

Submitted: 15/12/2016

Accepted: 30/03/2017

Published: 14/04/2017

## Biosecurity and geospatial analysis of mycoplasma infections in poultry farms at Al-Jabal Al-Gharbi region of Libya

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### Abstract

Geospatial database of farm locations and biosecurity measures are essential to control disease outbreaks. A study was conducted to establish geospatial database on poultry farms in Al-Jabal Al-Gharbi region of Libya, to evaluate the biosecurity level of each farm and to determine the seroprevalence of mycoplasma and its relation to biosecurity level. A field team of 7 Veterinarians belongs to the National Center of Animal Health was assigned for data recording and collection of blood samples. Personal information of the producers, geographical locations, biosecurity measures and description of the poultry farms were recorded. The total number of poultry farms in Al-Jabal Al-Gharbi Region is 461 farms distributed in 13 cities. Out of these, 102 broiler farms and one broiler breeder farm (10 houses) which were in operation during team visit were included in this study. Following collection of blood, sera were separated and tested by Enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies against Mycoplasma (General antigen for *M. gallisepticum* and *M. synoviae*). The seroprevalence of Mycoplasma in the region was 28% (29 poultry farms out of 103 were infected). About 50% (23 out of 47) of poultry farms located in Garian city were infected with Mycoplasma and one significant cluster of Mycoplasma infection in the city was identified. Low level of biosecurity was found in poultry farms of the region. Out of the 103 farms included, 63% of poultry houses has a ground of soil and 44% of them has uncoated walls which may influence the proper cleaning and disinfection. Almost 100% of the farms are at risk of exposure to diseases transmitted by wild birds such as avian influenza and Newcastle disease due to absence of wild birds control program. Although, 81% of the farms have entry restrictions, only 20% have disinfectants at entry which increase the risk of exposure to pathogens. The results of this study highlight the weakness points of biosecurity measures in poultry farms of Al-Jabal Al-Gharbi region and high seroprevalence of mycoplasma. Data collected in this study will assist the Veterinary authorities to apply effective disease control strategies.

**Keywords:** Biosecurity, Geospatial analysis, Mycoplasma, Poultry.

### Introduction

The severe economic, social and public health consequences of infectious diseases in poultry require effective plans for rapid detection and response. Geospatial database of farm locations and biosecurity measures are essential to control disease outbreaks. Attributed farm locations are needed because many infectious disease mitigation strategies, such as culling or ring vaccination, explicitly rely on spatial relationships between susceptible animal population and pathogen reservoirs (Bruhn *et al.*, 2012).

Biosecurity is the prevention or control of pathogenic microorganisms from contacting animal or human populations. In the context of modern poultry production, it is essentially keeping the birds separate from the agents causing the disease. Used properly, biosecurity will also minimize the effect of disease and contain the spread of disease, if found (Dorea *et al.*, 2010).

Avian mycoplasmosis is caused by several pathogenic mycoplasmas of which *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the most important; they are the only ones listed by the OIE. MG causes chronic respiratory disease of domestic poultry, especially in the presence of management stresses and/or other respiratory pathogens. MS causes synovitis and/or airsacculitis. Infections of mycoplasma can yield significant losses in performance and associated economics to all sectors of the poultry industry worldwide (Evans *et al.*, 2005).

In the 1999 outbreak of MG in North Carolina, Vaillancourt *et al.* (2000) found a direct connection between an MG-positive farm and a previous case when the MG-positive grower helped another grower who had MG and returned to his farm without changing clothing and boots which reflects a failure in biosecurity. Silva *et al.* (2015) found high seroprevalence rates of mycoplasma (MG & MS) in

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backyard and commercial chickens which emphasize the need of keeping chicken flocks free from disease using effective biosafety systems.

There is lack of information on poultry industry in Libya in term of geospatial data. Moreover, to our knowledge, no detailed study has been carried out to determine the level of biosecurity that is practiced by poultry producers in Al-JabalAl-Gharbi region of Libya, and much less the relationship of such practices with mycoplasma infections. The present study was conducted for this reason.

### Materials and Methods

#### Data collection

Prior to this study, there was no database on poultry production in this region. A field team of 7 Veterinarians belongs to the National Center of Animal Health was trained and assigned for collection of data using a questionnaire containing personal information of the producers, geographical information, information of the farm, biosecurity measures and some other questions on previous diseases and control measures adopted. Blood samples were collected for the detection of antibodies against MG and MS. One hundred and two broiler farms and one broiler breeder farm (10 houses) which are located in 10 cities were included in this study (Table 1).

**Table 1.** Number of the poultry farms evaluated for the level of biosecurity and mycoplasma seroprevalence.

City	Frequency
Garian	47
Alasabaa	3
Kelaa	1
Yafrin	11
Rayaina	12
Zentan	6
Rujban	2
Alharaba	1
Nalut	16
Gadamis	4
Total	103

All of these farms were in operation during visit and evaluated according to the level of biosecurity practice and the presence of antibodies against Mycoplasma taking into account the relationship between biosecurity level and Mycoplasma infection. Eleven biosecurity variables were included which are related to either proper cleaning and disinfection or protection of the flock from external pathogens. The biosecurity variables evaluated were shown in Table 2. Every variable was coded as good biosecurity practice (code=1) and bad biosecurity practice (code= 0).

**Table 2.** Names and description of biosecurity variables investigated.

NO	Biosecurity variable (name)	Description (Code)
1	GROUND	Ground of the poultry house (Soil=0, Concrete=1)
2	WALL	Walls of the poultry house (uncoated brick=0, coated brick=1)
3	DIS BETWEEN FARMS	Distance between farms ( $\leq 1000$ meter=0, $>1000$ meter=1)
4	OTHER BIRD SPECIES	Other birds species in the farm (yes=0, no=1)
5	ENTRY RESTRICTION	The presence of a gate (no=0, yes=1)
6	ENTRY DISINFECTANTS	Disinfectants at the gate for cars (no=0, yes=1)
7	HOUSE DISINFECTANTS	Foot bath with disinfectants in front of the house(s) (no=0, yes=1)
8	COVERALL CLOTHS	Presence of clothes for visitors and workers (no=0, yes=1)
9	BIRD DISPOSAL	How dead birds are disposed (thrown away=0, burned and buried=1)
10	WILD BIRD CONTROL	Control of wild bird (no=0, yes=1)
11	RODENTS CONTROL	Control of rodents (no=0, yes=1)

#### Serological analysis

Twenty blood samples were collected from each poultry farm (each house) at the end of the production cycle. After collection, the blood samples were sent to the Veterinary Laboratory of the region. Sera were separated and tested by Enzyme-linked immunosorbent assay (ELISA) using Mycoplasma (MG-MS) commercial kit (Biochek®, Netherlands).

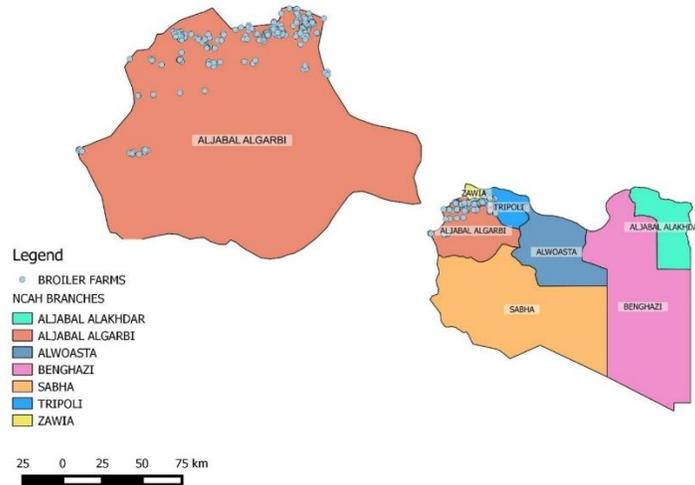
#### Spatial analysis

In this study a map showing all seven branches (regions) of the National Center of Animal Health was designed using QGIS 2.8.1 software (<http://www.qgis.org>) (Fig. 1).

The poultry farms of Al-Jabal Al-Gharbi region were also shown on the map based on their coordinates.

The spatial aggregation of positive farms was investigated through a purely spatial scan statistic, using a Bernoulli probability model (Kulldorff, 1997), which is particularly suitable for presence/absence or positive/negative data.

Farms were considered positive based on at least one positive ELISA result. A cluster of positive farms was considered significant when the simulated P-value was  $\leq 0.05$  (Dwass, 1957). The identified cluster was ranked on the basis of a likelihood function (Kulldorff and Nagarwalla, 1995; Kulldorff, 1997).



**Fig. 1.** A map showing the 7 branches of the National Center of Animal Health and poultry farms of Al-Jabal Al-Gharbi region.

**Table 3.** Number of poultry farms in different cities in Al-Jabal Al-Gharbi region.

City	No. of farms/houses at operation during study				No. of empty farms (out of operation during team visit)	Total
	Broilers	Layers	Breeders	Quails		
Nalut	16	1	0	0	144	161
Garian	60	3	1	1	59	124
Yafrin	13	1	0	0	51	65
Rayaina	17	1	0	0	27	45
Ruhaibat	0	0	0	0	15	15
Gadamis	11	0	0	0	1	12
Zentan	9	0	0	0	2	11
Jadu	0	0	0	0	7	7
Alasabaa	5	0	0	0	2	7
Mezda	0	0	0	0	6	6
Rujban	3	0	0	0	1	4
Alharaba	1	0	0	0	2	3
Kelaa	1	0	0	0	0	1
<b>Total</b>	<b>136</b>	<b>6</b>	<b>1</b>	<b>1</b>	<b>317</b>	<b>461</b>

**Table 4.** Number and percentage of poultry farms showed good biosecurity practice at every biosecurity variable.

Biosecurity variable	Garian *n=47	Alasabaa n=3	Kelaa n=1	Yafrin n=11	Rayaina n=12	Zentan n=6	Rujban n=2	Alharaba n=1	Nalut n=16	Gadamis n=4	Total n=103
Ground	20	0	0	2	6	3	0	1	4	2	38 (37%)
Wall	37	1	0	5	8	1	0	0	6	0	58 (56%)
Dis. Between farms	13	3	1	3	1	6	1	1	8	2	39 (38%)
Other bird species	44	3	1	11	10	6	2	1	12	4	94 (91%)
Entry restriction	45	3	1	2	12	6	2	1	11	0	83 (81%)
Entry disinfectants	9	0	0	5	1	1	0	0	5	0	21 (20%)
House disinfectants	45	3	1	10	12	6	2	1	16	4	100 (97%)
Coverall cloths	5	0	0	5	0	5	0	1	1	0	17 (17%)
Bird disposal	14	0	1	4	2	5	1	0	13	4	44 (43%)
Wild bird control	1	0	0	0	0	0	0	0	0	0	1 (0.97%)
Rodents control	3	0	0	9	6	1	1	0	2	0	22 (21%)

\*n= number of poultry farms that participated.

The data were handled by SaTScan™ version 9.1.1 (<http://www.satscan.org>), while the output was visualised in ESRI™ ArcMap® version 10.1 (<http://www.esri.com/>).

### Results and Discussion

The total number of poultry farms in Al-Jabal Al-Gharbi Region is 461 farms distributed in 13 cities (Table 3). Poultry farms showed low level of biosecurity especially the ground of the houses, distance between farms, the presence of disinfectants at the farm entry, the use of coverall cloths, disposal of dead birds and control of wild birds and rodents (Table 4). 63% of poultry houses has a ground of soil and 44% of them has uncoated walls which may influence the proper cleaning and disinfection. Almost 100% of the farms are at risk of exposure to diseases transmitted by wild birds such as avian influenza and Newcastle disease due to absence of wild birds control program (Keawcharoen *et al.*, 2011). Although, 81% of the farms have entry restrictions, only 20% have disinfectants at entry which increase the risk of exposure to pathogens that may be transmitted by vehicles carrying feed, day old chicks, etc.. Biosecurity and biosurveillance measures have been largely successful at minimizing MG outbreaks among the breeding stock of the turkey and chicken industries (Evans *et al.*, 2005).

Table 5 presenting the number of poultry farms infected with Mycoplasma. The prevalence of Mycoplasma (MG-MS) in the region was 28% (29 poultry farms out of 103 were infected).

**Table 5.** Number of poultry farms infected with Mycoplasma.

City	MG-MS**
Garian (*n=47)	23
Alasabaa (n=3)	0
Kelaa (n=1)	1
Yafrin (n=11)	1
Rayaina (n=12)	0
Zentan (n=6)	1
Rujban (n=2)	0
Alharaba (n=1)	1
Nalut (n=16)	1
Gadamis (n=4)	1
<b>Total (n=103)</b>	<b>29 (28%)</b>

\*n= number of poultry farms that participated.

\*\*MG-MS= Mycoplasma Gallisepticum and Mycoplasma Synoviae (Mycoplasma general antigen).

About 50% (23 out of 47) of poultry farms located in Garian city were infected with Mycoplasma (MG-MS). This may due to the extensive production in the city. This study showed presence of other bird species mainly backyard chickens in 94% of the poultry farms (Table 4).

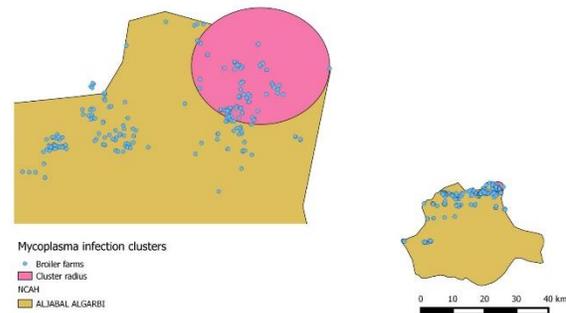
Backyard chickens could play important role in mycoplasma spread. Buchala *et al.* (2006) tested 15

free range chicken farms and found seroprevalence of 73% for MG and 100% for MS.

Pereira (2005), in a study on the prevalence of antibodies against MG and MS in 160 backyard chickens in Uberlândia, found that 102 (63.8%) were positive for MG. In the 1999 outbreak of MG in North Carolina, Vaillancourt *et al.* (2000) found a direct connection between an MG-positive farm and a previous case when the MG-positive grower helped another grower who had MG and returned to his farm without changing clothing and boots which reflects a failure in biosecurity. This may indicate that birds reared in farms where biosecurity programs are implemented are less contaminated than those reared in environments with no biosecurity measures (Silva *et al.*, 2015).

Spatial analysis scanning for clusters with high rates using the Bernoulli model was conducted using SaTScan Software. Geographical localization of Mycoplasma infection clusters in Al-Jabal Al-Gharbi region are shown in Figure 2.

Spatial analysis identified one significant cluster of Mycoplasma infection ( $P < 0.01$ ) (Fig. 2; Table 6). This cluster is mostly located within Garian's administration borders.



**Fig. 2.** Geographical localization of Mycoplasma infection clusters in Al-Jabal Al-Gharbi region.

**Table 6.** Characteristics of Mycoplasma cluster.

Cluster ID	Radius (km)	No. of farms included	No. of cases observed	No. of cases expected	P-value
1	21.52	29	18	2.14	0.0013

Geographic information system (GIS) and spatial analyses constitute a useful approach that supports the generation of hypotheses on drivers for disease diffusion based (Pfeiffer *et al.*, 2008). This study showed that all poultry farms of Al-Jabal Al-Gharbi region had bad biosecurity practice which may be a good reason for wide spread of mycoplasma infections. However, there is still a possibility of vertical transmission of mycoplasma from infected parents to their progeny.

The results of this study highlight the weakness points of biosecurity measures in poultry farms of Al-Jabal Al-Gharbi region. The seroprevalence rates of mycoplasma found in the present study emphasize the need of keeping chicken flocks free from disease using effective biosecurity system. Veterinary authorities has to inforce the application of biosecurity measures to improve birds' health and welfare.

Training of producers on the importance of biosecurity and its applications is of great value. This may be achieved by conducting seminars, using media and leaflets.

#### **Conflict of interest**

The authors declare that there is no conflict of interest.

#### **Acknowledgments**

The authors acknowledge the assistance of the Veterinarians and Veterinary Laboratory Staff of Al-Jabal Al-Gharbi region for collection of data and testing of blood samples.

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