, figures



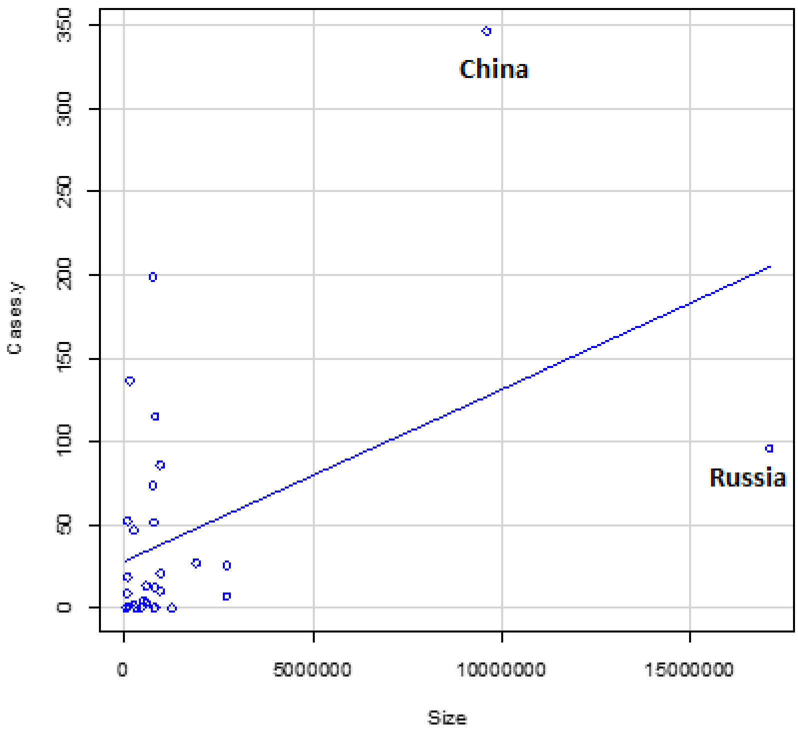
Appendix 1 figure 1. Relation between human anthrax cases and number of cattle.



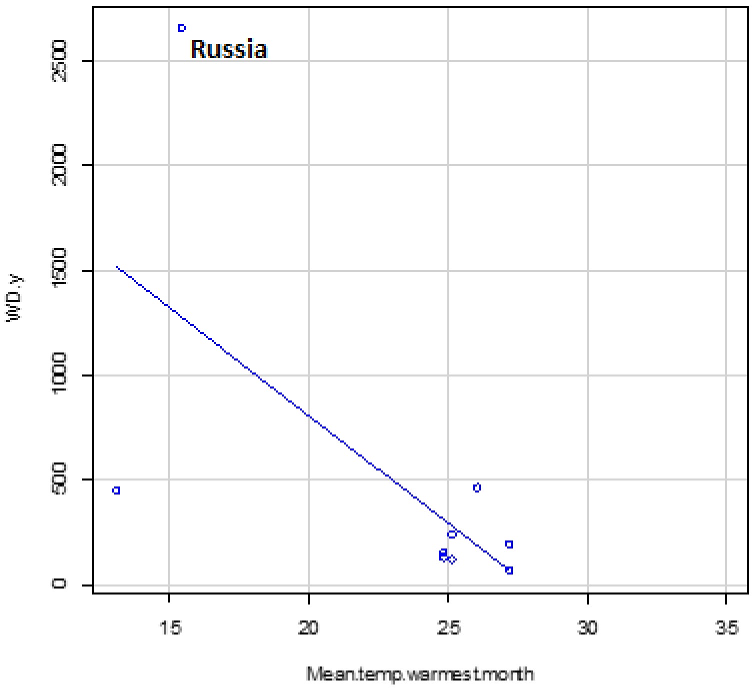
Appendix 2 figure 2. Relation between human anthrax cases and human anthrax deaths.



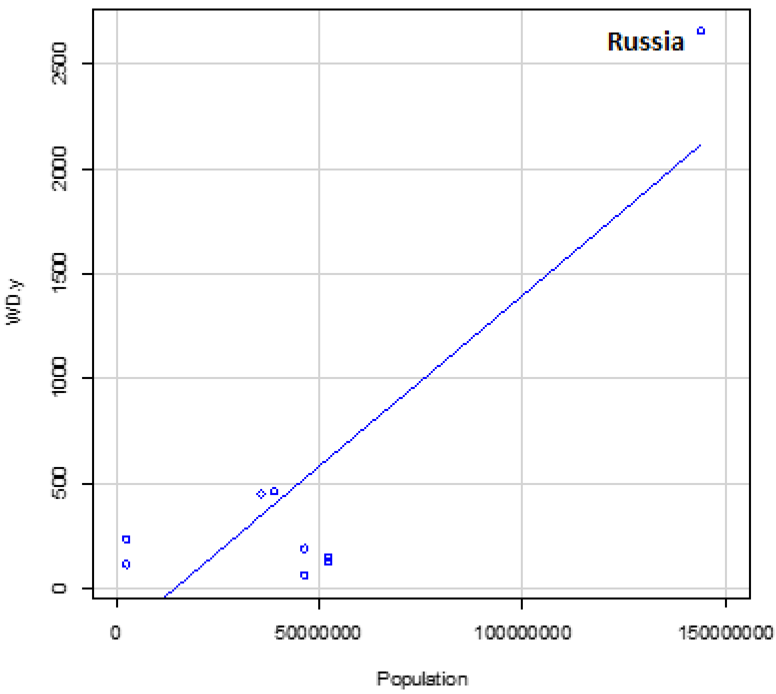
Appendix 3 figure 3. Relation between human anthrax cases and total population size.



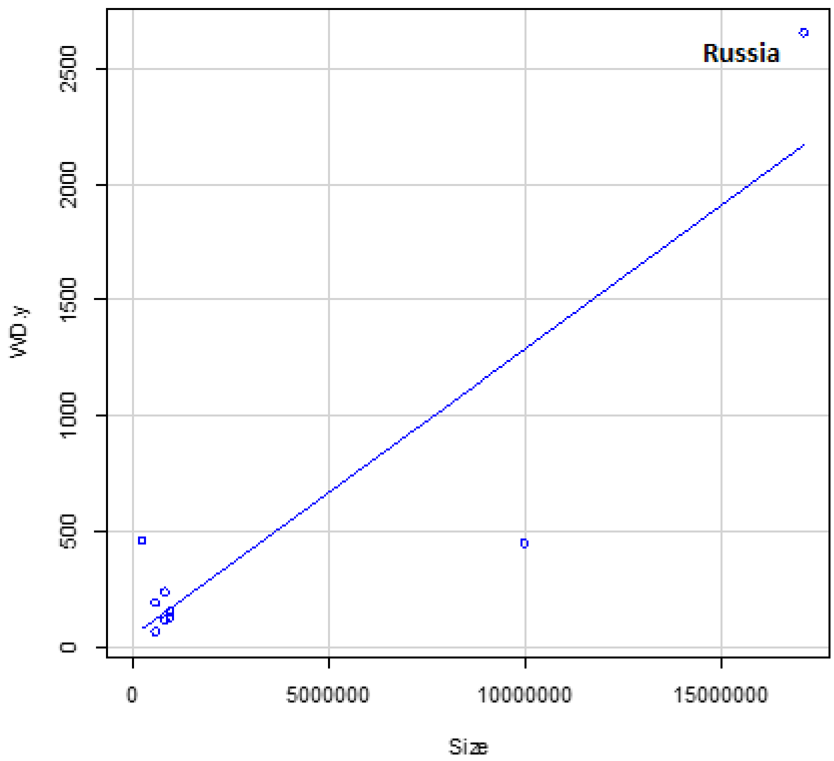
Appendix 4 figure 4. Relation between human anthrax cases and country size



Appendix 5 figure 5. Relation between wildlife anthrax deaths and the mean temperature of the warmest month.

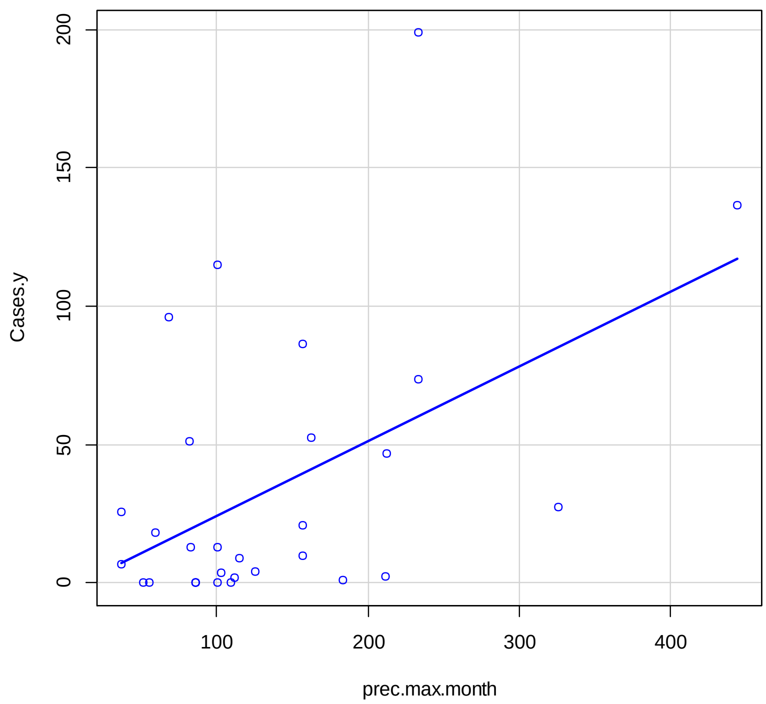


Appendix 6 figure 6. Relation between wildlife anthrax deaths and total population size.



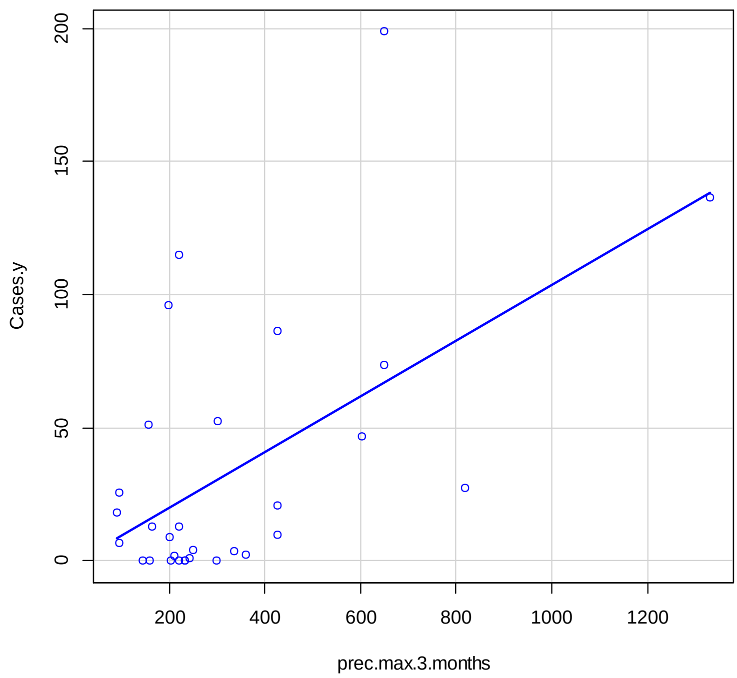
Appendix 7 figure 7. Relation between wildlife anthrax deaths and country size.

Excluding China, only showing new relations:



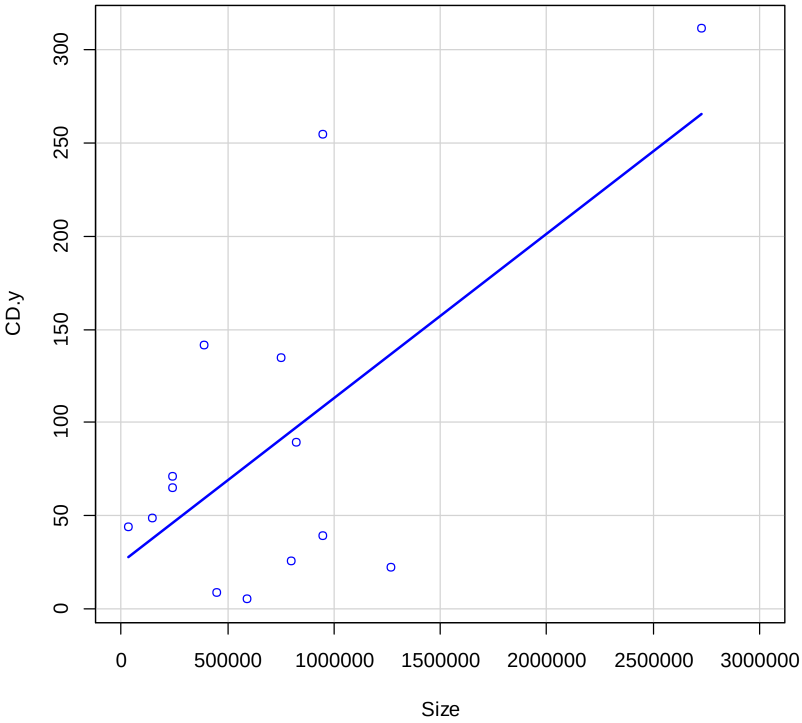
Appendix 8 figure 8.

Relation between human anthrax cases and maximum precipitation in 1 month.



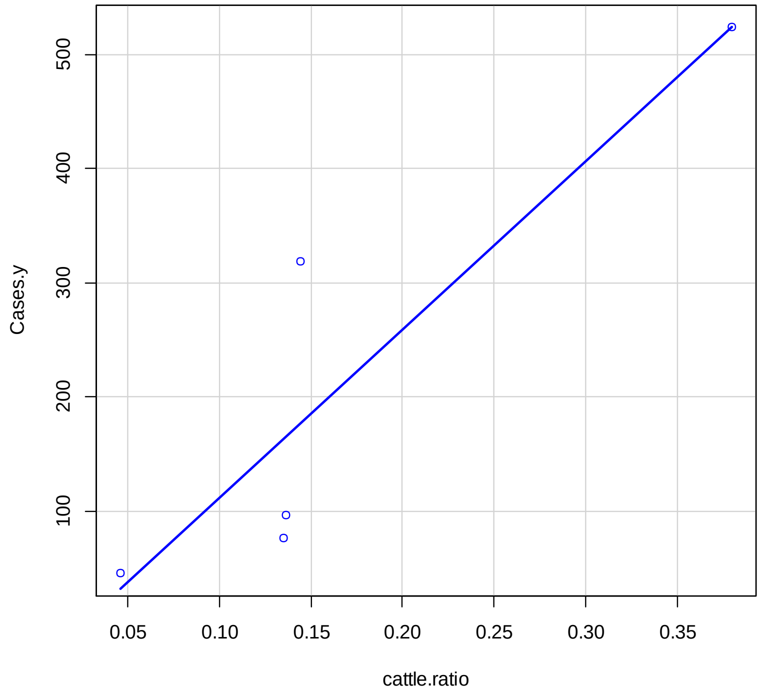
Appendix 9 figure 9.

Relation between human anthrax cases and the maximum precipitation in 3 months.

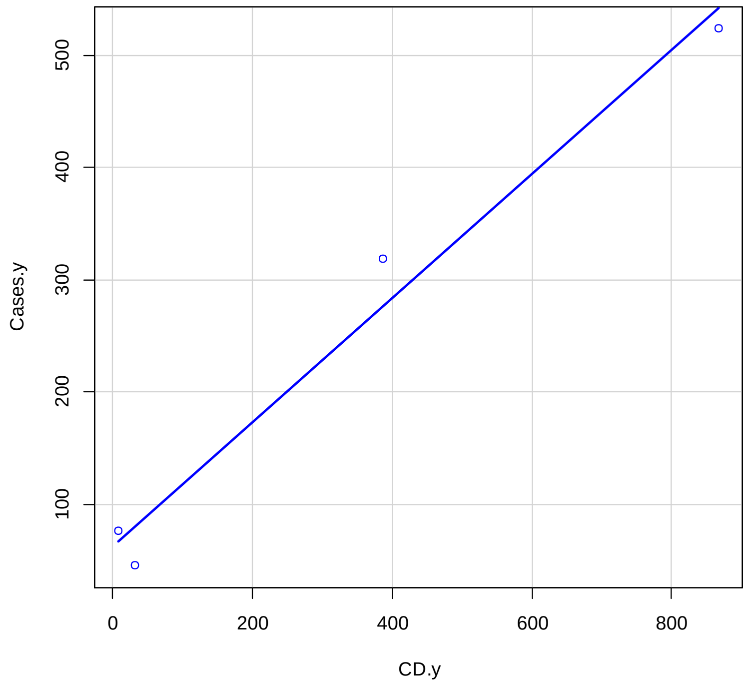


Appendix 10 figure 10. Relation between cattle anthrax deaths and country size.

Continents:

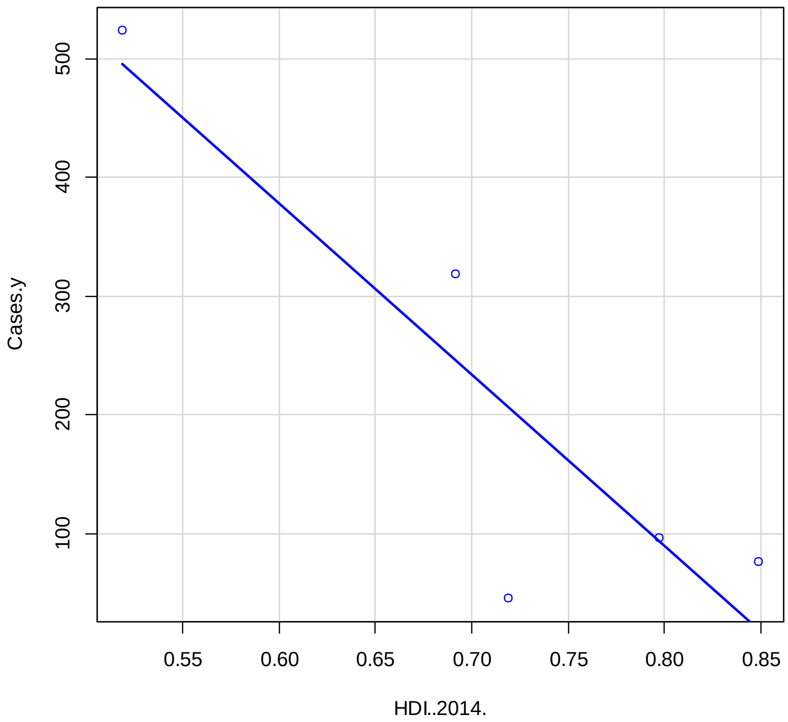


Appendix 11 figure 11. Relation between human anthrax cases and cattle ratio. Continents.



Appendix 12 figure 12.

Relation between human anthrax cases and cattle anthrax deaths. Continents.



Appendix 13 figure 13. Relation between human anthrax cases and HDI. Continents.

Appendix, tables

Appendix 14 table 4. Dataset of anthrax outbreaks. CD=cattle deaths, WD= wildlife deaths. Empty squares means no data.

Countries	Time	Years	Cases	Deaths	CD	WD
Azerbajian	1984-2010	27	498	0		
Bangladesh	2009-2010	2	273	0	98	
Canada	2012	1				451
Georgia	2000-2013	14	736	5		
Ghana	2003-2012	10	22	6	652	
Ghana	2005-2016	12			851	
Greece	2014	1	1	0		
China	2005-2013	9	3115		2261	
Hungary	2014	1	9	0		
Indonesia	2008-2017	10	273	8		
Kazakhstan	2016-2018	3	21	4		
Kazakhstan	1937-2005	69	1765	75	21498	
Kenya	2014-2017	4	15	4	20	768
Kenya	1999-2014	16				1014
Lesotho	2005-2016	12			526	
Namibia	1968-2011	44			3944	
Namibia	2017	1	0	0		241
Namibia	2018	1	13	0		115
Namibia	2019	1	115	0		117
Netherlands	2014	1	0	0		
Niger	2019	1	0	0	22	
Pakistan	2017	1	0	0	26	
Poland	2014	1	0	0		
Romania	2014	1	2	0		
Russia	2016	1	96	1		2657
Spain	2014	1	4	0		
Sweden	2014	1	0	0		
Sweden	2016	1	0	0	9	
Tanzania	2013-2016	4	345	24	1019	
Tanzania	2012	1	10	2		153
Turkey	1990-2007	18	926	26		
Uganda	2004	1				462
United Kingdom	2010	1	47	14		
Ukraine	1994-2001	8	105	5		
Zambia	1999-2007	9	1790	83	1216	
Zambia	2016-2018	3	221	8		
Zimbabwe	1995-2015	21			2978	

Appendix 15 table 5. Dataset of anthrax outbreaks calculated to single years. CD=cattle deaths, WD= wildlife deaths. Empty squares means no data.

Countries	Cases.y	Deaths.y	CD.y	WD.y
Azerbajian	18,44444	0		
Bangladesh	136,5	0	49	
Canada				451
Georgia	52,57143	0,357143		
Ghana	2,2	0,6	65,2	
Ghana			70,91667	
Greece	1	0		
China	346,1111		251,2222	
Hungary	9	0		
Indonesia	27,3	0,8		
Kazakhstan	7	1,333333		
Kazakhstan	25,57971	1,086957	311,5652	
Kenya	3,75	1	5	192
Kenya				63,375
Lesotho			43,83333	
Namibia			89,63636	
Namibia	0	0		241
Namibia	13	0		115
Namibia	115	0		117
Netherlands	0	0		
Niger	0	0	22	
Pakistan	0	0	26	
Poland	0	0		
Romania	2	0		
Russia	96	1		2657
Spain	4	0		
Sweden	0	0		
Sweden	0	0	9	
Tanzania	86,25	6	254,75	
Tanzania	10	2		153
Turkey	51,44444	1,444444		
Uganda				462
United Kingdom	47	14		
Ukraine	13,125	0,625		
Zambia	198,8889	9,222222	135,1111	
Zambia	73,66667	2,666667		
Zimbabwe			141,8095	