ROLE OF THE TRADING NETWORK IN THE DIFFUSION OF NEWCASTLE DISEASE IN THE LAKE ALAOTRA REGION, MADAGASCAR: A SOCIAL NETWORK ANALYSIS.

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SUMMARY

First reported in 1946, Newcastle disease (ND) is one of the major constraints of poultry farming in Madagascar. The trading network is thought to be the major pathway for transmission of this disease. This study aimed to describe the poultry commercial network in the Lake Alaotra region and assess the potential role of its components and its structure in the diffusion of ND virus. Several methods were combined to acquire data: classical survey, participatory epidemiology and disease surveillance. Social network analysis methods were used to analyze data. Network topology was scale-free, with 347 nodes and 1448 links. Hierarchical clustering showed six classes of nodes which were associated with ND outbreaks (p=0.004).

The originality of this study was having an almost complete network in developing countries with a measure of diseases. This is the first step of analysis, further studies would concern modelling the dynamics of ND within network taking into account virus strains which circulate.

INTRODUCTION

Newcastle disease (ND) is an infectious and highly contagious disease due to Newcastle disease virus (NDV) namely avian paramyxovirus type 1 (APMV-1) which belongs to the family of *Paramyxovirdae* and of genus *Avulavirus*. It affects several wild and domestic bird species but chickens are among those which are most sensitive. It is still one the major constraints to the development of poultry farming in a developing country.

The poultry industry holds an important place in Madagascar. According to Food and Agriculture Organization of the United Nations, there are 34.4 million domestic poultry (FAOSTAT, 2008), most of them being in smallholder production systems representing two-thirds of the rural population (Ocean Consultant, 2004). ND was first reported in Madagascar in 1946 (Rajaonarison, 1991). Well controlled by vaccination in industrial production, it causes high mortality in smallholder production systems. A study undertaken in 1999 in the peri-urban area of Antananarivo, the capital, showed that ND was responsible for 44% of poultry mortality

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(Maminiaina et al, 2007). Besides ND, two avian pathogens are known to circulate in Madagascar: *Pasteurella multocida,* the agent responsible of fowl cholera (FC) and avian influenza virus (AIV). A study undertaken by Porphyre (1999) determined that 14.9% (n=204) of chickens and 2.9% (n=175) of palmipeds showed serological evidence of AIV circulation. The same study (Porphyre, 1999) determined a seroprevalence rate of FC at 70.6% (*n=187*) in chickens and 25% (*n=140*) in palmipeds. FC and ND are registered as priority diseases by the national veterinary services. Leading to high morbidity and mortality rates with very similar clinical signs, these three diseases may be easily confounded in the field and the respective clinical impact of these 3 diseases remain unevaluated.

In Madagascar, the poultry industry related to smallholder production systems is complex, involving different types of actors in relation with each other: farms, collectors, live-birds markets and consumers. Farms can be classified according to their commercial practices (contact with collectors and markets). Some collectors are linked to farms where they buy poultry, collecting point and/or markets. Markets could be classified according to their size which is closely related to their administrative level (village market, municipal market, regional market and market in the capital). Generally, locally bred poultry are slaughtered at home by the consumer who buys directly from a market or eat their own birds.

The Role of the trading network or one of its components (e.g. market) in spreading AIV (Kung et al, 2003; Liu et al, 2003; Senne et al, 2003; Trock et al, 2003; Webster, 2004; Garber et al, 2007; Amonsin et al, 2008; Dent et al, 2008; Van Kerkhove et al, 2009; Yee et al, 2009; Soares Magalhães et al, 2010) and NDV (Kung et al, 2003; Sánchez-Vizcaíno et al, 2010) has already been established. In Madagascar, it is empirically hypothesised by poultry stakeholder that commercial network is the major pathway for transmission of ND and/or FC. In Madagascar, a typology study performed in the Lake Alaotra region (data not published) showed that farms could be described and classified according to a combination of risk factors including commercial practices, breeding types and environmental vicinity and that the risk of infection by NDV was statistically linked to some of these factors.

The aim of this study is to describe the poultry commercial network in Lake Alaotra region, analyze its structure and assess the potential role of its components and this structure in the diffusion of NDV.

MATERIALS AND METHODS

Study area

The study area is a landlocked region in the middle-eastern Madagascar. This is the basin of Lake Alaotra, the largest wetland area in Madagascar. It is contained in the centre, at 750 meters above sea level, 23.000 hectares (ha) of swamps, over 70.000 ha of rice paddies and 20.000 ha of open water (Ferry et al, 2009). The whole area is surrounded by hills reaching over 1300 metres of altitude. In the east it is bordered by the rainforest and in the west there are vast sparsely populated plateaus. The main channel of communication with the outside is the main road that connects with Andilamena in the north and with other regions of the island in the south. However, the region is densely populated because of the importance of agriculture and livestock.

The poultry population is high, estimated at 1.260.000 in 2001 (Andilamena included), with the largest population of domestic geese in Madagascar (UPDR/MAEP. 2003). Poultry flows are important within the region. Exchanges with the outside are formed mainly by the supply of the largest port city on the island (Toamasina) and the capital (Antananarivo). Trade with Andilamena in the north, which is also a very isolated area, and the east and west could be considered as negligible. Finally 35 municipalities in Ambatondrazaka and Amparafaravola districts were included in the study area. Only two municipalities of Amparafaravola (Tanambao-Besakay and Soalazaina) were excluded because they were far from other municipalities and have negligible connections with them.

Network Data collection

Two methods have been combined to collect data and to get a trading network as complete as possible. Firstly, a questionnaire survey involving professional traders was carried out from December 2009 to July 2010. Professional traders buy and sell poultry permanently (throughout the year) or temporarily. This group includes collectors (or middlemen) and stallholders in markets. A list of all known live poultry markets and collection points was established and all of them were visited. In each market or collection point, all identified traders were included in the survey. The questionnaire included questions about the origin and/or destination of poultry, frequencies of activity, number of birds treated and flow variation during year.

In a second step, a participatory approach (Jost et al, 2007) was conducted from December 2009 to November 2010. This survey involved the community animal health worker (CAHW) of the region and the chiefs of fokontany. Fokontany are administrative units constituted by one or some neighboring villages. The CAHW are farmers elected by the members of their village and trained for several weeks by “Agronomes et Vétérinaires Sans Frontières” (AVSF, a nongovernmental organization) for basic care, to help veterinarians providing a local service to farmers. Each of them is in charge of two or three fokontany. However, the network of CAHW has worked with only 60% of existing fokontany. To complete data and to compare answers, chiefs of fokontany in every municipality - the municipality being the administrative unit constituted by several fokontany- were asked to participate to the survey. Meetings were organized with them in collaboration with the local officials such as Mayors. For every municipality and CAHW’s association, two meetings were conducted. Network data targeted concerned villagers trading practices: the places where they usually buy and sale their poultry. After announcement of general subject i.e. trading network and poultry diseases, meeting always began with an open discussion. Individual semi-structured interview (SSI) and focus group were organized after the open discussion to focus on targeted data. Before its validation, all information collected by individual SSI was discussed again in focus groups. Our knowledge of the study area combined with direct observation and secondary data from literature were also used. At the final stage, triangulation was done to validate each data point. It consisted of allowing time for collecting all information from different sources (informants, literature and direct observation) and cross-checking them, at final stage, to correct any uncertainty or variations amongst responses.

Disease occurrence

Disease occurrence was recorded using two different methods: the above mentioned participatory epidemiology (Jost et al. 2007) and the second was a classical disease surveillance network. The same outbreak definition, established at the beginning of the study, was used for both methods. The targeted disease of interest was ND so “outbreak” declaration was validated when the following conditions were met: in at least two farms in a village there was an acute mortality (more than 30% of birds in flocks), or continual mortality (at least one bird per day during three days), with nervous signs (e.g. torticolis) and/or respiratory or gastrointestinal signs (diarrhoea). However, this case definition cannot differentiate ND from FC and avian influenza so RT-PCR was performed from brain and/or cloacal and tracheal swabs. Fokontany were chosen as the epidemiological unit so each fokontany was considered infected if there was at least one village within it where an outbreak occurred during the study period.

Poultry diseases were the second topic during participatory meetings. Participants described: (i) occurrence of poultry diseases in their fokontany since December 2009 and (ii) the clinical signs associated with each disease. All declarations were registered but an assessment of correspondence with outbreak definition was done after each meeting before validating each declaration as an outbreak.

The disease surveillance network of ND was implemented in collaboration with local veterinary services. The surveillance started in December 2009. CAHW or the chief of each fokontany declared, by phone, when there was outbreak. Correspondence with outbreak definition was assessed and a mobile team went to collect data and samples when a declaration was validated. Sample size targeted within each outbreak was at least 10 birds to obtain an absolute accuracy of 5% with a threshold prevalence rate of 30% (Thrusfield et al, 2001). During sampling, priority was given to diseased or recently deceased birds (less than one day). Implementation of surveillance network depended on collaboration of CAHW and chiefs of fokontany. It started in December 2009 with only CAHW on the south and east sides of the lake. It covered the whole study area in July 2010 after several meetings to convince the chiefs of fokontany and the other CAHW.

Data analysis

A social network analysis method (Wasserman and Faust, 2009) was used to describe and analyze the network in R 2.12 software (R Development Core Team, 2010). As data directions of links were known, a network with directional relations was computed. Outbreaks detected by participatory epidemiology were used in statistical analysis and proportion of outbreaks detected by the classical disease surveillance and confirmed by laboratory analysis was used to investigate specificity of declaration from fields.

Definition of network elements: Farms were grouped per village because poultry within villages share same environment and contact with trading network. According to our field knowledge, it was assumed that people in neighboring villages (fokontany) go to the same places to buy or to sell poultry. As it was impossible to get data for every village, fokontany were chosen as the epidemiological unit and node. Villages which were far from the other villages of its fokontany were considered as an independent fokontany or were put into the nearest fokontany. Ties were all movements (human with poultry) connecting fokontany. In addition to poultry farms, there were, live birds market inside some fokontany (with a maximum of one market per fokontany). Farms located within these fokontany were likely to be more exposed to commercial exchanges. This characteristic was used as an attribute of nodes. The occurrence of an outbreak within fokontany was considered also as an attribute. Destination, or only receiver nodes, which were located outside the study area were withdrawn from the analysis.

Network parameters, topology and outbreak occurrence: To describe the network, centrality parameters (degree, betweenness), clustering coefficient and density were first calculated. Their values and the distribution of degree explained the topology of the network. Simplified definitions of these parameters, in accordance with those already given in literature (Martínez-López et al, 2009; Wasserman and Faust, 2009), could be given:

(i) Degree is a measure of centrality of each node. It represents the number of ties connected with node. For directed network, there are three kinds of degree: the indegree is the number of ties received by node; the outdegree is the number of ties sent by node and the freeman degree is the sum of both in and out degree.

(ii) Betweenness is another centrality measure of node. It represents a measure of how a node lies in the middle of two other nonadjacent nodes. It means that a path which connects the two nonadjacent nodes has to pass through the node between them.

(iii) Clustering coefficient is an indicator of the importance of clusters present in network. It measures the sum of the proportion of nearest neighbouring nodes that are directly connected.

(iv) Density measures the proportion of observed contacts compared with all possible contacts among nodes. It indicates how the network is connected.

To assess the potential link between the occurrence of an outbreak and the centrality parameters of nodes, generalized linear models were implemented where the dependant variable was the occurrence or not of an outbreak in the nodes and explanatory variables were the centrality parameters. Since nodes were not independent, each parameter was tested individually. To assess the significance of model coefficients, random permutation tests were performed. Several random permutations of the matrix were done and coefficients of models were calculated for each permutation. The proportion of coefficients which were higher than for the observed matrix was calculated. The coefficient was considered as significant when this proportion was smaller than 5%.

<u>Positional analysis and outbreak occurrence</u>: Positional analysis consists on simplifying the network data set. This simplification consists on classifying nodes within positions identified by their structural equivalence. Two nodes are structurally equivalent if they are identically tied to and from all other nodes in the network (Wasserman and Faust. 2009). The following steps were performed for positional analysis and its association with outbreak occurrence:

(i) Measure of structural equivalence with euclidean distance.

(ii) Representation of network positions which consisted on partitioning nodes into classes where each class was constituted by structurally equivalent nodes. Partition of nodes was performed using hierarchical clustering of matrix of euclidean distances calculated above. Ward’s algorithm was used to aggregate nodes into class thus minimizing within-class dissimilarity and maximizing between-classes dissimilarity. The output of hierarchical clustering was a dendrogram or tree diagram which represents the series of partition. A cut-off level within the tree diagram was chosen to get interpretable and realistic classes. Description of obtained classes was done using values of centrality parameters and attributes within each class.

(iii) The association among nodes characteristic (according to clustering) and occurrence of outbreak was assessed by comparing the number of outbreak among classes using chi squared test.

RESULTS

The participatory approach concerned 40 CAHW and 35 municipalities in the study area. Two-hundred and thirty-one traders were interrogated on 21 markets and 20 collection points. Disease surveillance network recorded and investigated 35 outbreaks in 27 fokontany but the participatory approach revealed 134 outbreaks. Up to now, samples from 17 fokontany were assessed in the lab and 15 fokontany were confirmed to be infected with ND which corresponded to 18 outbreaks confirmed out of 24 assessed.

Network topology

Three-hundred and forty-seven nodes and 1448 links were identified. Fig 1 shows the network structure. There is no isolate node and some nodes are more connected than others, so it is a connected and heterogeneous network, meaning that the majority of nodes are weakly linked and few nodes are highly linked with others. The distribution of degrees follows a power law distribution (Fig 2). Table 1 shows network parameters and a clustering coefficient that is low (0.11). This network heterogeneity with the power law distribution of degrees and the low clustering coefficient confirmed that the avian commercial trade network of the Lake Alaotra region is a scale-free network (Barabási and Albert, 1999).

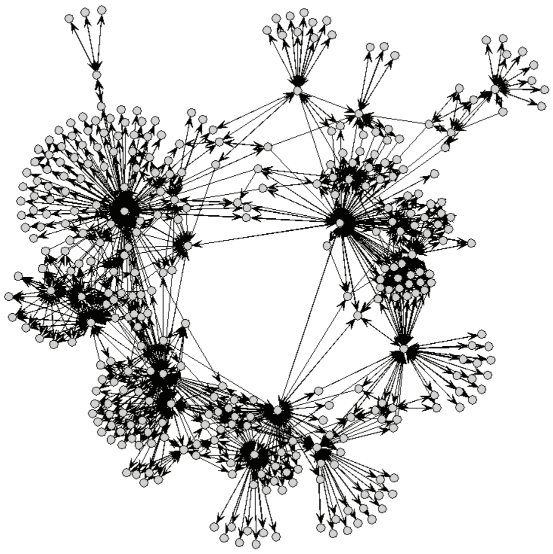

Fig. 1 Structure of the network of poultry commercial trade in the Lake Alaotra region. Madagascar

Table 1. General parameters of network

PARAMETERS	VALUES
Number of nodes	347
Number of links	1448
Clustering coefficient	0.11
Density	0.01
Average degree	8.35
Average betweenness	775.97

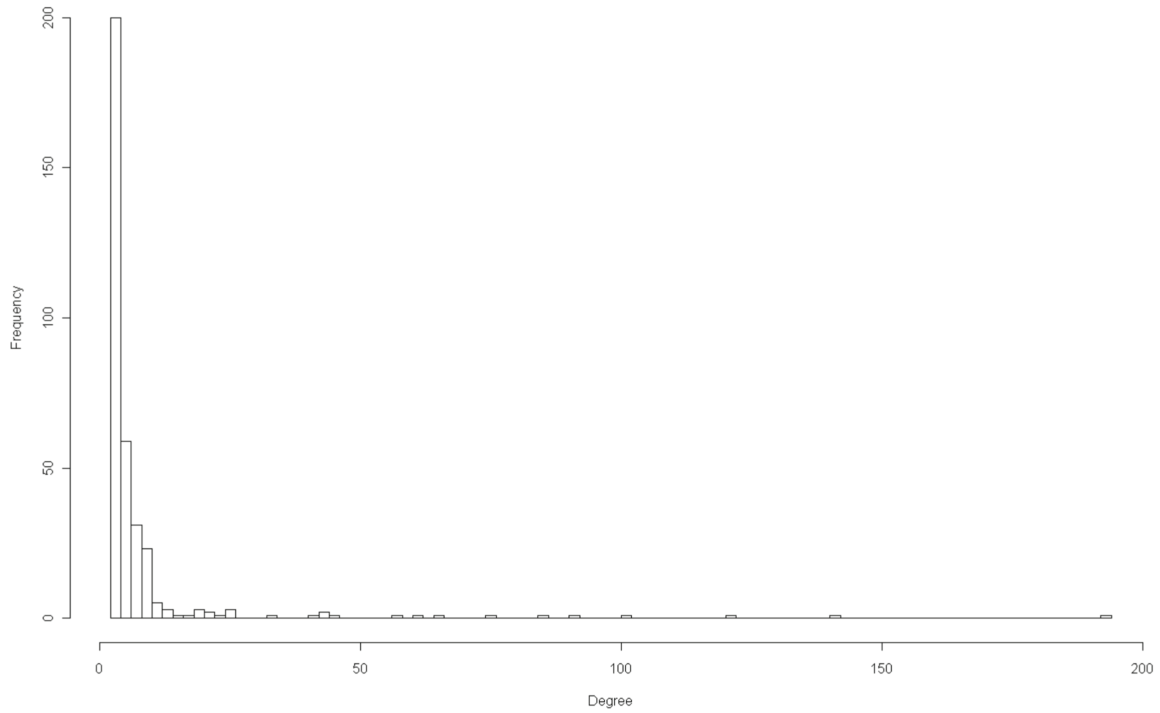

Fig. 2 Distribution of degree per nodes

Centrality measures and outbreak occurrence

Table 2 shows coefficients of each centrality parameter from binary logistic models and the related p-values from permutation tests. None of the parameters were significant indicating that occurrence of outbreak was not linearly associated with the logit of centrality parameters.

Table 2. Centrality parameters coefficients from logistic models

PAREMETERS	COEFFICIENTS	P-VALUE
Freeman degree	0.005	0.18
Indegree	0.008	0.24
Outdegree	0.015	0.12
Betweenness	$< 10^{-4}$	0.29

Hierarchical clustering and outbreak occurrence

Fig 3 shows the dendrogram from hierarchical clustering according to structural equivalence which was measured by euclidean distance. The horizontal line indicates a cut-off level putting in the same cluster all branches below. Six clusters were retained and table 3 shows their description in terms of different parameters.

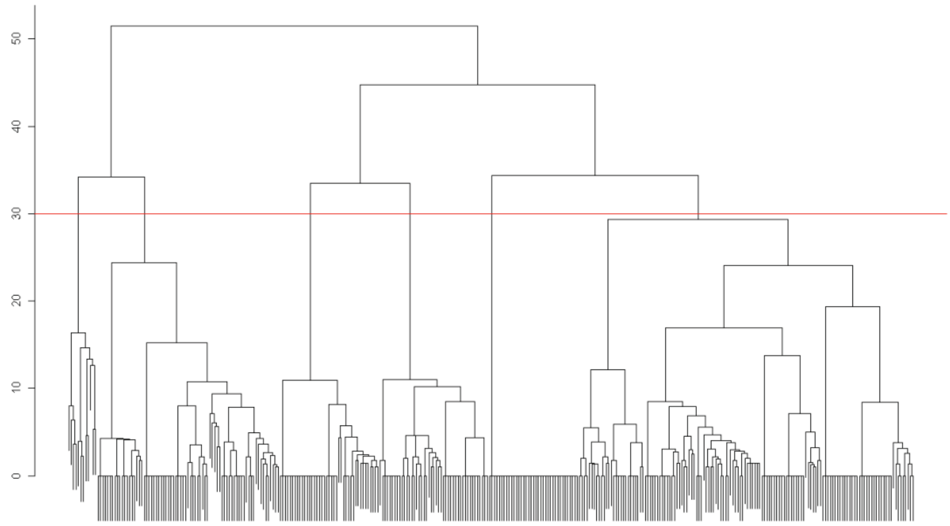

Fig. 3 Dendrogram of nodes representing assembling nodes by structural equivalence

Table 3 shows the description of clusters according to centrality parameters and attribute values. According to centrality parameters there was a hierarchy of their values among classes. Class 4 included the most connected nodes with highest betweenness and degree. All of them had big market (market 1) inside explaining this intensity of poultry commercial exchanges. It was the smallest class in terms of number of nodes inside but they were the hubs of the network. The second most connected class was class 6. There were some big markets and this class ranked second with 74 nodes. These two classes were those where outbreaks occurred more frequently during the period of study. Third place in terms of centrality was taken by class 3. It is the most common class with 138 nodes. 12% of them had small market inside. Frequency of outbreak was lower than the two former classes but it was still high with 41% of nodes infected. In terms of centrality, class 1 and class 5 were comparable. However, although there were more nodes having a big market inside in class 1, the frequency of outbreaks seemed to be lower than class 5. The last and the most peripheral class was the class 2. Its average betweenness was 0 meaning that none of the nodes of this class were necessary to connect two other nonadjacent nodes. However, the frequency of outbreak was higher than in class 1.

Chi-squared test comparing occurrence of outbreak, recorded by participatory epidemiology, among classes was highly significant ($p = 0.004$). It means that occurrence of outbreak was associated with position of nodes within the network.

Table 3. Cluster centrality parameters and outbreak occurrence

CLASS	NUMBER OF NODES	BETWEENNESS	FREEMAN DEGREE	INDEGREE	OUTDEGREE	FREQUENCY OF MARKET 1*(%)	FREQUENCY OF MARKET 2*(%)	FREQUENCY OF OUTBREAK (%)
1	41	55.6	5.5	2.6	2.9	5	7	22
2	37	0.0	2.5	1.5	1.0	0	5	27
3	138	203.0	5.0	2.3	2.7	3	12	41
4	12	17384.8	89.3	47.8	41.5	100	0	50
5	45	34.4	4.6	2.1	2.5	0	7	29
6	74	389.2	8.2	4.0	4.2	8	3	54

*Market 1: Biggest markets with regular presence of poultry trading.

*Market 2: Small markets with irregular presence of poultry trading.

DISCUSSION

In recent years, network analysis has increased its relevance in studying disease spread within complex inter-connected structures (Berthélemy et al, 2004; Berthélemy et al, 2005; Eames et al, 2008). There is also an increase in interest for this method in veterinary epidemiology (Martínez-López et al, 2009) and there were several studies about avian influenza and ND which used this method (Dent et al, 2008; Van Kerkhove et al, 2009; Soares Magalhães et al, 2010). However, in contrast to developed countries where there are generally good registration systems, there is a lack of data in developing countries making acquisition of network structures difficult. Consequently, most studies are based on a small sample of the population with resultant questions about validity of results. The originality of this study is that it was undertaken in an isolated area where it was possible to get almost complete network data combined with disease measures.

The combination of participatory approach and classical survey allowed the collection of a large set of data. It is safe to assume that almost 100% of existing nodes of this network were identified. As mentioned above. the study area is landlocked. Connections that directionally link the network with nodes located outside of the considered area as an output were withdrawn from analysis since it was assumed that they did not influence the circulation of a given pathogen. Other connections with external area could be considered as negligible because they were rare and included isolated villages outside the study area (i.e. in north. west and east). Apart from vaccination against ND and FC, which are facultative, there were no official control measures taken against these diseases even if an outbreak occurred. So, there was no modification of the network structure during an outbreak. Also, a survey of professional traders revealed that the flows of poultry they sell and frequency of activity change within year but places where they buy or sell poultry do not change. As the network was not valued, i.e. flows or poultry quantity exchanged were not considered, these cited modifications do not affect the network structure. This suggests that the structure of the network is stable considering the one year time-scale of study.

Results of this study show an important occurrence of poultry disease outbreak confirming their economic impact. The number of outbreaks declared corresponds to 37% of fokontany. But it should be remembered that there were several villages in each fokontany and it was declared as infected as soon as one village became infected. It means that at least 132 villages were infected.. It should be noted that the type of network identified (i.e. scale-free) is favourable for spreading pathogens (Barthélemy et al, 2004; Barthélemy et al, 2005).

Participatory epidemiology and formal disease surveillance network did not detect the same number of outbreaks. This is likely to be due to sequential implementation of the formal disease surveillance network. Furthermore, several meetings were necessary to encourage network compliance and to improve outbreak declaration from the observation posts. In addition, as field agents who declared cases were the same as those implicated in participatory approach, all outbreaks declared in formal surveillance were also declared during participatory meeting. Confidence was obtained after several meetings, during the participatory approach, so they could provide a complete list of outbreaks. The problem was that as diseased birds were not always seen by the mobile team, validation of outbreak declaration depended on clinical signs declared by Chiefs of fokontany or CAHW. Even if field's agents had a good knowledge of these diseases, it was impossible to distinguish ND, FC and avian influenza on the sole basis of clinical signs. Laboratory confirmation was necessary but this was only possible for samples from the formal disease surveillance. Thus, there was a lack of sensitivity for formal disease surveillance and a lack of specificity for participatory surveillance. A total of 15 fokontany out of 17 were confirmed infected after laboratory analysis implying that field agents had a good knowledge of disease and the outbreak definition was good. Without having means to get perfect data, both methods were complementary because participatory surveillance gave the overall importance of the three diseases and formal surveillance provided information on ND virus outbreaks. This study confirmed the relevance of using participatory epidemiology in developing country, as already shown elsewhere (Jost et al, 2007).

Results from logistic models and comparison of outbreaks within classes seem to be a paradox because none of the coefficients from logistic models was significant but the number of outbreaks was significantly different among classes. However, logistic models have just considered the centrality position of nodes without taking into account the way in which each node was connected to others nodes in the network. It appeared that this was not sufficient to explain the differences among nodes. Justly, hierarchical clustering was based on structural equivalence of nodes i.e. nodes that held the same position in the network (Wasserman and Faust. 2009) were classified in the same cluster. These nodes were interchangeable because they were linked with the same other nodes. However, description of classes revealed that there was a hierarchy of values of centrality parameters among clusters. Finally, it appeared that there was a highly significant association between positions of nodes in the network and occurrence of outbreak. Centrality parameters were not linearly correlated to the outbreak occurrence (more precisely to its link function) but these were still important indicators when coupled with the positions of network in the network.

This study is the first step of analyzing these data. Further analysis would permit the setting up of targeted disease surveillance by identifying the appropriate nodes. Furthermore, integration of values of links, virus strains and temporality of events (outbreaks and links) within each year would enable the modelling of diseases dynamics within the network and to simulate the effects of realistic control measures.

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