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1	Live bird markets chara	cterization and trading network analysis in Mali:						
2	implications for the survei	llance and control of avian influenza and Newcastle						
3		disease						
4								
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1 **ABSTRACT** (306 words)

Live bird markets (LBMs) play an important role in the transmission of avian influenza (AI) and 2 Newcastle disease (ND) viruses in poultry. Our study had two objectives: 1) characterizing LBMs in 3 4 Mali with a focus on practices influencing the risk of transmission of AI and ND, and 2) identifying which LBMs should be targeted for surveillance and control based on properties of the live poultry 5 trade network. Two surveys were conducted in 2009-2010: a descriptive study in all 96 LBMs of an 6 7 area encompassing approximately 98% of the Malian poultry population and a network analysis study 8 in Sikasso county, the main poultry supplying county for the capital city Bamako. Regarding LBMs' 9 characteristics, risk factors for the presence of AI and ND viruses (being open every day, more than 10 2 days before a bird is sold, absence of zoning to segregate poultry-related work flow areas, waste removal or cleaning and disinfecting less frequently than on a daily basis, trash disposal of dead birds 11 and absence of manure processing) were present in 80 to 100% of the LBMs. Furthermore, LBMs 12 tended to have wide catchment areas because of consumers' preference for village poultry meat, 13 thereby involving a large number of villages in their supply chain. In the poultry trade network 14 15 from/to Sikasso county, 182 traders were involved and 685 links were recorded among 159 locations. The network had a heterogeneous degree distribution and four hubs were identified based on 16 measures of in-degrees, out-degrees and betweenness: the markets of Medine and Waverma and the 17 18 fairs of Farakala and Niena. These results can be used to design biosecurity-improvement interventions and to optimize the prevention, surveillance and control of transmissible poultry 19 diseases in Malian LBMs. Further studies should investigate potential drivers (seasonality, prices) of 20 the poultry trade network and the acceptability of biosecurity and behaviour-change 21 recommendations in the Malian socio-cultural context. 22

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Graphical abstract

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Knowing the characteristics of live bird markets and live poultry trade networks allows improved

9 prevention, surveillance and control of transmissible poultry diseases in Mali.



1 Highlights

2	- LBMs have poor biosecurity and are supplied mainly with backyard poultry
3	- Some characteristics differ between LBMs of Bamako and of other regions
4	- Focusing on 4 LBMs will more efficiently detect AI and ND spread in Sikasso county
5	
6	Keywords
7	Poultry, live bird market, surveillance, Mali, avian influenza, Newcastle disease, West Africa

8

9 Abbreviations

AI avian influenza; GSC giant strong component; GWC giant weak component; HPAI highly
pathogenic avian influenza; ND Newcastle disease; LBM live bird market; LPT live poultry trader,
PDAM Project for the development of poultry production in Mali; SSS surveillance station staff

14 **1. Introduction**

15

Poultry have a key role in the livelihood of a large proportion of people living in Mali. In a country belonging to the 20 poorest in the world, with a gross annual national income per capita of US \$1,540 and about 80% of the 15,301,650 inhabitants depending on farming, herding and fishing for their subsistence (World Bank 2014), poultry are a source of cheap protein and income, thereby contributing to food security and poverty alleviation (Gueye 2000). Furthermore, poultry has an important role in sociocultural exchanges as it is used as a gift for friends and family, as a welcome meal for visitors and for ritual animal sacrifices (FAO 2006).

23

The national poultry population in Mali is estimated at around 33.9 million birds with two types of
poultry production coexisting, traditional village poultry and commercial poultry (DNPIA 2009).
Traditional backyard production represents 94% of the total number of poultry in Mali. It is practiced

by 40 to 80% of Malian people depending on whether they live in urban/rural environments and is aimed at subsistence or trade, mainly through local markets. Commercial production represents 6% of the poultry population. It is mainly located around urban areas and strongly depends on imports of inputs such as day-old chicks and embryonated eggs. Both types of poultry production have significantly developed over the last 20 years as a result of programs such as PDAM (Project for the development of poultry production in Mali) which was funded from 1998 to 2004 by the Arab Bank for Economic Development in Africa.

8

9 Diseases, in particular Newcastle disease (ND), are the most important constraint to traditional 10 poultry keeping in sub-Saharan Africa (Aboe et al. 2006, Gueve 1999, Sylla et al. 2003). Moreover, the arrival of highly pathogenic avian influenza (HPAI) virus H5N1 in West Africa in 2006 11 represented a potential threat for the developing Malian poultry sector. Several areas of Mali were 12 considered to be at high risk because they were located near the border of countries which experienced 13 HPAI virus H5N1 outbreaks or because they were visited by millions of palearctic and afrotropical 14 15 migratory birds potentially carrying avian influenza (AI) viruses (Cappelle et al. 2012, Gaidet et al. 2007, Molia et al. 2011). Factors related to poultry trade play a major role in the spread and 16 maintenance of transmissible avian diseases such as HPAI H5N1 or ND, especially the transport of 17 18 live birds to and from live bird markets (LBMs) (Alexander 1995, Capua and Alexander 2009, FAO 2011, Rasamoelina-Andriamanivo et al. 2014). Some of the characteristics of those LBMs (large 19 catchment areas, mixing of different domestic and sometimes wild bird species, and duration of stay 20 commonly longer than a day) have been found to support the dissemination and genetic reassortment 21 of HPAI virus H5N1 strains (Chen et al. 2009, Nguyen et al. 2005, Wang et al. 2006, Webster 2004). 22 23 LBMs are therefore important in AI surveillance and control.

24

In Mali, two types of markets are distinguished based on their frequency: markets *per se* are open daily and are generally located in the largest towns of a *circle* (Malian administrative division

equivalent to a county) whereas fairs are held less frequently, usually once per week, and are located 1 in a small number of large villages within a *circle*. Marketing of poultry can occur in either general 2 3 food markets or fairs, or in markets or fairs specialised in the sale of live birds. From this point on, 4 LBMs will exclusively refer to live bird markets that are held daily, and will not refer to live bird fairs. Some of the Malian LBMs were improved between 1998 and 2004 through the PDAM program 5 6 (with improvements such as construction of cement buildings and/or tiled stalls, provision of water 7 access and/or iron cages) but no inventory of the market infrastructure, number of traders or biosecurity practices was available at the time when our study was conducted. Our first objective was 8 9 therefore to describe the characteristics of Malian LBMs with a focus on practices influencing the 10 risk of transmission of AI and ND viruses between LBMs and the maintenance/amplification of these viruses within LBMs. 11

Furthermore, surveillance of live bird markets and fairs by EPIVET-Mali (the National Veterinary 12 Epidemiological Surveillance network of Mali) is based on convenience sampling despite it being 13 widely accepted that risk-based sampling is a more cost-effective method for conducting surveillance 14 15 and control interventions (Stärk et al. 2006). Studies using network analyses have increasingly been used in veterinary epidemiology to explain the transmission of infectious agents by characterizing the 16 pattern of animal movements and identifying important hubs of transmission (Webb and Sauter-Louis 17 18 2002, Christley et al. 2005, Bigras-Poulin et al. 2006, Ortiz-Pelaez et al. 2006, Dent et al. 2008, Rasamoelina-Andriamanivo et al. 2014). The second objective of our study was therefore to identify 19 which markets and fairs should be targeted for surveillance and control based on properties of the 20 contact network for live poultry traders (LPTs). 21

- 22
- 23
- 24 **2. Materials and Methods**
- 25

26 **2.1 General characteristics of markets**

2 2.1.1 Study area

3

The study area consisted of the district of the capital city Bamako and of five of the eight regions of Mali (Kayes, Koulikoro, Sikasso, Segou and Mopti), all located in the southern half of the country (Figure 1). The regions in the northern half of Mali (Timbuktu, Gao and Kidal) were excluded from the surveys because they account for only 2% of the estimated total poultry population (DNPIA, 2009), consist mainly of the Sahara desert, are difficult to access and are unsafe owing to the presence of al-Qaeda in the Islamic Maghreb.

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Figure 1: Study areas for surveys of live bird markets in Mali, 2009-2010: black lines mark the boundaries of regions; grey lines mark the boundaries of *circles* (Malian equivalent to a county); light grey filling represents the study area for the characterization of live bird markets; crosshatch filling represents the study area for the network analysis of live bird markets and fairs.

1 2.1.2 Study design

2

Approval for the study was obtained from the Ministry of Livestock. Discussions were held with representatives of the DNSV (National Directorate of Veterinary Services), DNPIA (National Directorate of Animal Production and Industry), PDAM, and FIFAM (Federation of the Malian poultry farming stakeholders) to establish a list of LBMs known in the study area. A LBM was defined as a location where live birds are traded daily, whether this location is part of a general food-market or is a location specialising in the sale of live birds.

9 The data collection consisted of two phases. A first cross-sectional survey was conducted between 10 July and October 2009 in all known markets of the district of Bamako. A second cross-sectional survey was conducted between April and August 2010 in all known markets of the regions of Kayes, 11 Koulikouro, Sikasso, Segou and Mopti. For each market, the corresponding surveillance station staff 12 (SSS) was identified. A SSS is a technician or veterinarian working for the veterinary services and in 13 charge of animal health surveillance in a given geographic area. SSS are well integrated into the 14 15 community in which they work and have a much more detailed knowledge of the conditions and constraints of livestock farming in their area than animal health officers in Bamako. They are 16 therefore key persons to contact in order to obtain access to markets and gain the trust of market 17 18 sellers. Before going to a market, meetings were held with the corresponding SSS to explain the 19 objectives of the survey and discuss the timing of the visit to the market. During the visit to the market, the SSS introduced the survey team to the market sellers. A visit to a market was aimed at 20 completing a questionnaire which had been pilot-tested in three markets of Bamako in July 2009. 21

22

The questionnaire included observational data and data gathered by interviewing the market chief (or the market seller with the longest experience in the market when no market chief was identified). Oral consent was obtained from all persons interviewed prior to their participation in the study: the objectives of the study and the types of questions that would be asked were explained to them; they were informed that their participation in the study was on a voluntary basis, that they could refuse to answer any question they did not wish to answer and that no negative consequence would arise from refusal to participate in the study or to answer a specific question; they were also assured that any published results or reports would only mention information at the market level and not at the individual level and that individual information would not be communicated to the government tax authorities of Mali. None of the market chiefs and sellers refused to participate in the study or to answer any question.

8

9 Information was collected on 48 variables providing a description of general aspects, health and 10 biosecurity, poultry supply and poultry sales at the market. The questionnaire was written in French 11 but because a large number of market sellers spoke little or no French, all interviews were conducted 12 by a single interviewer who was fluent in both French and Bambara and who had been previously 13 trained in relation to interviewing skills.

14

15 2.1.3 Data management and analysis

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Data were stored in an Excel spreadsheet table (Microsoft) and descriptive statistical analyses were conducted using R (R Development Core Team 2012). Chi-squared test and Fisher's exact test were used to compare characteristics of markets in Bamako and in the five study regions (with the significance level set at 0.05).

- 21
- 22 2.2 Network analysis

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24 *2.2.1 Study area*

The study area for the network analysis survey was selected based on results from the first part of the 1 LBM survey in 2009: Bamako was the area with the highest poultry meat consumption in Mali and 2 3 Sikasso was identified as the most common and most important source of poultry for markets in 4 Bamako (see section 3.1). Because surveys to generate data suitable for network analysis require interviews with all actors in the network and because of the limited resources and time available for 5 6 the study, we decided to focus our investigations on the structure of the network of contacts resulting 7 from poultry trade from and towards the *circle* of Sikasso (Figure 1). Our target population consisted 8 therefore of all live bird markets and fairs in the Sikasso circle.

9

10 2.2.2 Study design and data collection

11

Approval for the study was obtained from the Ministry of Livestock. Two LBMs were known in the 12 Sikasso circle (the market of Medine and the market of Wayerma) but no list of live bird fairs or 13 LPTs existed at the time of our study. A LPT was defined as someone selling poultry that he/she did 14 15 not breed. LPTs included sellers and middlemen. A seller was defined as a LPT who had a stand at a market or a fair and a middleman was defined as a LPT who did not have a stand at a market or a fair. 16 During a preliminary phase of the study in April 2010, we interviewed staff of the veterinary services 17 18 and animal production services in the Sikasso *circle* to establish a list of live bird fairs. We also designed and tested the questionnaire to be used for data collection. Because LPTs had very little time 19 to answer our questions, the questionnaire was shortened so that it could be completed in 5 to 10 20 minutes. It included data on the type of poultry trading activity, the period of the year with the highest 21 poultry trading activity, the means of transport of poultry, the main difficulty encountered in poultry 22 23 trading activities, the origins and destinations of the traded poultry, and the average number of poultry sold/purchased in each location per month within the year preceding the interviews. 24

During the data collection phase between May and July 2010, we interviewed all LPTs present in the
pre-identified live bird markets and fairs and attempted to identify fairs not present in the original list.

Similar to what was done for the market characteristics survey, all field investigations were prepared and conducted in collaboration with the SSS of the area in order to facilitate data collection and oral consent was obtained from all LPTs interviewed prior to their participation in the study. The questionnaire was written in French but because a large number of LPTs spoke little or no French, all interviews were conducted in French by a single interviewer with translation in Bambara by the SSS.

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2.2.3 Data management and analysis

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9 A directed weighted network was built where a node was a location (either a market, a fair, a village 10 producing poultry or a selling-point on the road-side), and a link represented the commercial movements of poultry between two given locations, with the link weight being equal to the mean 11 number of traded poultry per month in the last year. We used network analysis methods previously 12 applied in veterinary epidemiology (Dube et al. 2011, Martinez-Lopez et al. 2009). We calculated 13 centrality indices at the node level to rank them and discuss their role in the network. For each node 14 15 i (with i = 1 to the total number of nodes n), we calculated the in-degree (total of the average numbers of poultry sent per month from nodes that trade towards node i during the year prior to the interview). 16 the out-degree (total of the average numbers of poultry traded per month out of node i during the year 17 prior to the interview), the shortest-path betweenness (extent to which node i belongs to the shortest 18 paths between all pairs of nodes excluding node i), and the random-walk betweenness (extent to 19 which node i belongs to the paths between all pairs of nodes excluding node i if the choices of 20 consecutive nodes in the path are made at random). For each of the four centrality measures, nodes 21 22 were assigned the rank they had when all nodes were sorted by decreasing order for that centrality 23 measure. The sum of the four ranks was then calculated for each node.

We also calculated measures of cohesion which are indices for determining the level of connectivity in the network: the size (number of nodes), the density (proportion of existing links among all possible links in the network), the average geodesic distance (mean of the shortest path lengths among all

connected pairs of nodes), the diameter (the length of the longest path between connected nodes), and
the global clustering coefficient (average of the proportion of existing links among all possible links
between all nodes directly connected to node i). These measures were all calculated based on a
directed unweighted graph.

5 Some difficulties were encountered in the data collection process and were dealt with as follows. 1) 6 Unknown number of poultry traded: the number of poultry traded per month was assigned the median 7 value of the number of poultry traded for all poultry trade transactions. 2) Uncertainty regarding the 8 market of destination when the destination was a city which had two markets: the destination of these 9 links was modelled using a Bernoulli process of probability n1/(n1+n2), with n1 the number of links 10 known to end in market 1 of the city, n2 the number of links known to end in market 2 of the city. A success meant that the simulated link ended in market 1, and a failure meant that it ended in market 11 2. These random samplings were repeated 1000 times generating 1000 different networks and 12 providing 1000 different sets of network parameters. 3) Uncertainty regarding the market of origin 13 when the origin was a city which had two markets: the origin of these links was modelled using a 14 15 Bernoulli process of probability n1/(n1+n2), with n1 the number of links known to originate from market 1 of the city, n2 the number of links known to originate from market 2 of the city. A success 16 meant that the simulated link originated from market 1, and a failure meant that it originated from 17 18 market 2. These random samplings were repeated 1000 times generating 1000 different networks and providing 1000 different sets of network parameters. 19

20

We analyzed subgroups and calculated the number of strong components of the network (the maximal connected subregions of the network in which all nodes are mutually accessible by following the direction of the links) and identified the giant strong component (GSC, the largest strong component in the network). We also calculated the number of weak components (the maximal connected subregions of the network in which all nodes are linked, not taking into account the direction of the links) and identified the giant weak component (GWC, the largest weak component in the network). Finally, we identified cutpoints (nodes whose deletion increases the number of components in the
 network). Data were managed with Excel 2007 (Microsoft) and analyzed using the packages "igraph"
 (Csardi and Nepusz, 2006) and "sna" (Butts 2014) of R (R Development Core Team 2012).

4

5 **3. Results**

6

7 **3.1** General characteristics of markets

8

9 A total of 96 markets were investigated, of which 55 were in the district of Bamako and 41 in the five 10 study regions (seven in Kayes, ten in Koulikoro, nine in Sikasso, nine in Segou and six in Mopti) 11 (Figure 2). During the investigations, data was also collected about the potential existence of other 12 markets that were not included in the list established through the group discussion, but no other 13 market was identified.

LBMs were rather small, with 12 sellers on average (interquartile range 4-15), and had very basic 14 infrastructure. Only 26% had access to electricity and 63% to water. Access to water was sometimes 15 limited to access to a tap in a neighbouring shop and was not provided directly at the market. Eighteen 16 17 (19%) of the markets had benefited from improvement work (provision of iron cages with waste-18 collection trays, and/or construction of tiled or iron stalls, and/or access to water and electricity) funded by the PDAM Program. These PDAM markets were significantly more likely to have access 19 to electricity and water (56% and 89%, respectively) than non-PDAM markets (19% and 56%, 20 respectively) (p=0.01 by chi-squared test and p = 0.005 by Fisher's exact test, respectively). There 21 were two PDAM markets in Bamako, four in the Sikasso region and three in each of the regions of 22 Kayes, Koulikoro, Segou and Mopti. 23





Figure 2: Location of live poultry markets in the district of Bamako (a) and in the five study regions
(b) in Mali

5 The biosecurity standard of the LBMs was in general poor. Health inspection of birds by market 6 sellers upon bird delivery to the market was only performed in 5% of the markets. No formal health 7 inspection by representatives of the veterinary services was performed upon bird delivery to the

market. Different species of birds were kept together in cages in 80% of the markets. Sick and dead birds were not removed systematically from cages since they were observed to contain some sick and dead birds during the visits of the LBMs. Disinfection practices were insufficient with no daily cleaning of cages (only 4.3 times per week in average) and use of disinfectants in only 16% of the markets. Free-roaming birds were also seen in 33% of the markets.

6 LBMs were supplied mainly with indigenous breed village backyard poultry (98.8% of the total 7 number of birds). Birds from commercial farms were only sold in 42% of the markets, represented 8 only 1.2% of birds and consisted mainly of old laying hens (broiler farms tended to sell their birds on 9 site at the farm or directly to restaurants). Markets were supplied on average 4.4 times per week by 10 different suppliers, with each supply averaging 582 birds (interguartile range 150-650). The majority of the supply was done by middlemen. Additional supply was provided by market sellers themselves 11 (in 69% of the LBMs) and by villagers who brought their birds directly without using the services of 12 a middleman (only outside of Bamako, in 82% of the LBMs). An average of 1667 birds (interquartile 13 range 450-2525) were sold every week per market, of which 77.1% were chickens, 15.0% Guinea 14 15 fowl, 7.0% pigeons, 0.7% ducks and 0.1% geese and turkeys.

16

Some characteristics differed between markets in Bamako and those in the five regions (Table 1). 17 18 LBMs in Bamako were more numerous, had better access to water, had fewer sellers, practiced less health inspection or disinfectant use, had more dung on the ground and had a wider catchment area. 19 Birds sold at the market in Bamako were reported by market chiefs and sellers to originate from all 20 five regions, with Sikasso being the first supplying region followed by Segou and Koulikouro. Poultry 21 supply to Bamako was organized mainly by trucks which transported poultry three to four times a 22 week to a specific delivery point near the downtown bridge of "L'Amitié". From this delivery point, 23 cages with poultry were then dispatched by push carts to all markets in the city. Additional supply to 24 Bamako markets was provided by middlemen and sellers transporting poultry in cars. On the other 25 hand, markets in the five regions were mainly supplied with birds from the same circle while 45% of 26

them received birds from other circles of the same region and 27% also received birds from other 1 regions. Two markets (Yelimane in the Kayes region and Yorosso in the Sikasso region) were 2 occasionally reported to be supplied with birds originating from other countries (Mauritania and Ivory 3 4 Coast, respectively). Poultry supply to LBMs in the five studied regions was more frequent and involved a larger number of middlemen and sellers, with birds being transported mainly by bike 5 (46%), car (27%), or motorbike (14%). More LBMs in Bamako were equipped with a slaughter area 6 7 than in the five studied regions, reflecting a higher percentage of already-slaughtered birds brought 8 home by bird purchasers in Bamako. Finally, the type of poultry sold and the peak sales periods varied slightly between Bamako and the five regions. More Guinea fowl and less ducks were sold in the five 9 10 regions than in Bamako. The most commonly cited peak sales periods were December and Ramadan for both Bamako and the five regions but an additional peak sale period, the "hivernage" (between 11 June and September) was mentioned in 23% of the LBMs of the five regions. 12

Table 11: Characteristics of live bird markets (LBMs) in the district of Bamako and five regions (Kayes, Koulikouro, Sikasso, Segou and Mopti) of Mali, 2009-2010,

2 and p-value for statistical testing (MW = Mann-Whitney test, Chi² = chi-squared test, Fisher = Fisher's exact test) of the difference between LBMs of Bamako and of

the five regions

	All LBMs	LBMs in	LBMs in 5	p-value (test)
	(n=96)	Bamako (n=55)	regions (n=41)	
GENERAL INFORMATION				
Mean number of poultry sellers	11.9	8.4	16.8	<0.001 (MW)
Mean % of men among poultry sellers	90	98	80	<0.001 (MW)
% of LBMs with water or nearby access to water	63	78	41	<0.001 (Chi²)
% of LBMs with electricity	26	29	22	0.430 (Chi ²)
POULTRY HEALTH AND BIOSECURITY				
% of LBMs with health inspection of poultry upon arrival	5	0	12	0.011 (Fisher)
Mean # of sick birds observed during the visit	2.8	3.1	2.4	0.052 (MW)
Mean # of dead birds observed during the visit	1.1	0.4	2.1	0.119 (MW)
Frequency of cage cleaning (per week)	4.3	4.6	3.9	0.221 (MW)
Frequency of ground cleaning (per week)	6.3	6.9	5.4	<0.001 (MW)
% of LBMs where disinfectant is used when cleaning	16	5	30	0.001 (Chi²)
% of LBMs where different poultry species are kept together	80	75	87	0.119 (Chi ²)
% of LBMs with no/little/medium/a lot of dung observed on the ground	0/71.6/28.4/0	0/62/38/0	0/85/15/0	0.013 (Chi²)
% of LBMs where poultry are seen roaming freely	33	31	35	0.675 (Chi²)
% of LBMs where sellers bring back home unsold poultry	21	4	45	<0.001 (Chi²)
POULTRY SUPPLY IN THE LAST YEAR				

Mean number of times a market is supplied per week	4.4	3.9	5.1	0.010 (MW)
Mean number of poultry in one supply	582	343	886	0.017 (MW)
% of LBMs where market sellers supply poultry themselves	69	62	80	0.057 (Chi²)
% of LBMs where middlemen supply poultry	96	93	100	0.136 (Fisher)
% of LBMs where commercial farmers supply poultry	42	42	42	0.947(Chi ²)
% of LBM where biggest supplier is market seller themselves/middlemen	26/73	17/83	38/62	0.023 (Chi²)
% of LBMs where poultry is supplied from other regions	67	96	27	<0.001 (Chi ²)
% of LBMs where poultry is supplied from other countries	2	0	5	0.175 (Fisher)
POULTRY SALES IN THE LAST YEAR				
Mean number of chickens sold per week	1349	1432	1242	1.000 (MW)
Mean number of Guinea fowls sold per week	263	204	333	<0.001 (MW)
Mean number of pigeons sold per week	120	76	144	0.935 (MW)
Mean number of ducks sold per week	13	34	10	0.009 (MW)
Mean number of turkeys sold per week	0.1	0.0	0.1	-
Mean number of geese sold per week	1.0	0.0	1.0	-
Mean number of days before a bird is sold	2.9	3.0	2.7	0.192 (MW)
% of LBMs equipped with poultry slaughter area	76	95	50	<0.001 (Chi²)
Mean % of chickens sold alive	48.2	21.9	82.4	<0.001 (MW)
Mean % of Guinea fowls sold alive	46.3	18.9	81.6	<0.001 (MW)
Mean % of pigeons sold alive	71.9	64.4	78.1	0.025 (MW)
Mean % of ducks sold alive	86.3	100.0	84.8	0.514 (MW)

1 **3.2** Network analysis

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3 Four markets and 22 fairs were visited and a total of 182 poultry traders were interviewed of which 4 81 were middlemen and 101 sellers. All middlemen bought poultry in villages and sold them at fairs to sellers. They transported poultry on foot (1%), by bike (57%), motorbike (38%) or car/truck (4%). 5 They mentioned as peak sale periods "hivernage" (between June and September) (86%), and/or 6 7 celebrations (end of the year and Ramadan) (11%) and/or other periods (5%). They mentioned as the 8 main challenges associated with their activity a lack of operating funds (78%), bird mortality during 9 transport (5%), bird diseases (5%), difficulties in resale (1%), or other causes (11%). Sellers bought 10 poultry from middlemen (85%) and/or from other market sellers (79%) and/or in villages (3%). They sold poultry at home (6%), and/or at a fair or market (72%), and/or on roadsides (4%) and/or to 11 Bamako (26%). They transported poultry by bike (6%), motorbike (22%) or car/truck (72%). They 12 mentioned as peak sale periods "hivernage" (43%), and/or celebrations (58%) and/or other periods 13 (5%). They mentioned as the main challenges associated with their activity a lack of operating funds 14 15 (44%), bird mortality during transport (24%), bird diseases (5%), difficulties in resale (6%), or other causes (21%). 16

Six hundred and eighty five poultry trade transactions (links) originating and/or ending in the *circle* of Sikasso involved 159 locations (Figure 3), including 105 villages in which poultry are raised, 28 markets (of which 22 in the city of Bamako, 2 in the city of Sikasso, 1 in the city of Koutiala and 3 in Ivory Coast), 24 fairs, 1 roadside selling point and 1 commercial farm.

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2 3

The majority of transactions followed the global pattern of middlemen collecting birds in villages to supply fairs from where markets in Sikasso were supplied by fair or market sellers. Some market sellers in the markets of Sikasso (especially in the market of Medine) thereafter supplied markets in Bamako or to a much lesser degree in Ivory Coast. Exceptions to that general pattern nevertheless occurred with fairs being supplied by other fairs, markets in Sikasso being supplied directly by villages and markets in Bamako being supplied by two fairs.

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11 The number of poultry traded was unknown for six transactions and was assigned the median value (200) of the number of poultry traded across all poultry trade transactions. Three-hundred and twenty-12 13 six transactions consisted of poultry sent to the city of Sikasso (which has two markets: Medine and Waverma), including 125 transactions to the market of Medine, 34 transactions to the market of 14 Wayerma, and 147 transactions for which the poultry trader did not know whether the poultry arrived 15 at the market of Medine or the market of Wayerma. The destination of these 147 links was modelled 16 using a Bernoulli process of probability 125/(125+34). Hundred and twenty-four transactions 17 consisted of poultry sent from the city of Sikasso, including 36 transactions from the market of 18 Medine, 20 transactions from the market of Wayerma, and 68 transactions for which the poultry trader 19

did not know whether the poultry came from the market of Medine or the market of Wayerma. The 1 origin of these 68 links was modelled using a Bernoulli process of probability 36/(36+20). These 2 random samplings were repeated 1000 times generating 1000 different networks and providing 1000 3 4 different sets of network parameters which are summarized in Table 2. All 1000 generated networks had a size of 159 locations. Their median density was 0.79%, their median average geodesic distance 5 6 was 4.6, their median diameter was 5.0 and their median global clustering coefficient was 0.041. The 7 structure of the network was characterised by a small subset of nodes (hubs) connected to a large 8 number of nodes, while the majority of nodes had small degrees.

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Table 2: Minimum, median, mean and maximum values for network parameters for 1000 generatedpoultry trade networks to/from the *circle* of Sikasso, Mali, 2010

	Minimum	Median	Mean	Maximum
Size	159	159	159	159
Density	0.76%	0.79%	0.79%	0.81%
Average geodesic distance	3.8	4.6	4.3	4.7
Diameter	4.0	5.0	4.6	5.0
Global clustering coefficient	0.033	0.041	0.041	0.050

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The five nodes which had the lowest sum of ranks for the four centrality measures were the same for
the 1000 networks and are listed in Table 3. They included the markets of Medine and Wayerma and
the fairs of Farakala, Niena, and Kafana (Figure 4).

1 Table 3: Minimum, median, mean and maximum values for centrality measures (a) and for ranks of centrality measures (b) of the five nodes with the

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lowest sum of ranks of centrality measures for 1000 generated	l poultry trade networks to/from the <i>circle</i> of Sikasso, Mali, 2010
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a)	Indegree				Outdegree				Shortest-path betweenness				Random-walk betweenness			
	Min	Median	Mean	Max	Min	Median	Mean	Max	Min	Median	Mean	Max	Min	Median	Mean	Max
Medine market	69,014	77,054	76,694	83,314	32,120	39,540	39,428	45,880	1,637	2,240	2,239	2,692	3.98e-5	3.98e-5	3.98e-5	3.98e-5
Wayerma market	7,840	12,332	12,503	17,584	14,460	22,200	22,265	31,780	437.5	967.1	971.5	1,657.8	3.98e-5	3.98e-5	3.98e-5	3.98e-5
Farakala fair	6,140	6,140	6,140	6,140	4,360	4,360	4,360	4,360	621.0	621.0	630.2	692.7	23.7e-5	23.7e-5	23.7e-5	23.7e-5
Niena fair	3,680	3,680	3,680	3,680	8,320	8,320	8,320	8,320	252.0	262.0	267.1	317.3	18.1e-5	18.1e-5	18.1e-5	18.1e-5
Kafana fair	1,555	1,555	1,555	1,555	1,680	1,680	1,680	1,680	273.0	280.0	293.6	415.2	24.2e-5	24.2e-5	24.2e-5	24.2e-5

b)	Rank for indegrees			Rank for outdegrees				Rank for shortest-path betweennesses				Rank for random-walk betweennesses				
	Min	Median	Mean	Max	Min	Median	Mean	Max	Min	Median	Mean	Max	Min	Median	Mean	Max
Medine market	1	1	1	1	1	1	1	1	1	1	1	2	5.5	5.5	5.5	5.5
Wayerma market	2	3	3	4	2	2	2	2	1	2	2	3	5.5	5.5	5.5	5.5
Farakala fair	6	6	6	6	9	9	9	9	2	3	3	3	2	2	2	2
Niena fair	7	7	7	7	5	5	5	5	5	7	6.6	7	4	4	4	4
Kafana fair	16	16	16	16	19	19	19	19	5	5	5.6	7	1	1	1	1





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Figure 4: Location of live poultry fairs and markets in the circle of Sikasso, Mali (Fairs and markets highlighted in red correspond to the five nodes which had the lowest sum of ranks for the four centrality measures of the poultry trade network)

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6 The results of the analysis of components and cutpoints were identical for all 1000 generated networks. While all nodes were connected with the same GWC, the GSC only included 4 nodes. The 7 8 GSC included the fairs of Farakala, Kafana, Niena, and Doumanaba and the two strong cutpoints 9 were Farakala and Kafana. The size of the GWC was highly affected by the deletion of the nodes identified as the most central. It decreased by 16% when the market of Medine was deleted from the 10 network, by 45% when the markets of Medine and Wayerma were deleted, by 59% when the markets 11 12 of Medine and Wayerma and the fair of Farakala were deleted, by 75% when the markets of Medine and Wayerma and the fairs of Farakala and Niena, by 75% when the markets of Medine and Wayerma 13 and the fairs of Farakala, Niena and Kafana were deleted. 14

2	On average, a total of 56,360 poultry were sent per month from the <i>circle</i> of Sikasso to the markets
3	in Bamako (of which 6,000 (10.6%) were from the fair of Niena and 1,600 (2.8%) from the fair of
4	Finkolo; depending on the randomly generated network, the contribution of the market of Medine
5	varied from 29,760 (52.8%) to 42,120 (74.7%) with a median of 36,960 (65.6%) and the contribution
6	of the market of Wayerma varied from 6,640 (11.8%) to 19,000 (33.7%) with a median of 11,800
7	(20.9%)). On average, a total of 3,600 poultry per month was sent from the <i>circle</i> of Sikasso to the
8	markets in Ivory Coast (of which 1,200 (33.3%) were from the market of Medine and 2,400 (66.6%)
9	from the market of Wayerma).

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12 **4. Discussion**

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14 **4.1 General characteristics of markets**

This is the first study to describe the characteristics of Malian LBMs with a focus on practices influencing the risk of transmission of AI and ND. To the best of our knowledge, only one similar study has been conducted in East Africa (in Uganda, Kirunda et al. 2014) despite the fact that the circulation of HPAI virus H5N1 has been demonstrated in LBMs in Nigeria and Egypt (Abdelwhab et al. 2010, Miko et al. 2013).

Our assessment of the situation of Malian LBMs is quite alarming because all the main risk factors previously found to be associated with the presence of LPAI viruses or of HPAI virus H5N1 in LBMs were present in the vast majority (80% or more) of the markets; these factors were: being open every day, overnight poultry storage, absence of zoning to segregate poultry-related work flow areas, waste removal or cleaning and disinfecting less frequently than on a daily basis, slow and trash disposal of dead birds, and absence of manure processing (Bulaga et al. 2003, Fournié et al. 2011, Garber et al. 2007, Indriani et al. 2010, Kung et al. 2003, Lau et al. 2007, Leung et al. 2012, Martin et al. 2011,

Trock et al. 2008). One important risk limiting factor was that very few ducks and geese were sold – 1 two species known to play an important role in the maintenance and dissemination of HPAI virus 2 3 H5N1 (Aly et al. 2008, Hulse-Post et al. 2005, Phan et al. 2013, Sturm-Ramirez et al. 2005). The poor 4 biosecurity standard of LBMs has been described in other developing countries where it was partly attributed to 1) the lack of financial means for infrastructure and equipment that allow efficient 5 6 cleaning and disinfection and 2) a lack of awareness of biosecurity issues or poor compliance by 7 poultry traders with good practice guidelines (Abdullahi et al. 2010, Fasina et al. 2009, Kirunda et al. 8 2014, Samaan et al. 2011, Van Kerkhove et al. 2009). In Mali, the situation is made worse by the fact 9 that markets have a wide catchment area. Indeed, Malian people prefer consuming indigenous breed 10 village chickens because their meat is considered tastier. Their value chain therefore involves a supply circuit of LBMs from a large number of villages. Our study also documented transboundary supply 11 since two Malian markets occasionally imported birds from Mauritania and Ivory Coast. A 12 particularly large catchment area is associated with the markets in Bamako which represented more 13 than half of the total number of LBMs in the country and which were supplied by all five regions of 14 15 our study area. Bamako is indeed the highest poultry consumption area in Mali. FAO estimates that poultry consumption per capita in Mali is approximately four times higher in urban populations than 16 in the general population (FAO 2013). 17

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19 **4.2 Social network analysis**

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Our study also allowed us to better understand the contact structure of poultry trade to and from the *circle* of Sikasso which is the biggest supply *circle* for the markets in Bamako. This type of information is crucial for developing strategies for disease surveillance, prevention and control by targeting markets and fairs that are hubs for poultry trade flows.

The general structural characteristics of the Sikasso poultry trade network (with villages supplying fairs which in turned supplied markets, some of which then supplied the capital city) and the very

limited involvement of commercial poultry farms in the network that we observed in our study were
 also described in other SNA studies conducted in Cambodia, Ethiopia and Madagascar (Rasamoelina Andriamanivo et al. 2014, Vallee et al. 2013, Van Kerkhove et al. 2009).

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5 The structure of the network, with a small subset of nodes (hubs) connected to a large number of nodes while the majority of nodes had small degrees, has consequences on disease control 6 7 interventions that can be applied to markets and fairs since such disease transmission networks are 8 robust to random interventions but vulnerable to interventions targeting hubs (Barabasi and Bonabeau 9 2003). Indeed, we found that the size of the GWC, which provides an estimate of the upper bound of 10 the maximum epidemic size in case a pathogenic agent reaches the network (Kao et al. 2006), could be decreased by 75% just by removing four nodes (the markets of Medine and Wayerma and the fairs 11 of Farakala and Niena). Such a structure of poultry trade flows has also been observed in Madagascar 12 (Rasamoelina-Andriamanivo et al. 2014). 13

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15 The method we chose to identify influential nodes used a combination of centrality measures including degree which is an egocentric measure, and betweenness which require knowing the overall 16 network to be calculated (Wasserman and Faust 1994). In-degree, out-degree and shortest-path 17 18 betweenness have been used previously in other studies conducted in developing countries to inform surveillance, prevention and control of HPAI virus H5N1 or ND (Fournié et al. 2014, Martin et al. 19 20 2011, Poolkhet et al. 2013, Rasamoelina-Andriamanivo et al. 2014, Soares Magalhaes et al. 2010, Soares Magalhaes et al. 2012, Vallee et al. 2013, Van Kerkhove et al. 2009). We also used random-21 22 walk betweenness because it better captures the stochastic nature of the diffusion of a contagious 23 disease agent in a network (Newman 2005, Rasamoelina-Andriamanivo et al. 2014).

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Our results have to be interpreted taking into account several limitations of our study. The missing
values amongst the poultry trade movement data is the main limitation as has been the case in all

other published network analysis studies conducted in developing countries. Indeed, information on 1 animal movement in these countries usually has to be collected through field surveys, in contrast to 2 many developed countries where it is readily available in national databases due to traceability 3 4 obligations (Bigras-Poulin et al. 2006, Dent et al. 2008, Ribbens et al. 2009). We interviewed all LPTs 5 that were present at the fairs and markets but it is likely that we missed some LPTs that were absent 6 on the day of the market/fair visit. This may have caused an underestimation of the degrees for these 7 markets/fairs and may have affected the measures of betweenness (Scott 2000). Nevertheless, 8 considering the overall heterogeneous degree distribution structure of our network, it is unlikely that 9 the identified top five hubs would change if we added information for the LPTs that we were not able 10 to interview.

The lack of knowledge about the exact market of destination/origin for almost half of the transactions to/from the city of Sikasso was due to the fact that some LPTs knew the person they sold to/bought from but were not sure whether poultry transited through the market of Medine or the market of Wayerma. We chose to account for this missing information by generating 1000 different networks based on probabilities derived from data with known market of destination/origin in the city of Sikasso. Although this only gave us a range of possible values for the different network parameters, it did not change the markets and fairs that were identified as the top five most influential nodes.

Some caution should also be exercised regarding the weights of the network links. Although the presence of the SSS greatly facilitated interviews with LPTs - who are often very busy and reluctant to answer questions, as seen elsewhere (Fournié and Pfeiffer 2013, Soares Magalhaes et al. 2010) and despite it having been explained to them that their anonymity would be ensured, we cannot be certain that they did not underestimate the amount of poultry they traded per month over the last year before the survey for fear the data we produced would be used by the Malian government tax authorities.

Poultry trade patterns may vary across seasons and this is particularly true in Southeast Asia where
Chinese New Year or Khmer New Year (in Cambodia) constitute periods where there is a major

increase in poultry trade and consumption (Pfeiffer et al. 2007, Soares Magalhaes et al. 2012, Van 1 Kerkhove et al. 2009). Seasonality was not properly captured in our survey since for each poultry 2 3 trade transaction between two locations, we asked LPTs about the average number of poultry that had 4 been traded in each month during the 12 months prior to the survey. Nevertheless, we also asked LPTs about peaks in poultry trading activities and they identified peaks mostly during hivernage and 5 celebrations related to the end of the year or to religious events such as Ramadan. Whether this 6 7 seasonality results in a change of the poultry trade network structure remains unknown. Results from 8 network analysis studies conducted in Cambodia and Ethiopia show that it was mostly the number of 9 traded poultry that changed over seasons and not so much the structure of the poultry trading network 10 (Vallee et al. 2013, Van Kerkhove et al. 2009) whereas in China, the centrality measures and the geographical extent of poultry trade increased in February-March (Soares Magalhaes et al. 2012). 11

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4.3 Implications and perspectives

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15 Our results can be used to design biosecurity-improvement interventions and to optimize the prevention, surveillance and control of transmissible poultry diseases in Malian live bird markets and 16 fairs. Much remains to be done in Mali to reduce the frequency of practices that increase the risk of 17 18 transmission of AI and ND (Molia et al. 2015). Several critical control points in LBMs have been identified in low-resource settings (Samaan et al. 2011) but the nature and the applicability of 19 recommendations is likely to change depending on each country's epidemiological and socio-20 economic situation. Among the measures that have proved to effectively decrease the prevalence and 21 dissemination of HPAI virus H5N1 (Fournié et al. 2014, Kung et al. 2003, Leung et al. 2012, Sims 22 23 2007), some may be relatively easy to implement in Mali through communication campaigns: 1) preventing the mix of birds of different species and from different origins in the same cages; 2) 24 preventing free-roaming of poultry in LBMs; 3) systematically removing and appropriately disposing 25 of sick and dead birds; and 4) increasing the health inspection of supplied birds (visual inspection and 26

refusal of sick birds). Other measures such as adopting daily cleaning and disinfection would be more 1 easily adopted if the infrastructure was improved (iron cages with waste-collection trays, tiled or iron 2 3 stalls, access to water and electricity) and equipment were provided (brushes, disinfectant, etc). 4 Participatory interventions combining infrastructure changes with behaviour-change education 5 successfully improved the biosecurity of two markets in Indonesia (Samaan et al. 2012). Finally, 6 some measures would be quite difficult to implement such as introducing a market rest day. This 7 measure would indeed not be useful for fairs since they are held a maximum of once per week and it would encounter major resistance from LPTs of markets, as few of them (5%) consider poultry 8 9 diseases to be an important factor potentially affecting their business.

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In terms of optimization of surveillance on markets and fairs, the current strategy of convenience 11 sampling should be replaced by sampling targeted at markets and fairs that have high centrality 12 measures in the poultry trade network (hubs), or at least more resources should be allocated to those 13 hubs than to other nodes. The same applies to control interventions (such as movement restrictions) 14 15 in case of an outbreak of HPAI (Dent et al. 2011). For the circle of Sikasso, four markets and fairs (Medine, Wayerma, Farakala and Niena) were identified as hubs whose removal from the network 16 would decrease the maximum epidemic size by 75%, assuming that the trade network is the main 17 18 mechanism for HPAI virus transmission. Removal of a node from the network may entail temporary closure of the market/fair (with the risk of inducing the emergence of a new unknown poultry trade 19 structure) or less drastically through more effective enforcement of heath inspection and disinfection 20 procedures. Additionally and although it only ranked ninth in our classification based on centrality 21 degrees, the fair of Finkolo would also potentially be a target of interest because it had direct trade to 22 23 Bamako.

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Similar studies should be conducted in *circles* other than Sikasso to obtain a network of poultry trade
at the country level and to optimize national surveillance and control plans. Nevertheless, the question

remains about whether there would be a network structure change during an outbreak or just a change
 in the intensity of the flows.

3 Further studies should also investigate in more detail potential drivers of the poultry trade network 4 structure such as seasonality or prices. Anthropological and other studies should also assess the 5 acceptability and feasibility of biosecurity and behaviour-change recommendations which may differ 6 based on age, gender, education and religion (Kirunda et al. 2014, Naysmith 2014). Finally, testing 7 the association between ND outbreaks and network parameters would allow the verification of the 8 role of hubs as amplifiers and disseminators of ND virus. Nevertheless, obtaining reliable, sensitive 9 and specific data on ND outbreaks is difficult in Mali as the animal health surveillance network faces 10 many challenges, in particular a low reporting of disease by poultry owners (Molia et al. 2012).

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