

Spatial clustering of pathology submissions during the initial introduction and spread of avian influenza H5N1 in poultry in Nigeria in 2006-2007

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Keywords

Avian influenza, Disease spread, HPAI-H5N1, Nigeria, Pathology submission, Poultry, Spatial clustering, Surveillance.

Summary

Highly pathogenic avian influenza (HPAI) virus H5N1 spread throughout Nigeria between 2006 and 2007. Bird samples collected across the country were submitted through the free-of-charge (FOC) program to the National Veterinary Research Institute, Vom (NVRI-Vom) laboratory. The present article describes the spatial distributions and evaluated clustering of the FOC submissions from poultry farms at the global, local, and focal levels between 2006 and 2007 epidemic in Nigeria. Spatial statistics evaluating clustering of the FOC submissions were implemented using the *Moran's I* test, the purely spatial cluster analysis with the SaTScan Poisson model, and the Bithell's linear score test. A significant global clustering of the FOC submissions was observed. Significant local clusters of submissions were observed in the North-East, North-Central, and South-West zones. There was significant decline in FOC submissions with increasing distance from NVRI-Vom. These results indicated that the geographic area of influence of the FOC submission program in Nigeria was limited to regions closer to the diagnostic laboratory. This work provides a detailed insight into the surveillance activities during the HPAI outbreaks in Nigeria, and should assist policy-makers and field veterinarians to improve the effectiveness of national eradication plans in the face of any outbreak of animal diseases.

Distribuzione geografica dei campioni infetti dal virus H5N1 di influenza aviaria sottoposti ad analisi durante le fasi di introduzione e circolazione del virus in Nigeria tra il 2006 e il 2007

Parole chiave

Campioni sospetti, Clustering spaziale, Diffusione della malattia, Influenza aviaria, HPAI-H5N1, Nigeria, Pollame, Sorveglianza.

Riassunto

Tra il 2006 e il 2007 tutta la Nigeria è stata interessata dal virus H5N1 di influenza aviaria ad alta patogenicità. Questo articolo ne descrive la distribuzione e valuta i cluster a livello globale, locale e centrale sulla base dei campioni, provenienti da aziende di pollame, sottoposti al programma *free-of-charge* (FOC) del laboratorio Vom (NVRI-Vom) del *National Veterinary Research Institute*. Per la valutazione dei cluster, le statistiche spaziali si sono basate sull'indice di Moran e l'analisi spaziale è stata condotta con il modello Poisson di SaTScan e il metodo di Bithell. Quest'analisi ha evidenziato un significativo cluster globale e rilevanti cluster locali nelle zone Nord-Orientali, Nord-Centrali e Sud-Occidentali mentre, con l'aumento della distanza dal NVRI-Vom, è stato riscontrato un notevole calo dei campioni inviati. L'area geografica coinvolta nel programma FOC in Nigeria era dunque limitata alle regioni più vicine al laboratorio diagnostico. Lo studio fornisce una visione dettagliata delle attività di sorveglianza durante le epidemie HPAI in Nigeria e dovrebbe aiutare i responsabili politici e i veterinari a migliorare l'efficacia del piano nazionale di eradicazione a fronte di qualsiasi epidemia di malattie degli animali.

Introduction

Avian influenza (AI) is a highly contagious disease caused by Influenza A viruses, which are segmented negative-strand RNA viruses in the family *Orthomyxoviridae* (Cattoli and Terregino 2008). Influenza A viruses can be highly virulent to both humans and birds and can infect a broad range of hosts (Cardona *et al.* 2009). Highly pathogenic avian influenza viruses (HPAIVs) cause systemic disease in poultry, often resulting in high mortality in turkeys and chickens (Yee *et al.* 2009). Since it was first detected in Guangdong Province, China in 1996, the H5N1 HPAIV has spread beyond Asia into the Middle East, Europe, and Africa causing the deaths of 447 humans across 15 countries (WHO 2015). Highly pathogenic avian influenza is 1 of the 100 terrestrial and aquatic animal diseases listed by and notifiable to the World Organization for Animal Health (OIE) by member countries. Outbreaks and spread of HPAI have been associated worldwide with the movement of live poultry through trade and with the migration of wild birds (Sims *et al.* 2005).

In February 2006, an outbreak of H5N1 HPAI was reported in commercial poultry in Northern Nigeria. This is the first outbreak ever reported in Africa. It rapidly spread throughout the country killing more than 1.2 million domesticated birds (Fasina *et al.* 2009, Meseko *et al.* 2010, Fasina *et al.* 2011) in 25 of the 36 States and the Nigerian Federal Capital Territory (FCT). The outbreaks had been described as arising from multiple sources and introduction of the disease was strongly linked to poultry trade, movement of infected bird, live bird markets, inappropriate disposal and poorly implemented control measures, as well as migratory birds (Cecchi *et al.* 2008, Ducatez *et al.* 2006, Fasina *et al.* 2009). The presence of a reassortant H5N1 virus with new genetic characteristics was reported in at least 7 States in Nigeria in February 2007 (Monne *et al.* 2007).

In the wake of the 2006–2007 outbreaks, in order to improve the existing disease surveillance and outbreak reporting system, the Federal Government of Nigeria, implemented a free-of-charge (FOC) diagnostic submission program. The program had the goal to facilitate early notification and submission of sick or dead birds' specimen to the National Veterinary Research Institute, Vom (NVRI-Vom) from all regions across the country, and prompt diagnosis of the H5N1 HPAIV. The objective of this study was to describe the spatial distribution and examine clustering of the FOC submissions during the 2006–2007 HPAI H5N1 epidemics in Nigeria, to guide on-going and future control surveillance strategies. We hypothesized that submissions of diagnostic samples through the FOC program to the NVRI-Vom were randomly distributed across the country.

Materials and methods

Study area

The study focuses on the entire territory of Nigeria, which is divided into 36 states and the FCT. Nigeria has the biggest national poultry population in Africa, which is estimated to be 140 million, 25% of which is kept in the commercial production system, 15% in the semi-commercial, and 60% as backyard or rural poultry (Ortiz *et al.* 2007, AICP-Nigeria 2012). As part of the HPAI control plan in Nigeria, poultry farmers were enlightened on the need to report cases of unusually high mortality in their flock to the nearest veterinary authority. In every state, desk officers were appointed under the Nigerian Avian Influenza Control and Human Pandemic Preparedness and Response Project (NAICP) to investigate field suspicions, collect appropriate specimen, and submit to NVRI-Vom for laboratory confirmation at no cost to the farmer (Oladokun *et al.* 2012). Farmers could also send samples (whole carcass or live birds) from suspected flocks to the NVRI-Vom on their own, at no cost. The NAICP also conducted census of all poultry farms in the country at the local and state government levels (Figure 1).

Data sources and case definition

We used data of the FOC submission program compiled by the NVRI-Vom during the H5N1 HPAI (HPAI hereafter) epidemic in Nigeria from January 2006 to December 2007. The data contained information such as the name and location of farm, date of submission, type of bird, flock size, mortality recorded, and whether the farm or poultry premises was positive or negative for HPAI. The number of farms that submitted samples to the FOC program during each year was obtained. Repeated submissions from the same farm were recorded only once based on the information related to the first submission. Also, data of all poultry farms compiled from across the country by the NAICP during the HPAI epidemic were obtained and used to represent the population at risk in this study. Farms registered by NAICP included premises where poultry were intensively (commercial) or extensively (backyard and rural poultry) managed. Poultry farms that submitted samples to the NVRI-Vom from 2006 to 2007 as part of the FOC service were selected as cases. These were farms with clinical history of unusually high mortality and/or signs suggestive of HPAI infection, but not necessarily confirmed positive for HPAI H5N1 or Newcastle disease, the latter being a probable differential diagnosis. Poultry farms registered by NAICP were used to represent population at risk.

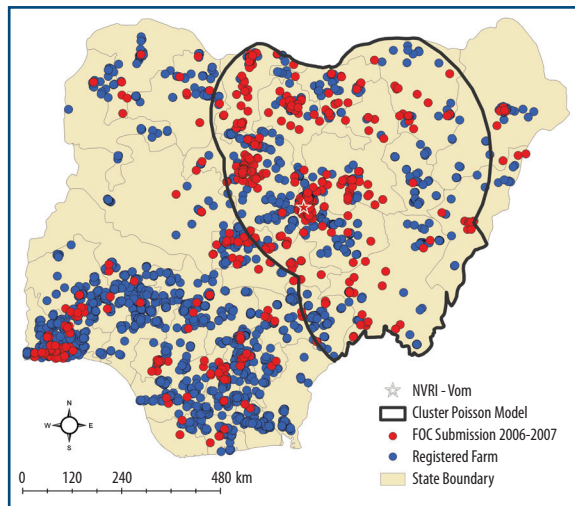


Figure 1. Map of Nigeria showing locations of registered and submitting farms, diagnostic laboratory (NVRI-Vom), and the identified local clusters of free-of-charge poultry diagnostic submissions during the highly pathogenic avian influenza epidemic in 2006-2007.

A subset of the FOC submission data, representing submissions from the commercial, backyard and rural poultry farms based on Adene and Oguntade classification (Adene and Oguntade 2006), were selected and used in the analysis. The commercial poultry farms included the highly integrated large-scale farms with very high bio-security system, the large to medium scale farms with moderate to high bio-security system, and the low scale farms with low to minimal bio-security systems. The backyard and rural poultry farms are subsistence and comprise of extensively managed mixed stocks of improved and local breeds with minimal bio-security systems. Submitting farms were only counted once during each year.

Spatial distribution

The spatial distributions of FOC submissions were evaluated in relation to the population of poultry farms (registered by the NAICP) during 2006-2007. Also, spatial distributions of HPAI H5N1 positive farms were evaluated in relation to the number of FOC submissions during this period. The data were compiled in Microsoft Excel. With FOC submissions stratified by geopolitical zone, the observed (O), expected (E), and the observed/expected (O/E) submission ratio based on registered farms were computed. This was also computed for the HPAI H5N1 positive farms. The Chi square test of homogeneity was used to test the level of significance of the differences between the observed and expected FOC submissions, and the observed and expected HPAI H5N1 positive farms for each of the 6 geopolitical zones (North-West, North-Central, North-East, South-West, South-South, and South-East) in

Nigeria. The null hypothesis tested is that the FOC submissions and HPAI H5N1 positive farms from the geopolitical zones are equally proportioned (homogeneous) at $\alpha=0.05$.

Global cluster analysis

The *Moran's I* test was used to assess the presence of significant spatial autocorrelation of the FOC submissions among the 36 Nigerian states and the FCT. The null hypothesis was that there was no clustering of FOC submissions, alternative hypothesis was that there was clustering. *Moran's I* is a measure of how related the values of a variable are based on the locations where they were measured. The dataset used included the number of FOC submissions from the 36 states and FCT aggregated over the study period, and the states' latitude and longitude coordinates. An inverse distance matrix was generated using the coordinates, each off-diagonal entry $[i, j]$ in the matrix is equal to $1/(\text{distance between point } i \text{ and point } j)$. This was implemented in R statistical software using functions in the "ape" library (Paradis et al. 2004).

Local area cluster analysis

Spatial local area clustering was examined using the Poisson spatial model in the SaTScan Spatial Scan Statistic Software v 9.3 (Kulldorf 1997). The model assumed that FOC submissions were randomly distributed under null hypothesis. The alternative hypothesis claimed elevated submissions inside a region as compared to the outside region. Cases, defined as numbers of farms that submitted samples as part of the FOC program, were geo-located in the centroid of each state. The number of farms registered per state by the NAICP was used as the population at risk. The purely spatial scan statistic test imposed circular window on the map and allowed its centre to move over the country so that, at any given position, the window was centered on each of the several possible submitting states across the country. The radius of the circular window varied continuously from zero to maximum radius so that the window's coverage would not exceed 50% of the total population at risk. To detect clusters, a likelihood function was applied to the windows, comparing the observed and expected number of cases inside and outside the window. A cluster was reported with its level of significance, location, radius, observed and expected cases, log likelihood ratios, and relative risk. The criterion for reporting secondary clusters was set at the default – geographically overlapping clusters are not reported. The statistical significance of clusters was evaluated through Monte Carlo simulation (9999

iterations). A $P < 0.05$ was considered significant for all one-sided tests. Map of FOC submissions and all significant clusters detected were generated using ArcMap 9.3 (ArcGIS, ESRI, Redlands, CA, USA).

Focussed area cluster analysis

The Bithell's linear score test (Bithell 1995) was used to test the association between the proximity of submitting farms (aggregated by states) to the NVRI-Vom laboratory and the proportion of FOC submissions. The dataset included cases, controls, and distances from the geographical centroid of each state to the NVRI-Vom. The test calculated the linear rank score test statistic and evaluate its significance based upon the observed (O) and expected (E) numbers of submissions. A significantly large value indicated evidence of spatial clustering around the pre-specified point source of the situation under study; i.e., there was a significant decline in risk with increasing distance from the pre-specified point source (here, NVRI-Vom). The statistical significance of clusters was evaluated through Monte Carlo simulation (9999 iterations).

Results

Sample submission

From January 1, 2006 to December 31, 2007, the NVRI-Vom received FOC submissions from 1,293 locations in the FCT and across 33 of the 36 States in Nigeria. The locations included commercial, backyard, and rural poultry farms; residential/business premises where wild birds were captured alive or found dead; live bird markets and animal control points at international borders. For this study, a subset of 1,006 locations (78%) representing commercial, backyard, and rural poultry farms was used. A total of 4,356 poultry

farms or premises (comprising commercial, backyard, and rural poultry flocks) were registered by the NAICP across the country during this period, representing the number of active poultry farms/premises between 2006 and 2007.

Spatial distribution

Approximately 86% (861/1006) of FOC submissions to NVRI-Vom during 2006–2007 H5N1 HPAI outbreaks in Nigeria originated from farms in the Northern part of the country; while 14% (145/1006) were from farms in the Southern part. Based on the number of registered farms, the percentage of farms that submitted samples through the FOC submission program was higher in the North compared to those submitted from the South (Table I). There were significant differences ($P \leq 0.001$) between the observed and expected submissions (homogeneity hypothesized) across the regions. The O/E ratios were consistently higher in the Northern area compared to the Southern area. The comparison of the number of farms that submitted samples to NVRI-Vom through the FOC submission program with the number of farms that were confirmed positive for H5N1 virus shows that approximately 77% (229/299) of H5N1 positive farms were in Northern Nigeria (Table II). The O/E ratios were higher for North-West and South-West zones. Approximately, 15–47% of farms that submitted samples through the FOC program from the Northern area were confirmed HPAI H5N1 positive, while 28–62% of farms that submitted samples from the Southern area were confirmed HPAI H5N1 positive.

Global cluster

The *Moran's I* test showed that there was significant ($P = 0.0028$) spatial autocorrelation present in the FOC submissions across the states.

Table I. Distribution of poultry farms and free-of-charge poultry diagnostic submissions to the National Veterinary Research Institute - Vom laboratory during the H5N1 HPAI outbreaks between January 2006 and December 2007 by geopolitical zones.

Zone	Number of Farms			Observed/Expected
	Registered	Submitting Samples (%)	No. Expected	
North-central	1348	460 (34.1)	311.3	1.48
North-east	415	144 (34.7)	95.8	1.50
North-west	622	257 (41.3)	143.7	1.79
South-east	440	33 (7.5)	101.6	0.32
South-south	562	26 (4.9)	121.5	0.21
South-west	1005	86 (8.6)	232.1	0.37
Total	4356	1006 (23.0)		

Table II. Distribution of H5N1 HPAI positive farms and free-of-charge poultry diagnostic submissions to the National Veterinary Research Institute - Vom laboratory during the H5N1 HPAI outbreaks between January 2006 and December 2007 by geopolitical zones.

Zone	Number of Farms			Observed/Expected
	Submitting Samples	H5N1 Positive (%)	No. Expected	
North-central	460	67 (14.6)	136.7	0.49
North-east	144	43 (29.9)	42.8	1.00
North-west	257	119 (46.3)	76.4	1.56
South-east	33	9 (27.3)	9.8	0.92
South-south	26	8 (30.8)	7.7	1.04
South-west	86	53 (61.6)	25.6	2.07
Total	1006	299 (29.7)		

Table III. Local spatial clusters of free-of-charge poultry diagnostic submissions to the National Veterinary Research Institute - Vom laboratory in Nigeria during the H5N1 HPAI outbreaks between January 2006 and December 2007.

Cluster	Cluster Location and Size			Registered		Submissions		P-value	
	Center	Latitude	Longitude	Radius (km)	Total	Observed	Expected		
Poisson Model									
1	Bauchi	10.8074	9.9547	324.8	1420	563	257.8	2.18	<0.0001
2	Kano	11.7312	8.4760	191.1	400	278	72.6	3.83	<0.0001
3	Nasarawa	8.4528	8.1985	0.0	34	32	6.2	5.19	<0.0001
4	Taraba	8.0169	10.8262	240.2	843	235	153.0	1.54	<0.0001
5	Lagos	6.5178	3.5682	0.0	70	35	12.7	2.75	<0.0001

Local area cluster

Five significant local spatial clusters of FOC submissions were identified with the Poisson model. The most likely cluster ($P \leq 0.001$) was centered in Bauchi State (North-East area) and had a radius of 324.77 km. In this cluster, a total of 563 farms submitted samples through the FOC program, this was found to be 2.18 times greater than the expected submissions computed to be 257.75 farms from among the 1,420 registered farms (farm at risk) within the area covered by the cluster. The second, third, fourth, and fifth most likely clusters ($P \leq 0.001$) were centered in Kano (North-West), Nasarawa (North-Central), Taraba (North-East), and Lagos States (South-West), respectively (Table III).

Focussed area cluster

There was a significant ($P = 0.0001$) increase in the number of FOC submissions based on the proximity of submitting poultry farms to NVRI-Vom. As the distance increased from the poultry farm to the NVRI-Vom, there were fewer samples submitted.

Discussion

To the best of our knowledge, this study was the first to describe the spatial distribution and clustering of the FOC diagnostic submission program during the HPAI H5N1 outbreaks in Nigeria. The results of this study allowed us to analyse the geographical distribution of the submissions at the local and focal levels. Spatial statistics in the analysis of disease spread or outbreak surveillance data are becoming standard practice in the study of infectious diseases (Soberano *et al.* 2009).

The large proportion of FOC submissions observed from Northern Nigeria may involve backyard / family poultry and urban / peri-urban semi-commercial poultry production system, which are extensively practiced in this area (Ekong *et al.*, 2012). The backyard production system in Nigeria is subsistent and mostly extensive, consisting of multiple species

with both local and improved breeds, with little or no bio-security (Adene and Oguntade 2006). The urban and peri-urban semi-commercial production has minimum to moderate bio-security; and is predominately composed of broilers and egg production for the urban markets. Fewer FOC submissions were observed in Southern Nigeria, where poultry production is mainly at commercial and semi-commercial scale. The large-scale commercial poultry production operates on farms that are usually vertically or horizontally integrated with other allied businesses, and managed with state-of-the-art equipment and operations. These farms breed and hatch their own commercial day-old chicks and sell to other farms across the country (AICP-Nigeria 2012). Bio-security practices on these farms are mostly high. The bulk of commercial poultry farms in Nigeria are based in the South-West zone, especially the states nearer to Lagos -industrial capital of Nigeria. It is estimated that over 65% of Nigeria's commercial poultry is in the 5 states of Lagos, Ogun, Oyo, Osun, and Ondo; while another 25% is based in South and South-East geo-political zones. Overall, about 10% of Nigeria's commercial poultry is bred in the 15 North-central, North-West, and North-East states (Adene and Oguntade 2006).

The general significant clustering of FOC submissions revealed by the *Moran's I* was well supported by the presence of significant local clusters of submissions as determined by the Poisson spatial scan analysis. The observed local clusters extended over the states of Bauchi, Gombe, Jigawa, Plateau, Kano, Yobe, Kaduna, Adamawa, Borno, Katsina, Taraba, and Nasarawa, which indicated higher number of submissions from these states as compared to the population (farms) at risk.

These results give an assessment of the spatial clustering of the submissions to NVRI-Vom in relation to the poultry farms in Nigeria. The secondary local clusters of submissions identified within limited areas in Lagos states may likely be due to the presence of farm settlement areas in this region, where there is a large concentration of poultry farmers

whose system of production is largely backyard / semi-commercial poultry production. The findings of the local clustering were well supported by the focused cluster test, which revealed a significant decline in submissions with increasing distance from NVRI-Vom. The study found the impact of the FOC submission program to be within a limited geographic area, mostly in states near the NVRI-Vom. This might be due to lack of adequate, general publicity about the FOC submission program to farmers and the public, resulting in submissions generating from the same geographic area. It could also indicate the unwillingness on the part of farmers to transport suspected cases over long distances to the NVRI-Vom laboratory.

However, during the 2006-2007 outbreaks of HPAI H5N1 in Nigeria, clusters of infected poultry farms were identified within average distance of 300 km radius in the North-West, South-West, and North-East areas (Ekong *et al.* 2012). Compensation plan, based on the age of depopulated bird and prevailing market price, was put in place to encourage farmers to report suspicious cases. Farmers' and the public perception of the reality associated with the disease could also be a factor accounting for less FOC submissions from these areas. Some believed HPAI was not different from Newcastle disease and as such should not be paid any special attention.

A potential bias in the study could be the use of the NAICP database to determine estimates for the population at risk. This might be less representative of the true population, because some poultry premises may not have been registered. The registration of poultry farms was carried out by AI project desk officers under the NAICP in different states across the country. These officers, including the AI control team within the states, were mostly responsible for the collection, transport of suspected samples from the farms to the NVRI-Vom, the depopulation, and decontamination of infected farms. To encourage further early reporting of suspicions, farmers were paid compensation for their losses only if their premises had been confirmed positive by NVRI-Vom and their flock depopulated by the AI control team. Improved efforts should be made towards the continuous and mandatory registration of all poultry farms and premises across Nigeria and total poultry population census compiled. The spatial analyses conducted in this study were at the state-level, thus reflected aggregated areas of submission within the country. Secondly, the definition of cases (FOC submissions) in this study was based on clinical history or signs suggestive of HPAI H5N1. These signs are consistent with those observed in other poultry diseases especially Newcastle disease, which is the single most probable differential diagnosis for HPAI H5N1. Some of the farms that submitted samples to NVRI-Vom through the FOC submission program

could have been confirmed positive or negative for H5N1 or Newcastle disease, all these farms were captured in our case definition based on clinical history and signs.

Although the FOC program for poultry farmers has helped to increase the total number of submissions of suspected cases from wider region in the country, this study demonstrates that the program has a limited geographic area of influence. Increasing the availability of information about the FOC program to the public, involving all the animal diagnostic laboratories, veterinary faculties, and teaching hospitals, alongside to states' and private veterinarians in the surveillance and monitoring of livestock diseases may generate increased submissions from all localities at risk. In this study, a large percentage (15-62%) of farms that submitted samples through the FOC program were confirmed positive for HPAI H5N1, which suggests that continuous monitoring of laboratory submission databases from across the country, as a form of syndromic surveillance system, may be beneficial to HPAI surveillance in Nigeria. These measures have great potential to facilitate early identification, detection, control, and possible eradication of high-impact poultry diseases in Nigeria. Implementation of similar types of programs in other countries may need to consider the limited geographical area of influence gained due to the program, to utilize more efficiently resources to identify and manage highly transmittable diseases in the future.

To improve the efficacy of the avian influenza surveillance program, the availability of the information about the FOC submission program needs to increase potentially by publicizing it through the national media-printed and electronic, distribution of posters and flyers to farmers at states and local levels, and dissemination on social media and telecommunication (cell phones) networks. Also, the submission of specimens could be made more convenient for farmers by allowing the NVRI zonal laboratories to serve as points of sample submission for the areas within their zones of influence. Samples could then be transported to the NVRI-Vom laboratory for diagnosis. The local laboratories should be equally upgraded to manage better sample processing and analysis. This could increase the number of submissions to the laboratories and give rise to a better surveillance program to detect promptly AI and other livestock diseases. The farmers should be continuously educated about the importance of early reporting of disease outbreaks in their flocks to the veterinary authorities for timely detection and control, thereby limiting economic losses and public health issues that might be related to the disease.

The FOC diagnostic submission program for poultry

flocks and wild birds implemented by the Federal Ministry of Agriculture and maintained by the NVRI since 2006 might have helped to improve the reporting of suspected outbreaks and the control of HPAI in poultry in Nigeria through early reporting, prompt diagnosis, and stamping out of affected birds in infected premises. This study, however, identified that the area of influence of the program appeared to be largely limited to regions closer to the diagnostic facility. This calls for the upgrading of all veterinary diagnostic facilities across the country for improved service delivery and consideration for implementing diagnostic submission strategies to identify and manage highly transmittable diseases.

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