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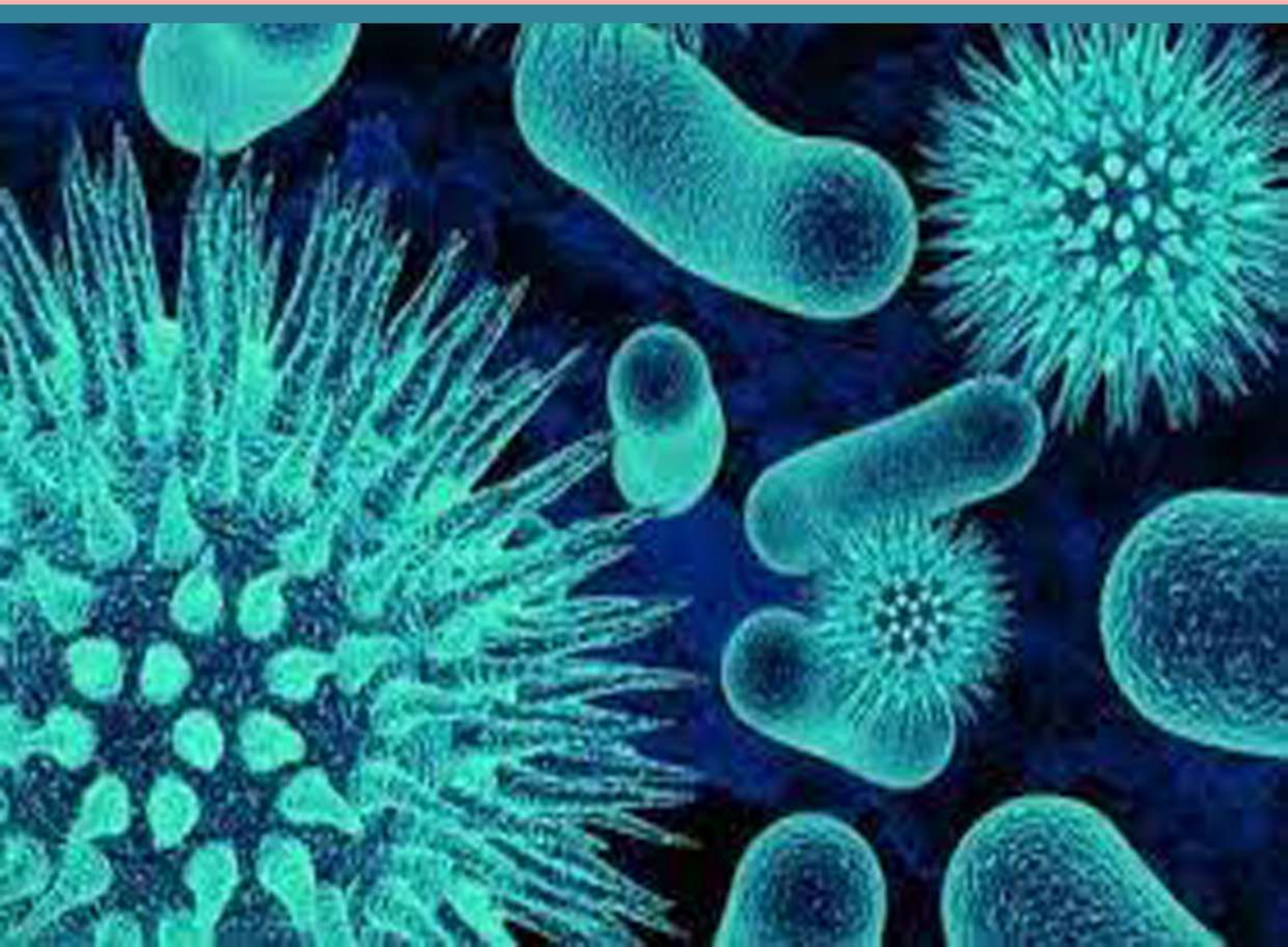

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Original Research

Determinants of Canine Rabies in Morocco: How to Make Pertinent Deductions for Control?

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ABSTRACT

Objective
This case control study aims to highlight risk factors to contract canine rabies, taking into account several factors like geographical affiliation, sex, age, season, socio-economic and demographic characteristics of the environment in which the canine population evolves.

Design
Case-control study at national level in Morocco included non-randomized comparison groups. This approach was chosen to address practical considerations and the data used correspond to a five years period. A group of 215 cases and 215 controls were needed for an odds ratio (OR) of 2.2 and the sample size was calculated for a 1:1 match, with a power of 80% and a risk of the first species at 5% (95% confidence interval).

Setting
Data collection is retrospective and the collection of information on the exposure of cases and controls was conducted in the same way.

Subjects
Target population was the general canine population of Morocco. Criteria related to age group (puppies, young, adult) and gender (female, male) have been considered in the selection of the target population.

Results
The Moroccan rural context characterized by a lack of basic infrastructures (education, health) makes that there are practices and cultural habits that favor the endemicity of rabies in some regions. Thus, the disease is associated with the presence of rural slaughterhouses and animal markets and landfills which appears offering unlimited food sources for stray dogs and then indirectly promotes their reproduction accordingly.

Conclusion
These findings support advocacy efforts for strengthening a global prevention system. In addition, socio-ecological surveys should be carried out to build understanding in depth for a better adaptation of the ongoing prophylactic programs.

Keywords
Rabies; Dogs; Case control study; Risk factors; Rural habitat; Municipal development index; Rural slaughterhouses; Weekly livestock markets.

Abbreviations
CDI: Communal Development Index; CHB: Communal Hygiene Bureau; LRAR: Regional Laboratory of Analysis and Research.

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INTRODUCTION

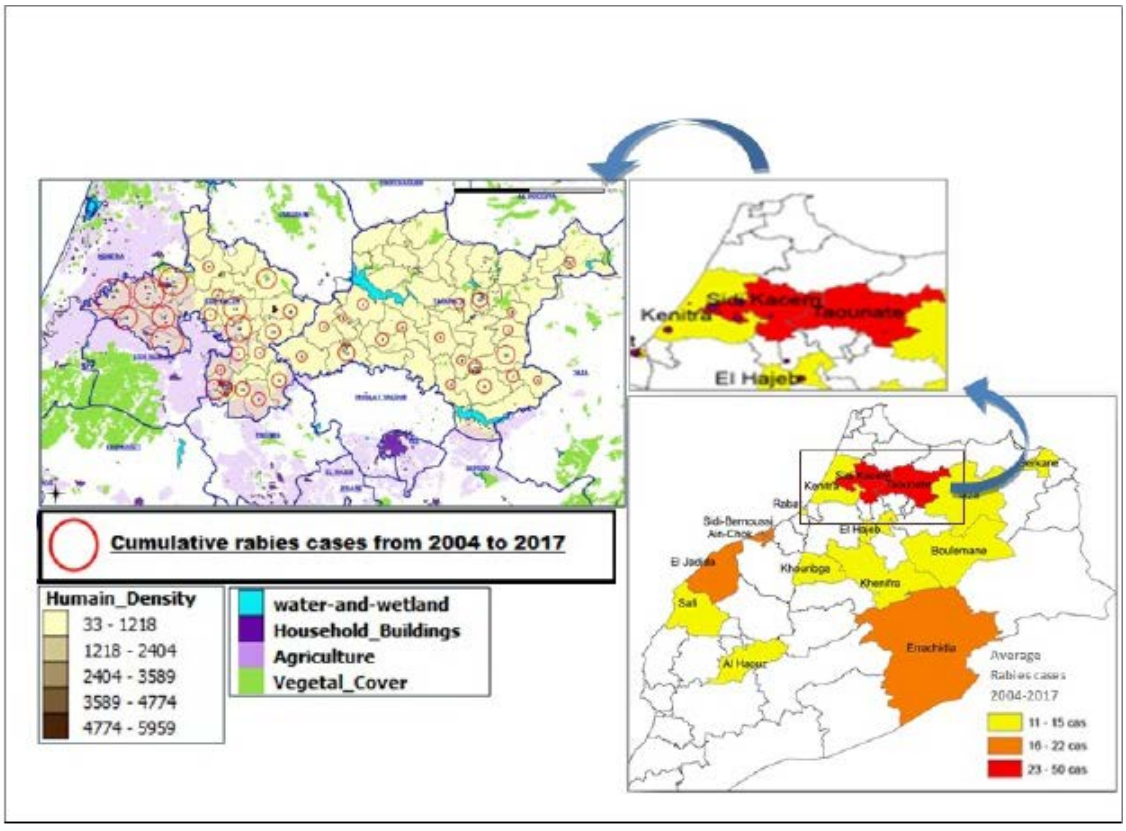
Rabies is a fatal viral infection that can infect all mammals, but domestic dogs cause over 99% of all human deaths from rabies among the world,[1] so the vast majority of human cases (>90%) result from the bites of rabid domestic dogs.[2] Although rabies can infect and be transmitted by a wide range of mammals, reservoirs comprise only mammalian species within the orders Carnivora (e.g. dogs, raccoons, skunks, foxes, jackals) and Chiroptera (bats). After a century of rabies control, Morocco registered an average of 301 animal cases and 21 human cases annually for the last decade (2005-2015).[3]

The reporting of the National Epidemiology Service[4] indicates that animal rabies is spread over almost the entire Morrocan territory and has been enzootic for several decades, all provinces are affected, but to varying degrees from 50 to a few cases per year with highest cases number in rich rural areas and around cities. It is unevenly distributed but in any case the dog is the vehicle and the main reservoir of the virus. Since 1986 in Morocco, an average of 384 domestic animal rabies cases are reported annually (1986 to 2016).[4] It has a high prevalence in rural areas with a percentage of 80% of the declarations. As a result of this prevalence, rabies causes a major public health problem: an average of 22 cases of human rabies is reported each year during the same period both in rural and urban areas.[4]

The prophylaxis carried out for a few decades (since the eighties) have not been able to achieve the expected objectives and animal rabies continues to spread consequently. The strategy adopted was based on elimination of the stray dogs and the vaccination of 70% to 80% of dogs belonging to owners. For logistic reasons, those actions took place in five successive phases corresponding to different geographical areas from 1986 to 1992 and became widespread in all country's provinces from 1993. Public awareness, staff training technical assistance and the provision of appropriate facilities and equipment have been the necessary means to achieve the objectives. Aware of the importance of this disease, the Ministry of Agriculture in collaboration with the Ministries of Health and the interior, developed a new strategy that was launched in 2003 in 2009 pilot areas which have been generalized in 2004. Despite several years of increased fighting, rabies continues enzootically in Morocco and causes a serious public health problem. The averages recorded each year remain high in relation to the efforts made in to control rabies. The success of any rabies control strategy is dependent on a good knowledge of the risk factors associated with the occurrence of the disease and the rigorous application of the proposed control measures and taking into account the epidemiological situation of the disease.[4]

From the foregoing, it appears that the descriptive study of the epidemiological situation of animal rabies in Morocco is characterized by very general indicators which therefore do not allow drawing up a very specific epidemiological analysis of the observed trends of the disease. Similarly, it should be noted that the geographical and temporal situation is very mixed in general.

Figure 1. *Geographic analysis of canine rabies cases by administrative levels (Provinces "Hot spots" and communes "clusters")*



From this descriptive spatial analysis, it appeared the fact that within the same province "hot spot", there is a tendency to observe-for several years-aggregations of canine rabies cases in some specific localities rather than others. From this finding that our assumptions was initiated on the conditions or determinants that make a dog more likely to inoculate the rabies virus in one specific place than in another like showed in Figure 1.

In parallel, the geographic analysis of the epidemiological data at the communal level made it possible to spatialize the communes which recorded each year more than two cases of animal rabies (Figure 1). These definitions of geographic «clusters» of animal rabies is the prelude of reflection on the determinant factors of canine rabies in Morocco and try to build understanding in depth on why the disease is more likely to be implanted in some given areas rather than others.

This led us to propose a case-control study aimed at highlighting risk factors for the canine population that predispose it to become enraged, according to geographical affiliation, season, sex, environment or age. Finally, we also studied the factors related to the socio-economic and demographic characteristics of the environment in which the canine population of the country evolves.

We attempt to describe rabies disease outside the classical purely sanitary paths and we have tried to introduce other explanatory factors (geographical and socio-economic) according to the fact that dogs-which are a reservoir of this disease-live according to a conditioned-based environment and rabies epidemiological trends are mainly influenced by the communities' behaviors in Morocco.

This study is the first conducted in Morocco for a sustained scrutiny on rabies analytic knowledge of the disease. The upshot is to employ statistics procedures for robust outputs, both to verify assumptions and attempt to make pertinent deductions on the reasons for failures of multiple national control plans during de last decades. The gathered data will help to build a new strategy with a focus on a "One Health" approach.[3]

This study is the first to clarify the risk factors for rabies in Morocco with the aim of improving prevention and adapting disease control recommendations based so far on vaccination measures and culling stray dogs.

METHODS

Choice of Method

The criteria that guided the choice for a case-control survey focused on the characteristics of the disease and the retrospective survey describing its annual variability. In general, the annual incidence of rabies in Morocco shows fluctuations that would be attributed, on one hand, to the cyclical phenomenon of rabies: period of recrudescence and lull, and on the other hand to the status of the different components of the national rabies control plan, including medical and control measures. Within these annual fluctuations, there are seasonal variations closely related to the cycle of reproduction of the canine species coinciding with the fall and spring period of the female's sexual activity. Indeed, the periods of recrudescence and calm observed of rabies trends explain the peaks observed between February-May and August-January. Given the difficulties in obtaining information on the numbers of the canine population and its structure, and moreover due to a possible spatial autocorrelation the case-control approach seemed a better possibility of quantifying the importance various risk factors for dog exposure to rabies virus. The characteristics of the determining factors studied (nature, number and frequency) but also logistical considerations in terms of cost and time lead us to propose the study plan below.

This case-control study included non-randomized comparison groups. This approach was chosen to address practical considerations (in terms of time and availability of retrospective data).

Retrospective studies are often referred to as "experimental batch/control group studies." The normal procedure here is to retrieve records of observed disease events in a population, to determine the presence or absence of the factor. The experimental batch can then be compared to a group of healthy individuals in which the frequency of the determining factor has been determined.[5]

It should be noted that the studies on experimental batch/control lot, in fact boil down to a comparison of the frequency of the determining factor in the two groups: that of the sick animals (cases) and that of the uninfected animals (controls).[5]

Type of Study and Period

There are several types of explanatory epidemiology surveys, but they all have the same objective and the same principles: the objective is to test the hypothesis of a causal relationship between exposure to a risk factor and an illness. The principle is to compare groups of subjects with different characteristics on two levels, the disease and the risk factor studied.[6] Compared with prospective studies, case-control studies have various advantages. The main reason is that the required data are already available, which saves time. There is also the fact that they relate to individuals already identified, which is in fact a privileged instrument for the study of low incidence diseases.[5]

The presence of two groups, both sick and non-sick or exposed and unexposed, is essential in order to draw an explanatory conclusion.[6] It is impossible to attribute a causal role to a factor by working only on a sick population, that is to say by not having witnesses. Indeed, a given factor can be frequently encountered in a healthy population, one cannot draw any conclusions of causality. This same factor can be also frequently encountered in the healthy population and therefore nothing can be concluded without this information in healthy subjects.[6]

Our case-control study included non-randomized comparison groups. This approach was chosen to address practical considerations (in terms of time and availability of retrospective

data) and the data used correspond to a five years period.

Population

The target population was the general canine population of Morocco. The population source is defined as any dog undergone with a laboratory diagnosis or veterinary observation for rabies-related reasons during the five years period from different regions.

The sample size was calculated for a case-control survey for a 1:1 match, with a power of 80% and a risk of the first species at 5% (95% confidence interval). To highlight an odds ratio (OR) of 2.2.

Sample Size

The sample size was calculated for a case-control survey for a 1:1 match, with a power of 80% and a risk of the first species at 5% (95% confidence interval). To highlight an OR of 2.2, a staff of 215 cases and 215 controls were needed.

Case Definition

The definition used for the case (rabid dog) was mainly based on laboratory diagnosis (biological tests and/or clinical criteria issued from 2007 regional laboratories in the whole country. In the study, only dogs that were diagnosed as rabies in the period (2003-2008: period of the pilot control plan) were included as cases. The validity of the choice of cases in this study is based on a definition that includes laboratory criteria associated with suggestive-based symptoms of rabies (based on the laboratory submission form).

We have accepted that the use of registers of regional veterinary laboratories will not ensure (even if they are exhaustive) representativeness of selected cases in relation to the general population (regional laboratory records generally only identified owned dogs whose heads or cadavers have been transferred to laboratories by official veterinary services. For stray dogs, the fatal outcome happen usually in the wild with loss of information and we cannot advance an estimate of this undiagnosed proportion accordingly)

- For a "certain case": dog for which the result of laboratory diagnosis was positive
- For a "probable case": dead dog with suggestive clinical signs of rabies

Excluded cases were those for which veterinary laboratory registrations or veterinary records were incomplete.

The cases are recruited in 53 different provinces of Morocco: Among the 817 confirmed cases of rabies during the last five years: 324 cases were excluded from the study due to lack of information on (commune) locality, age and sex. Five hundred and seven cases were included in the case-control study.

All 507 eligible cases were included in the descriptive study, an inclusion rate of 100%. The majority of cases were males (76%) and the median age was 18 months (between 1.5 months and 168 months).

Two hundred and forty-eight eligible cases (49%) were from urban areas and 259 cases were recruited from rural areas.

The different geographical areas listed in table below were divided into 3 groups: arid zone (11%), plain zone (69%) and mountain area (20%).

Sixty-seven percent of the cases are recorded during the estrous season of the bitch. Rutting period during which, there is a crowd of dogs that predisposes to contamination between individuals (proximity, fights). Only 33% of cases are reported outside this period.

Selection of Control Group

The constitution of the control group is a key element in this survey because of the risk of selection bias. The definition of the controls was also based on biological criteria revealed by the laboratory or clinics. Thus our control group is constituted from the dogs whose result of laboratory or a setting in veterinary observation reveal a negative result. Our control group consists of dogs listed from the registers of the laboratories or official veterinary services by respecting the fact that those animals must come from the same general dog population of the cases group.

The control group was matched for each case on the following criteria:

- The administrative unit (same province)
- Age according to the following age ranges (<1 year, 1 to 3 years, >3 years)
- The time unit (same quarter): given the seasonal variations of rabies reporting as indicated in the bibliographic section.

The selection of the control group was made from the lists of veterinary laboratories. The first two controls on the veterinary laboratory list where the case was diagnosed positive, meeting the matching criteria, were included. If none of the dogs on this list met all the criteria, the control group was searched successively in the registers of the veterinary service which covers the nearest area of case origin then failing in the lists of veterinary inspections.

Data Collection

Data collected was from each study dog was: i) Province of origin, diagnosis period (clinical or laboratory), sex, age, laboratory results or veterinary observation; ii) socio-demographic information: number of human population by province and commune, area of each commune, density of human population per km^2, communal development index (CDI); and iii) information on communal infrastructure: presence of slaughterhouses, landfills, municipal hygiene bureau, impoundment, weekly rural markets, and distance to an asphalt track in a commune.

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As in our study, the collection of data is retrospective and the collection of information on the exposure of cases and controls was conducted in the same way, data on the following factors was not possible to obtain: Canine population density, the erratic fringe of dogs compared to the population of dogs owned, vaccine status of dogs (absence of records).

We are referred to data from national animal disease surveillance system (for health data which are compiled regularly from the local veterinary services *via* SIPS's information system). Also, we used data from the planning authority (HCP) that provided us with the data on socio-economic factors (source: national census).

Data Entry

The data has been entered on access tables and their processing is performed on the EPIINFO 3.5.1 software.

Data Analysis Method

Factorial methods establish synthetic representations of large data tables, usually in the form of graphical representations. The purpose of these methods is to reduce the dimensions of the data tables to represent associations between individuals and between variables in small spaces. In this study, this analysis made it possible to guide the choice of exposure to the different risk factors tested.

The complete data analysis must include an univariate analysis and then a multivariate analysis. Univariate analysis consists of evaluating the gross relationship between rabies and each of the exposure factors, without adjusting for potential confounding factors.

Relative risk was estimated by the matched odds ratio (OR). The proportions were compared by chi2 test or Ficher's exact test.

The analysis was performed using the Microsoft Office Access software and EPIINFO version 3.5.1.

RESULTS

Description of Cases

The cases are recruited in 53 different provinces from all official regional laboratories and veterinary services registrations (National Office of Food Safety, ONSSA, Rabat, Morocco). Private veterinarians are also enrolled in this surveillance database by providing 13% of cases. We identified 831 cases for which rabies diagnosis (clinical and tests confirmed) was made between 2003 and 2008; 817 (98%) were confirmed cases and 14 (2%) were probable cases. Nearly half of the cases (54%) were diagnosed between December and April of this period.

Among the 817 confirmed cases of rabies: 324 cases were excluded from the study due to lack of information on (commune) locality, age and sex. Five hundred and seven (confirmed and probable) cases were included in the case-control study.

All 507 eligible cases were included in the descriptive study, an inclusion rate of 100%. The majority of cases were males (76%) and the median age was 18 months (between 1.5 months and 168 months) (Table 1A).

Table 1A. *Cases Distribution by Age*

Age Group	Males		Females		Total	
	N	(%)	N	(%)	N	(%)
<1 Year	135	(35)	43	(35)	178	(35)
1-3 Years	149	(39)	52	(42)	201	(40)
>3 Years	100	(26)	28	(23)	128	(25)
Total	384	(100)	123	(100)	507	(100)

A total of 248 eligible cases (49%) were from urban areas and 259 cases were recruited from rural areas. Distribution by habitat shows that 51% of cases come from rural areas and 49% from urban areas. The different geographical areas were divided into 3 groups: arid zone (11%), plain zone (69%) and mountain area (20%) (Table 1B).

Table 1B. *Casess Distribution by Geographica Areas*

Habitat	Nbr of Cases	(%)
Urban	248	(49)
Rural	259	(51)
Total	507	(100)

A proportion of 67% of cases are recorded during the estralous season of the female dogs. Rutting period during which, there is a crowd of dogs that predisposes to individual contamination between (proximity, fights). Only 33% of cases are reported outside this period (Table 1C).

Table 1C. *Cases Distribution by Habitat*

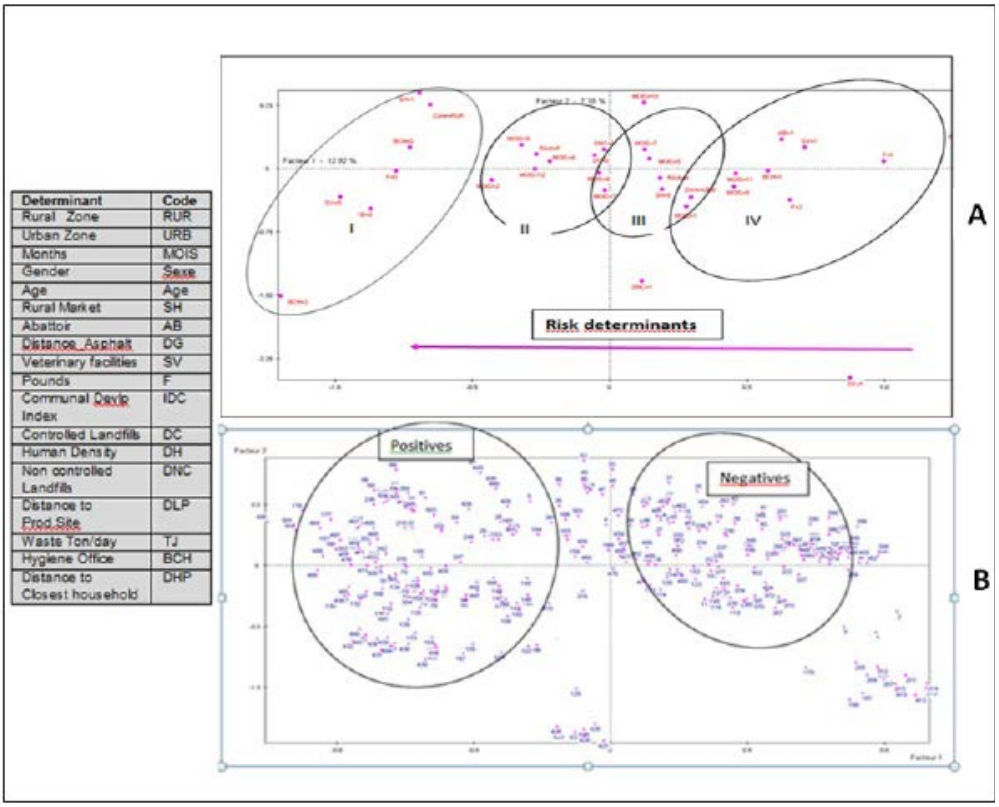
Geographic Areas	Nbr of Cases	(%)
Desert zone	53	(11)
Plain zone	349	(69)
Montanious zone	105	(20)
Total	507	(100)

Result of the Case-Control Analysis

Factorial methods establish synthetic representations of large data tables, usually in the form of graphical representations. The purpose of these methods is to reduce the dimensions of the data tables to represent associations between individuals and between variables in small spaces. In this study, this analysis made it possible to guide the choice of exposure to the different risk factors tested.

The two axes of the domain (factor 1 and factor 2) depend on the projection model used. In our study, the choice is made on factorial axis 2, which best explains the direction of exposure between cases and controls and the determining factors (Figure 2). Thus, we observed that it is more likely to have cases if one operates in an environment characterized by the absence of health and medical management structures (veterinary services,

Figure 2. *Projection of Factors (A) and Eligible Cases-Controls (B) on the Factorial Axes*



BCH, pound), belonging to the rural environment (Group I) and corresponding to a given season (Group II).

On the opposite side, we note that the controls group belongs much more to an environment with presence of veterinary structures and a well management of the wandering fringe of the canine population. This environment is in a generally developed in urban areas (absence of rural markets and dumps) and corresponding to a season which is outside the estrus period (Groups III and IV).

Comparison according to the intrinsic characteristics of dogs: Rabies is a disease that would be contracted regardless of the range age of dogs. There was no evidence of an association between a specific age group and the occurrence of rabies in dogs.

There was no evidence of a link between the sex of the dog (male/female) and the occurrence of rabies (OR=0.99 [95% CI: 0.65-1.51]). However, in rural areas, female dogs appear to be more prone to rabies than males in the same medium (OR=1.2) (but this risk was not statistically significant ([95% CI 0.51 -2.82], p=0.20). The estrus period, which favors dog gatherings in comparison with the anestrus period, seems to predispose dogs to the risk of contracting rabies (OR=1.42, p=0.04), but not significantly since the confidence interval contains 1 (95% CI 0.99-2.04).

Comparison according to spatial characteristics: The geographic area including a mountainous area is associated with rabies occurrence in dogs (OR=1.99 [95% CI: 1.02-3.91]). A dog's membership in the rural setting is associated with the occurrence of rabies (OR=1.92 [95% CI: 1.30-2.84]).

Comparison according to socio-economic aspects: Rabies Cases are notified by the veterinary services and are reported in the databases in relation to municipalities. Our purpose has been to evaluate the infrastructure of these communities and see for instance, if the presence of uncontrolled landfills or rural killings makes it more likely that dogs living in these communities will have rabies.

The risk is conceived for all the territory of the commune because the dogs move on all the geographical space which allows them the survival. Dogs are not static and do not have a fixed address in their geographical space. They move for their foods: one time on dump spots, another time in a rural slaughter or at any other food sources.

To evaluate the socio-economic aspects., we used the municipal development index (MDI) calculated from the data of the Office of the high commissioner for planning (HCP) and measured by three elements from the results of the national general census (GG) of September 2004 (Morocco). This is a composite index comprising three elements that report on the budgetary resources of municipalities, the state of equipment and collective infrastructure, and their level of access to basic public services (water, electricity and road network).

The interactive data corporation (IDC) is the result of the simple arithmetic mean of the rates of the three components.

Any weighting of the latter cannot be the same for all municipalities. Dogs in a territory with an IDC<35% are highly susceptible to rabies (OR=3.90 [95% CI: 1.47-10.72]). For dogs belonging to a communal area with an IDC between 35% and 70%, the association between this factor and the risk of occurrence of the disease is not statistically significant (p=0.06).

The presence of a weekly livestock market is associated with the occurrence of rabies (OR=1.95 [95% CI: 1.25-3.05], Table 2). However, this association between the souk and the disease is more common in urban areas than in rural areas even though the confidence interval contains 1 (OR=1.87 [95% CI: 0.93-3.77] p=0.05).

Table 2. *Risk and Protective Factors for Canine Rabies*

Risk Factors	Cases	(%)	ORmatched	IC95 %	*p* Value
Mountain	24	(11)	1.99	1.30-2.84	0.02
Rural Habitat	82	(38)	1.92	1.30-2.84	<0.001
IDC<35%	16	(7)	3.90	1.47-10.72	0.006
Rural Markets	59	(28)	1.95	1.25-3.05	0.001
Slaughterhouse	40	(35)	1.92	1.12-3.29	0.01
Distance/ Asphalt	70	(33)	1.76	1.17-2.65	0.004
Distance (rural)	64	(78)	4.40	2.27-8.59	<0.001
Distance (Plain)	62	(31)	1.82	1.18-2.80	0.004
N.controled Landfills	42	(20)	1.70	1.03-2.81	0.02
Not closured Landfills	16	(76)	4.27	1.16-16.49	0.01
Daily waste<100t/d	22	(76)	4.19	1.32-13.76	0.006
Distance/production	12	(46)	4.63	1.18-19.11	0.01
Low H.Density	42	(20)	2.05	1.22-3.44	0.003
Densité/IDC	17	(9)	4.71	1.71-13.65	0.03
Protective Factors	**Cases**	**(%)**	**ORmatched**	**IC95 %**	***p* Value**
Abattoir (IDC>70%)	86	(56)	0.63	0.41-0.96	0.02
Veterinary facilities	114	(53)	0.64	0.44-0.93	0.01
Pounds	81	(38)	0.57	0.39-0.82	0.001
Hygiene Office	124	(58)	0.65	0.44-0.94	0.01

Weekly markets are defined as markets for livestock which opens one day per week. The link with rabies problem is due to the fact that on the spot of these markets are organized activities of restoration and butchery (grills). For dogs, it's an ideal place to find food resources and therefore to gather dogs (where they fight for food and thus the possibility of rabies contamination between them). We have included livestock markets as factor to study because we have observed that communities hosting such kind of facilities are often subject to the presence of several cases of rabies (reported by local veterinary services).

The presence of a slaughterhouse appears to be protective against the occurrence of rabies (OR=0.67 [95% CI: 0.47-0.97] p=0.027). Slaughterhouses have been classified according to their condition into two types: municipal slaughterhouses (satisfactory sanitary state of the establishment) and killings (generally deplorable sanitary state of the establishment). It appears from this classification that rabies is significantly associated with the presence of slaughter (OR=1.92 [95% CI: 1.12-3.29] p=0.01).

The difference between the municipal abattoir and the rural killings is the volume of the meat preparation and processing activities. In general, the municipal abattoir that serves the urban population is by far the most functioning structure.

The second important point is that the municipal slaughterhouse is a structure that is managed by a technical service and that responds to a construction plan that meets sanitary standards (Fences around the facilities to prevent the introduction of dogs, cats and rodents, presence of a room specifically dedicated to the seizure of carcasses and offal which are unfit for consumption, presence of incinerator to burn the seizures and also septic zone for the burial of the remains). In this way, the municipal slaughterhouse is designed and operated in such a way that it does not allow stray dogs' access to food resources and therefore cannot contribute to their survival and reproduction.

Rural killing, however, is quite the opposite: no fence, mismanagement, unqualified staff, no standing operating procedure (SOP's) or procedures for denaturing seizures of carcasses and offal that are generally thrown out and are therefore available for feeding stray dogs.

For all these reasons that we have found that the presence of a rural killing in a commune is a predisposing factor for the presence of rabies in this place and therefore a high risk of rabies for the humans and the animals belonging to this area.

Considering that the level of clinical documentation improvement (CDI) can have a strong significance for the occurrence of rabies, the study of the exposure to the slaughterhouse factor according to the IDC did not allow to reveal an associa-

tion between the slaughterhouse determinant factor and disease (OR=2.93 [95% CI: 0.34-27.68] $p1$=0.23); (OR=0.78 [95% CI: 0.32-1.93] $p2$=0.56). The presence of a slaughter establishment at a communal area with an IDC>70% seems to allow protection against rabies (OR=0.63 [95% CI: 0.41-0.96] p=0.02).

The presence in the communal area of public veterinary services is considered a protective factor against the risk of occurrence of rabies (OR = 0.64 [95% CI: 0.44-0.93], p=0.01).

The existence of an important distance separating the communal periphery from an asphalt track is associated with rabies (OR=1.76 [95% CI: 1.77-2.65], p=0.004). This finding is strongly associated with the risk of rabies occurring in rural areas (OR=4.40 [95% CI: 2.27-8.59]), Table 3) and a geographical area mostly represented by plains (OR=1.82 [95% CI: 1.18-2.80]).

Table 3. *Results of the Modeling of Risk Factors Associated with a Canine Rabies*

	ORMat	P	OR	p
Weekly Rural Markets	1.95	0.001	7.50	0.006
Rural Habitat	1.92	<0.001	9.07	0.003
Human Density	2.05	0.003	8.76	0.003
Slaughterhouse	1.92	0.01	2.58	0.04

Distance has been proposed in order to see the accessibility of veterinary teams to these communes during vaccination campaigns of dog-to-owner. Municipalities that are far from a road or paved track are with a difficult access for veterinary teams and therefore dogs in this area are less likely to be vaccinated and protect against rabies. We agreed with the HCP experts to define two range: less than 5 km and greater than 5 km.

Pounds are defined as public facilities where abandoned dogs are temporarily kept until a fine is paid by the owner. The presence of municipal pounds is a protective factor against the risk of occurrence of the disease (OR=0.50 [95% CI: 0.34-0.75], $p1$=0.0004). A statistically significant association between the occurrence of rabies and the number of existing pounds in the communal area ($p2$=0.7 and $p3$=0.24) was not found.

Communal Hygiene Bureau (Hygiene communal office) which is a public facility in charge of common space management in terms of the fight against pests, capture of stray dogs, medical care of bitten people by feral dogs and the management of places where we keep stray dogs. The presence of Communal Hygiene Bureau (Hygiene communal office) is also demonstrated as a protective factor against rabies (OR=0.65 [95% CI: 0.44-0.94], p=0.01). Controlled landfills tend to be considered protective factors against the risk of rabies occurring. However, this association is not statistically significant since the numbers were very small (OR = 0.62 [95% CI: 0.26-1.47], p=0.24).

On the other hand, the presence of an uncontrolled garbage discharge increases the risk of contracting rabies in dogs belonging to a communal area containing this type of landfill (OR=1.70 [95% CI: 1.03-2 , 81], p=0.02).

The absence of closure for uncontrolled landfills appears to be the most important risk factor in the occurrence of rabies (OR=4.27 [95% CI: 1.16-16.49], p=0.01). Landfills with a daily tonnage of less than 100 T/day seem to be more associated with the occurrence of the disease than landfills with a large storage capacity (OR=4.19, 95% CI: 1.32-13), 76], p=0.006). The origin of this waste is generally provided by houses located around these sites-mainly on the outskirts of urban agglomerations - which explains why the existence of a reduced distance (less than 5 km) separating the landfill compared to production sites is more associated with the occurrence of the disease in dogs from this communal area (OR=4.63 [95% CI: 1.18-19.11], p=0.01).

Socio-Demography of the Environment

Human density: Low human density is considered associated with the occurrence of rabies (OR=2.05 [95% CI: 1.22-3.44], p=0.003) which confirms the rural character of rabies canine in Morocco.

Human density and CDI: Taking into account the human density factor according to the IDC, shows a strong association between a low density and the occurrence of rabies in a communal area with an IDC>50% (OR=4.71 [95% CI: 1.71-13.65], $p1$ (Ficher exact)=0.57 and $p2$= 0.03), which is particularly characteristic of human agglomerations at the edges of cities or the so-called semi-urban environment.

From the Univariate analysis of this study, several risk factors that contribute to the occurrence of rabies have been identified. In general, the occurrence of rabies remains globally dependent on the level of development of the environment as well as the state of the hygienic and sanitary infrastructures. Protective factors indicate a certain degree of availability in terms of health structures for management and the fight against the stray dog population, as well as the increased interest in upgrades to hygienic establishments of public interest (slaughterhouses). All those factors are summarized in summary Table2.

Multivariated Analysis

Multiple regressions are complicated by the presence of multicollinearity. Indeed, this study, like the majority of similar studies, involves explanatory variables that are correlated. A simple method for detecting too much correlation between independent variables is to make collinearity tests.

We investigate whether these risk factors influence the likelihood of rabies occurring. We want to determine, among these explanatory variables, the one that best explains this condition of occurrence. A multiple linear regression is realized and we notice that among the variables introduced: the variable distance with respect to the tar, uncontrolled discharges and IDC were particularly affected by this problem and do not contribute significantly to the regression as they present bad collinearity statistics (tolerance and VIF are far from 1 and outside the recommended limits (tolerance>0.3 and VIF<3.3) and therefore are highly correlated with each other. Only 4 of the 12 variables included in multi-varietal

modeling were independently associated with rabies: Weekly Rural Markets, Rural Habitat, Human Density, and Killing (Table 3).

DISCUSSION

Canine rabies was considered a major zoonosis studied as a priority in terms of public health in Morocco and actions were proposed in the areas of prevention, control and information for doctors, veterinarians and the general public. To our knowledge, this study is the first to specify the risk factors for dog exposure to rabies in Morocco with the aim of improving prevention and adapting the disease control recommendations so far based on measures of vaccination of dogs to owners and slaughter of stray dogs.

Of the exposures independently investigated for possible association with rabies and with the exception of the estrous season which favors dog assemblages predisposing to rabies contamination, none was an exclusively intrinsic exposure (age groups, sex). This situation allows us to conclude that the age group most prone to develop rabies, is represented practically by all the dogs having more than one year that they are of male sex or female. The fact that we have not found here an association between intrinsic factors and rabies in Morocco could be interpreted by the fact that there is an under declaration of cases of canine rabies as it has been recorded in recent years in favor of cases of ruminant rabies, to which can be added the cases of canine rabies stray dogs whose fatal outcome is usually in the wild and which in turn escape the current health monitoring system.

The close relationship between the density of the human population and that of the dog population is also highlighted as a risk factor, because the denser the human population, the denser the dog population too and the higher the risk of exposure to rabies.[3] The incidence of canine rabies in Morocco varies according to the geographical area and habitat environment. As has been highlighted in epidemiological studies and in our study, canine rabies is most often reported in rural areas. Canine rabies remains linked in Morocco to an essentially rural way of life of the population, which does not support the constraints of prophylaxis.[7] This situation is due to the existence of a large canine population that is inadequately vaccinated and whose movements are uncontrolled. For these reasons, especially in rural areas, this canine population has long been mistaken for a wandering population. They are in fact dogs owned by owners and subject to estimation.[8]

It is particularly prevalent in low-density urban areas and also in communal areas with a relatively satisfactory level of equipment, which confirms both the rural character of this disease highlighted by previous epidemiological studies and existence of new endemic areas of the disease especially around the urban centers. This situation is due to the fact that female dogs without landowners do not reproduce much and are not able to breed puppies.[9] These semi-urban agglomerations are generally the result of the rural exodus to the cities and thus preserve all the sociocultural practices related to the dog in rural areas. Stray dogs generally appear to be more numerous than they actually are both in urban and rural areas.[10,11] It seems that stray dogs can not constitute stable populations. The duration of the wandering period seems to correspond to a survival time.[12] This finding is reinforced by the study of the exposure linked to communal facilities such as weekly rural markets and uncontrolled landfills, which by their locations; generally on the outskirts of urban centers, offer inexhaustible food resources for this fringe of dogs. In the absence of existing data on the state of communal infrastructures that may be related to the risk of developing rabies. We have opted for a CDI. This approach seemed to us the most appropriate to evaluate, at the communal level, the situation of equipment and infrastructures (slaughterhouse, slaughter, furrow, controlled dumps, and communal hygiene office).

In this study, it was found that the presence of structures providing veterinary services (particularly focused on vaccination), management of the canine population in terms of collection and culling of stray dogs (pounds and Hygiene Office) constitutes a guarantee for the communal space against the risks of occurrence of rabies. The absence of these facilities at the level of a given communal area can lead to a decrease in the epidemiological observation thanks to an under-declaration at the level of the monitoring device, as well as a decrease in the level of realization of sanitary prophylaxis measures (vaccination and culling stray dogs). Epidemiological surveillance makes it possible to judge the effectiveness of the measures taken.[13,14] It also appears from this study that the presence of a huge distance separating the communal area from a practicable track limits the accessibility of vaccination, collection and slaughter teams to this place and is therefore a factor risk for rabies.

The study also concerned infrastructures that are presupposed to be indicators of rabies-related exposures, either directly (presence, absence), or through the relative variable of the level of development of the communal space in which these structures are located. Slaughterhouse exposure analysis indicates that the type of these establishments is a determinant of risk. Poorly equipped rural slaughterhouses (or slaughterhouses) (lack of fencing, lack of seizure management or denaturation pits) provide dogs with an easily accessible and sustainable food resource given the frequency of slaughter at these establishments. On the other hand, a high CDI regardless of the type of slaughterhouse (Urban slaughterhouse or Abattoir) is a guarantee against the occurrence of rabies. The index reflects the state of equipment and maintenance of the slaughter establishment, which is characterized by the presence of fencing around the establishment which limits the accessibility of the dogs inside the premises. Specific observations show that dogs without owners settle where the quantity and accessibility of the main resources are sufficient both in urban areas[9] and in rural areas.[15]

Weekly rural markets are also a risk factor for rabies because of the fact that these places are involved in slaughtering and marketing of carnivals, which are generally carried out in uncontrolled establishments, which, like killings subsistence resources for dogs. This situation is personalized by a state of rurality adjacent to the city and characterized by the abundance of stray dogs that provide dog populations that can be recruited as rabid in urban centers.

Indeed, unsupervised dogs looking for food are direct competitors of stray dogs.[15] They are not subject to any prophylaxis. They intervene in the transmission of infectious agents responsible for zoonoses such as rabies and hydatidosis.[16] Their promiscuity with humans and domestic animals makes them particularly dangerous.

The study of exposure to landfills that meet international design standards, storage and handling garbage is a protective factor in the risk of rabies occurrence. Uncontrolled and generally unfenced landfills appear to be particularly pre-disposing at the risk of contracting the disease. In urban areas, for example, the home range always has "feeding areas", usually made up of piles of garbage.[9] In rural areas, stray dogs settle around garbage dumps, groups of fixed and open bins. They seem to be forced to avoid their direct competitors of food: stray-owner dogs.[15]

In this study, it was shown that the risk of rabies is greater if the distance between the landfill and the garbage production sites is less than 5 km. This is particularly true of the semi-urban environment. urban area, which hosts most garbage dumpsites and whose resident human population does not benefit from a public waste management service, it seems obvious that the destination of most of the household waste produced in these conurbations made to these sites. This provides accessible and sufficient food resources for dogs belonging to these peri-urban agglomerations. In fact, the poorly guarded dog has a shelter in the home of his master but must obtain some or all of his food by his own means and has a refuge to raise the young.[17] The diet consists mainly of various kitchen waste recovered from residents or dumps.[9,17]

Like most zoonoses, rabies is often underdiagnosed, particularly in underprivileged areas, a state of affairs that reflects the limited capacity and coverage of health services. On the one hand, the subdiagnosis of zoonoses is further aggravated by the unequal geographical distribution of these conditions as well as by the difficulties inherent in the diagnosis of some of them.[18]

CONCLUSION

To limit the incidence of rabies, defined as a major zoonosis in Morocco, the prevention system must be strengthened. The results of this study revealed a shift in risk factors towards slaughter infrastructure, weekly rural markets and road networks.

It is also recommended to review the current regulations on the acquisition of dogs in rural and semi-urban areas to make owners aware of the obligation to keep their dogs tied at the farm level, to prohibit the release of puppies in the wild from numerous litters and to make the acquisition of sanitary documents mandatory for every dog in the household.

In such an epidemiological and socio-economic context, continuous and regular medical prophylaxis by the veterinary services existing in rural areas must be able to stop the deterioration of the epidemiological situation. A culling policy is less likely to interrupt transmission (particularly for owned dogs) as compared to a mass-vaccination strategy, and socially not acceptable. A much higher transmission rate, as suggested above, would affect the threshold coverage of vaccination. Among the compared intervention strategies, mass vaccination of 70% of the dog population is the most profitable and cost-effective intervention, sufficient to interrupt rabies virus transmission for at least 6 years.[19]

Socio-ecological surveys in different regions should allow a better estimate of the dog populations to be vaccinated and the adaptation of ongoing prophylactic programs.

All these measures, combined with a more rigorous application of the legal policy, should allow a significant improvement in the epidemiological situation of canine rabies in Morocco.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Compliance with Tobacco Control Policies and Global Youth Tobacco Survey: A Cross-Sectional Comparison between GYTS 2000 and 2015 in Maharashtra, India

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ABSTRACT

Background
Tobacco is the leading avertable cause of death in the world. Adolescents are the most vulnerable population to initiate the use of tobacco. Most adult users start tobacco use in their childhood or adolescence. The Global Youth Tobacco Survey (GYTS) was design to obtain information on tobacco use, exposure to tobacco smoke and other related indicators among adolescent. This study aims to compare the GYTS data between 2000 and 2015 for the state of Maharashtra in India.

Methods
The GYTS is a school-based two-stage cluster design survey of students aged 13-15 years. This was a self-administered cross-sectional survey conducted in Maharashtra using standardized GYTS questionnaire. To record student survey responses, in 2000 optically readable answer sheets and in 2015 tablets were used. For statistical data analysis SPSS 20.0 was used.

Results
Between 2000 and 2015 the prevalence of tobacco use reduced from 17% to 11% among boys and 14.5% to 8.5% among girls. Age of tobacco initiation has shifted from 11 years or younger (~70%) in 2000 to older than 11 years (~70%) in 2015. Proportion of students who received pocket money increased from 20.2% to 42.7% for boys and 18.7% to 33.8% for girls in 2015. Discussions about harmful effects of tobacco in schools and at home had reduced from 2000 to 2015. Distribution of free tobacco products decreased over the years, however sale of tobacco products to minors and sale around educational institutions continued.

Conclusion
Prevalence of overall tobacco use has decreased. Strengthening the existing Cigarettes and Other Tobacco Products Act (COTPA) laws and improving implementation and effective monitoring will not only reduce youth's tobacco use but might restrict future initiation among youths. Continuous tobacco education at home and at school will further strengthen the tobacco control efforts.

Keywords
Tobacco use; Tobacco control; Policy compliance; Pocket money; Family and friends.

INTRODUCTION

Tobacco was introduced to India by the Portuguese ~ 400 years ago, rapidly grew as a part of socio cultural milieu in various communities. India currently is the second largest producer and consumer of tobacco in the world, after China.[1] Tobacco kills up to half of its users.[2] The tobacco epidemic has been one of the biggest public health threats to the world killing more than 7 million people a year. Of the 1.1 billion people who smoke globally about 182 million (16.6%) live in India.[3] According to the Global Adult Tobacco Survey (GATS) 48% males and 20% females in India use tobacco.[4] It is predicted that tobacco will account for about 13% of all deaths in India by 2020.[5]

The World Health Organization (WHO) Framework Convention on Tobacco Control (FCTC) is the world's first public

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health treaty on tobacco control. India has ratified FCTC in 2004. The WHO FCTC encourages countries to improve and implement action plans to include public policies, such as bans on direct and indirect advertising of tobacco, rise in tobacco tax and its' rates, promotion of smoke-free public places and workplaces and placing health related warning labels on tobacco packaging. The WHO FCTC also encourages countries to set up surveillance systems in order to explore the distribution of the magnitude, determinants, patterns and consequences of tobacco consumption and exposure to tobacco smoke warning labels on tobacco packaging.[6]

India is a major stakeholder in global tobacco control activities. The country has taken several initiatives for tobacco control including legislative measures, ratification of the WHO FCTC and implementation of the National Tobacco Control Programme. India enacted various tobacco control measures in response to growing evidence of harmful and hazardous effects of tobacco.[7] In order to strengthen the tobacco control activities in India, the Government of India passed the India Cigarettes and Other Tobacco Products Act (COTPA) in 2003. The COTPA included many tobacco control provisions like prohibition of smoking in public places, prohibition of direct or indirect advertising of cigarettes and other tobacco products on billboards and in all media excluding at the point of sale. It also included prohibition of sale of tobacco products to minors and sale within a radius of 100 yards of any educational institution. It also emphasized on mandatory display of pictorial health warnings on tobacco product packages.[8]

During 2005 a ban on display of tobacco products, restrictions on point-of-sale advertisements and ban on vending machines sale were introduced. During 2008-2009, Cigarettes and other Tobacco Products (Packaging and Labelling) Amendment Rules, were implemented to ensure the display of health message "tobacco causes cancer", along with one of the specified warning images for all tobacco packages (including cigarettes, cigars, cheroots, bidis, pipe, hookah, chewing tobacco, snuff, and paan masala) with text warning "SMOKING KILLS" for smoked tobacco products and "TOBACCO KILLS" for smokeless tobacco products respectively. Bidis taxed at 9% and cigarettes taxed at 38% of price. Ban on smoking in public places and hotels, restaurants and airports can have designated smoking areas introduced. Ban on sale of tobacco products near educational institutions introduced. In December 2010, new pictorial warnings mandated for all tobacco products and amended to the rotation period of two years for the specified health warnings. Ban on foreign direct investment for manufacturing tobacco products introduced. In 2011, round 2 of pictorial warnings was mandated for all tobacco packages four new pictorial warnings were specified for smoked and smokeless tobacco products. Ban on sale of tobacco products in plastic pouches introduced. Round 3 pictorial warnings were mandated for all tobacco packages in 2013. Three new pictorial warnings were specified for smoked and smokeless tobacco products. The word "WARNING" appeared in red font above the text of the health-warning message. A ban on sale, manufacture, and storage of gutka and pan masala introduced. In 2014 specific rates of excise duty increases on cigarettes in the range of 13% to 94%, excluding 'other' types of cigarettes which did not change between 2013 and 2014. Two cigarette price tires 'filter exceeding 75 but not exceeding 85' and 'other' merged into one tier 'other'. In April, 2015, round 4 of pictorial warnings were mandated for all tobacco packages. Pictorial warnings were required to cover 60% of the package and text warnings to cover 25% of the package. Excise duty increases on cigarettes, cigars, cheroots and cigarillos in the range of 17% to 29% introduced.

The Global Youth Tobacco Survey (GYTS) was developed as a part of the Tobacco Surveillance System (GTSS)[9] to monitor tobacco use among school going children aged 13-15 years using standard protocol so that results will be comparable with any geographic part of the country or the world or overtime in the same geographic location.

Adolescents is a vulnerable group, usually get exposed to tobacco and tobacco related habits very early. The age of initiation of tobacco related habit has a direct influence on the survival and related diseases in the individuals.[10] The current study to the best of our knowledge is the first attempt which compares GYTS in the state of Maharashtra conducted before (in 2000) and after (in 2015) COTPA implementation of various aspects related to tobacco, such as prevalence, knowledge, attitude, policy compliance in adolescent population. Study Procedures to conduct GYTS 2000 was approved by ethics committee of Tata Memorial Hospital and GYTS 2015 by ethics committee of Healis-Sekhsaria Institute for Public Health.

MATERIALS AND METHODS

GYTS a school-based survey of students in grades 8, 9 and 10 was administered according to the standard GYTS methodology.9 In 2000 the survey was self-administered using questionnaires on paper and responses were recorded on optically readable answer sheets. While in 2015, the survey was self-administered using electronic tablets. All government and government-aided schools (~11,000 in 2000 and 15,940 in 2015) in Maharashtra were included in the sampling frame. The number of students, enrolled in grades 8, 9 and 10 in each school were obtained from the Department of Education, Maharashtra. A two-stage cluster sample design was used. At the first stage, schools (51 in 2000 and 26 in 2015) were selected with probability proportional to enrollment size. At the second stage, from the selected schools classes were selected using pre-selected random numbers. All the students in the selected classes were eligible to participate, allowing for anonymous and voluntary participation of students present on the day of the survey (2256 in 2000 and 1610 in 2015). Both the surveys were self-administered in the classroom and took around 45 to 60 minutes, including introduction to the survey tools to the participants. All the selected schools received instructions from department of education prior to study field interviewer's visit to selected schools.

For India the core GYTS questionnaire was suitably adopted to include tobacco use in the forms of bidi smoking and smokeless tobacco use. Same set of questions were use in both surveys and questionnaires translated into Marathi. All questions

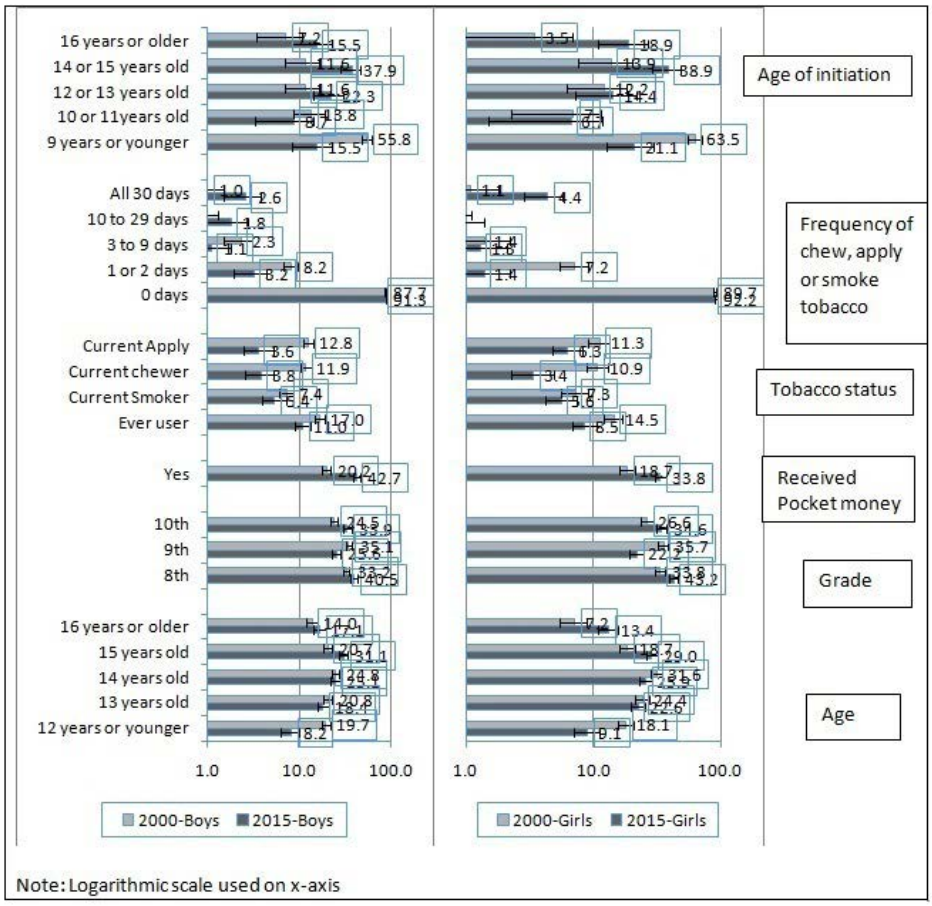
required answering (i.e. there was no skipping or branching pattern). The questionnaire was self-administered in classrooms with no identification information collected (name of student, class or school), maintaining complete anonymity. For statistical data analysis SPSS version 20.0 was used. Descriptive data by gender of the respondents were presented in tables. However, Odds Ratios (ORs) and 95% confidence intervals (CIs) was calculated for all respondents adjusted for age and gender. For multivariate analysis, survey respondent in year 2000 coded as "0" and in year 2015 as "1" included as dependent variable. While, other variables presented in rows included as independent variables. More details on India questionnaires and methodology is publish elsewhere.[10,11]

RESULTS

In this study, GYTS 2000 represents data before COTPA implementation and GYTS 2015 represents data after COTPA implementation for the state of Maharashtra. Gender information was missing for 133 records in 2000 survey data and 6 records in 2015, therefore excluded from the data analysis.

On comparison of GYTS 2000 and 2015 data (Figure 1), the prevalence of ever tobacco use has reduced from 17% (95% CIs; 14.9 to 19) to 11% (95% CIs; 9 to 13.4) among boys and from 14.5% (% CIs; 12.1 to 16.9) to 8.5% (95% CIs; 6.8 to 10.7) among girls. The age of tobacco initiation has also shifted from 11 years or younger (~70%) in 2000 to older than 11 years (~70%) in 2015. In GYTS 2015, 3.8% (95% CIs; 2.6 to 5.3) boys and 3.4% (95% CIs; 2.3 to 4.9) girls reported to have been currently chewing tobacco as compared to 11.9% (95% CIs; 10.2 to 13.7) boys and 10.9% (95% CIs; 8.8 to 13) girls in 2000. Similarly, 3.6% (95% CIs; 2.5 to 5.2) boys and 6.3% (95% CIs; 4.8 to 8.2) girls in 2015 reported of currently applying tobacco as compared to 12.8% (95% CIs; 11 to 14.6) boys and 11.3% (95% CIs; 9.2 to 13.5) girls in 2000. Even though overall current tobacco use in any form during past 30 days shows decline, daily (all 30 days) tobacco use shows an increase from 1% (95% CIs; 0.5 to 1.5) to 2.6% (95% CIs; 1.5 to 3.8) for boys and 1.1% (95% CIs; 0.4 to 1.8) to 4.4% (95% CIs; 2.9 to 5.8) for girls between 2000 and 2015 surveys respectively. Overall proportion of students receiving pocket money increased from 20.2% for boys and 18.7% for girls in 2000 to 42.7% for boys and 33.8% for girls in 2015.

Figure 1. *Sample Characteristics of Youth and Their Tobacco Use, Maharashtra GYTS 2000 & 2015*



Compared to 2000 survey responses (Table 1), in 2015 survey ~30% lower second hand tobacco smoke exposure at home and ~20% lower at places other than home was reported by students. Between 2000 and 2015 surveys, student reporting their both parents using tobacco (OR=0.36), only mother (OR=0.47) and only father (OR=0.71) using tobacco has decreased. In contrast, between 2000 and 2015 surveys, students reporting some of their closest friends chew or apply tobacco, has increased (OR=1.59). However, in both the surveys around 85% boys and 90% girls said that they would definitely refuse chewing or applying tobacco if offered by their best friends. Compare to 2000 survey responses ~20% lower number of students in 2015 survey thought that smoking was definitely not harmful to their health. Similarly, in 2015 twice (OR=1.97) the number of students than in 2000 survey reported that smoking for a year or two as long as you can stop was definitely not safe.

Table 1. *Harms of Tobacco Use & Exposure from Other Tobacco Use among Youth, Maharashtra GYTS 2000 & 2015*

Options	Year 2000						Year 2015						Odds Ratios* & 95%CI
	Boys (1357)			Girls (866)			Boys (788)			Girls (816)			
	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	
$Do your parents smoke, chew or apply tobacco?													
None	726	54.2	(51.6, 56.9)	478	56.1	(52.8, 59.8)	468	59.6	(56.2, 63.0)	543	67.4	(64.1, 70.5)	1
Both	124	9.3	(7.7, 10.8)	108	12.7	(10.4, 14.9)	35	4.5	(3.2, 6.1)	41	5.1	(3.8, 6.8)	0.36 (0.27, 0.48)
Father only	405	30.2	(27.8, 32.7)	219	25.7	(22.8, 28.6)	229	29.2	(26.1, 32.5)	163	20.2	(17.6, 23.1)	0.71 (0.61, 0.83)
Mother only	43	3.2	(2.3, 4.2)	29	3.4	(2.2, 4.6)	13	1.7	(1.0, 2.8)	18	2.2	(1.4, 3.5)	0.47 (0.30, 0.74)
My Guardians							14	1.8	(1.1, 3.0)	12	1.5	(0.9, 2.3)	-
I don't know	41	3.1	(2.1, 4.0)	18	2.1	(1.1, 3.1)	26	3.3	(2.3, 4.8)	29	3.6	(2.5, 5.1)	-
$If one of your best friends offered you a cigarette or a bidi or chewing tobacco, would you take it?													
Definitely not+	1140	84.5	(82.6, 86.4)	763	89	(86.9, 91.1)	664	85.1	(82.5, 87.5)	712	87.9	(85.5, 90.0)	0.90 (0.74, 1.09)
$Do any of your closest friends chew or apply tobacco?													
None of them	908	68.6	(66.1, 76.1)	705	82.7	(78.2, 83.5)	604	77.4	(74.4, 80.2)	741	92	(90.0, 93.7)	1
Some of them	101	7.6	(6.2, 9.1)	74	8.7	(6.8, 10.6)	153	19.6	(17.0, 22.6)	43	5.3	(4.0, 7.1)	1.59 (1.26, 2.00)
Most of them	298	22.5	(20.3, 24.8)	59	6.9	(5.2, 8.6)	15	1.9	(1.2, 3.1)	15	1.9	(1.1, 3.1)	0.11 (0.07, 0.16)
All of them	16	1.2	(0.6, 1.8)	14	1.6	(0.8, 2.5)	8	1	(0.5, 2.0)	6	0.7	(0.3, 1.6)	0.65 (0.34, 1.26)
$Do you think smoking is harmful to your health?													
Definitely not+	395	29.4	(27.0, 31.8)	212	24.7	(21.8, 27.6)	174	22.3	(19.5, 25.3)	171	21.3	(18.6, 24.3)	0.81 (0.70, 0.95)
$Do you think it is safe to smoke for only a year or two as long as you stop after that?													
Definitely not+	649	48.8	(45.8, 51.1)	361	42.2	(38.9, 45.5)	475	61.1	(57.6, 64.4)	486	60.4	(57.0, 63.8)	1.97 (1.72, 2.26)
$Do you think the smoke from other people's cigarettes or bidis is harmful to you?													
Definitely not+	267	20.3	(18.1, 22.4)	171	20.2	(17.5, 22.9)	142	18.1	(15.6, 21.0)	129	16.1	(13.7, 18.8)	0.94 (0.79, 1.12)
$During the past 7 days (one week), on how many days have people smoked in your home, in your presence?													
0 days	891	66.7	(64.2, 69.3)	637	74.2	(71.2, 77.1)	604	77.4	(74.4, 80.2)	633	78.8	(75.9, 81.5)	1
1 to 2 days	203	15.2	(13.3, 17.1)	124	14.4	(12.1, 16.8)	85	10.9	(8.9, 13.3)	55	6.8	(5.3, 8.8)	0.54 (0.43, 0.67)
3 to 6 days	113	8.5	(7.0, 10.0)	41	4.8	(3.3, 6.2)	34	4.4	(3.1, 6.0)	40	5	(3.7, 6.7)	0.64 (0.47, 0.86)
7 days	128	9.6	(8.0, 11.2)	57	6.6	(5.0, 8.3)	57	7.3	(5.7, 9.4)	75	9.3	(7.5, 11.6)	0.85 (0.67, 1.08)
$During the past 7 days (one week), on how many days have people smoked in your presence, in places other than home?													
0 days	711	53.3	(50.6, 56.0)	562	66.1	(62.9, 69.3)	474	60.8	(57.3, 64.1)	535	66.7	63.4, 69.9)	1
1 to 2 days	283	21.2	(19.0, 23.4)	161	18.9	(16.3, 21.6)	152	19.5	(16.9, 22.4)	131	16.3	(13.9, 19.1)	0.82 (0.69, 0.98)
3 to 6 days	169	12.7	(10.9, 14.5)	68	8	(6.2, 9.8)	80	10.3	(8.3, 12.6)	69	8.6	(6.9, 10.7)	0.84 (0.67, 1.05)
7 days	171	12.8	(11.0, 14.6)	59	6.9	(5.2, 8.6)	74	9.5	(7.6, 11.8)	67	8.4	(6.6, 10.5)	0.79 (0.63, 1.00)

Note: OR= Odds ratios, CI=Confidence interval, NA=not applicable. *year of survey was used as dependent variable with year 2000 coded as 0 & year 2015 was coded as 1 and adjusted for age and sex. +reference category used as sum of probably not, probably yes and definitely yes. $Totals may not add up due to inclusion of only valid cases.

Decrease was observed between 2000 and 2015 surveys (Figure 2), in any teaching about the dangers of smoking/chewing in their school [boys 67% (95% CIs; 64.4 to 69.5) *v/s* 59.6% (95% CIs; 56.2 to 63) and girls 64.4% (95% CIs; 61.2 to 67.6) *v/s* 56.8% (95% CIs; 53.3 to 60.1)], any discussions on why children of their age smoke/chew [(boys 53.9% (95% CIs; 51.2 to 56.6) *v/s* 34.6% (95% CIs; 31.3 to 38) and girls 45% (95% CIs; 41.6 to 48.4) *v/s* 29% (95% CIs; 26 to 32.3)] and any teaching about effects of smoking/chewing in previous year of school [(boys 69.3% (95% CIs; 66.8 to 71.7) *v/s* 53.1% (95% CIs; 49.6 to 56.6) and girls 66.1% (95% CIs; 62.9 to 69.3) *v/s* 48% (95% CIs; 44.6 to 51.4)]. Similarly, between 2000 and 2015 surveys (Table 2), 60% decrease reported by students in families discussing about the harmful effects of tobacco.

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Figure 2. *Tobacco in school curriculum, Maharashtra GYTS 2000 & 2015*

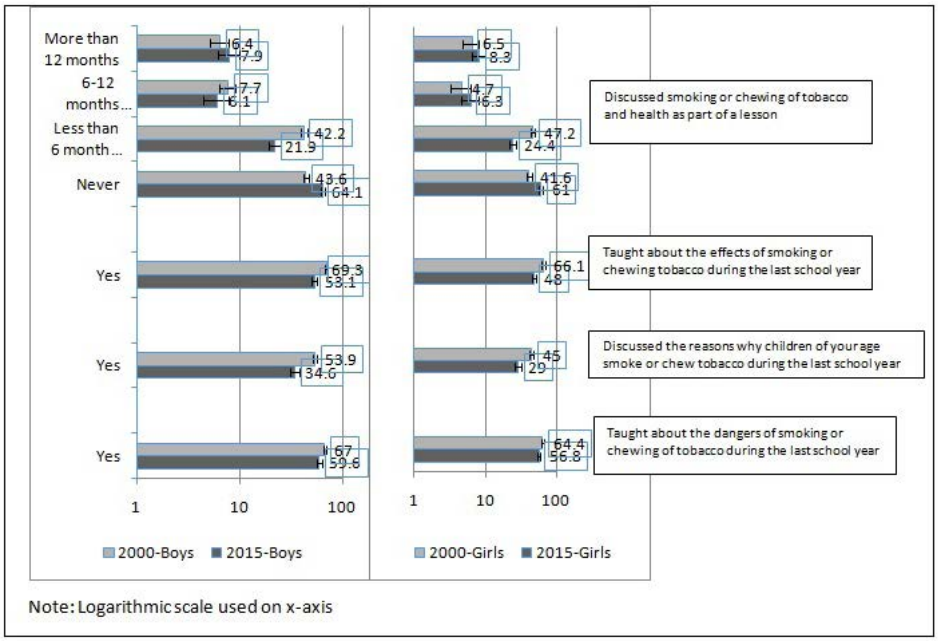

Table 2. *Perception And Attitude Towards Tobacco Use among Youth, Maharashtra GYTS 2000 & 2015*

Options	Year 2000						Year 2015						Odds Ratios* & 95%CI
	Boys (1357)			Girls (866)			Boys (788)			Girls (816)			
	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	
$During the past 30 days, did anyone ever refuse to sell you cigarettes because of your age?													
Yes	121	76.1	(69.5, 82.7)	72	84.7	(77.1, 92.4)	54	56.3	(46.3, 66.2)	45	55.6	(44.7, 66.4)	1
No	38	23.9	(17.3, 30.5)	13	15.3	(7.6, 22.9)	42	43.8	(33.8, 53.7)	36	44.4	(33.6, 55.3)	2.82 (1.79, 4.44)
$During the past 30 days, did anyone ever refuse to sell you bidi /any other smoking tobacco product because of your age?													
Yes	97	78.9	(71.6, 86.1)	71	86.6	(79.2, 94.0)	59	61.5	(51.7, 71.2)	51	62.2	(51.7, 72.7)	1
No	26	21.1	(13.9, 28.4)	11	13.4	(6.0, 20.8)	37	38.5	(28.8, 48.3)	31	37.8	(27.3, 48.3)	3.03 (1.83, 5.00)
$Has anyone in your family discussed the harmful effects of smoking or chewing tobacco with you?													
Yes	944	71.2	(68.8, 73.6)	563	66.6	(63.4, 9.8)	374	47.7	(44.2, 51.2)	385	47.8	(44.4, 51.3)	0.39 (0.34, 0.45)
$At any time during the next 12 months (one year), do you think you will smoke a cigarette or a bidi or chew a tobacco product?													
Definitely not+	1128	84.6	(82.7, 86.6)	761	89.3	(87.2, 91.4)	650	83.1	(80.3, 85.6)	711	88	(85.6, 90.1)	0.81 (0.67, 0.98)
$When you watch TV, videos, or movies, how often do you see actors smoking?													
Never	92	6.9	(5.6, 8.3)	58	6.9	(5.2, 8.6)	75	9.5	(7.7, 11.8)	115	14.3	(12.1, 16.9)	1
A lot	552	41.6	(38.9, 44.2)	372	44.3	(41.0, 47.7)	258	32.8	(29.6, 36.2)	245	30.5	(27.4, 33.8)	0.42 (0.33, 0.54)
Sometimes	404	30.4	(27.9, 32.9)	255	30.4	(27.3, 33.5)	278	35.4	(32.1, 38.8)	299	37.2	(34.0, 40.6)	0.68 (0.53, 0.88)
I never watch TV, videos, or movies	280	21.1	(18.9, 23.3)	154	18.4	(15.7, 21.0)	175	22.3	(19.5, 25.3)	144	17.9	(15.4, 20.7)	-
$When you watch TV, videos, or movies, how often do you see actors chewing tobacco?													
Never	155	11.6	(9.9, 13.3)	103	12.1	(9.9, 14.3)	126	16.1	(13.7, 18.9)	154	19.1	(16.5, 22.0)	1
A lot	398	29.9	(27.4, 32.3)	306	35.9	(32.7, 39.1)	199	25.5	(22.6, 28.7)	190	23.6	(20.8, 26.6)	0.51 (0.41, 0.63)
Sometimes	480	36	(33.4, 38.6)	286	33.6	(30.4, 36.7)	294	37.6	(34.3, 41.1)	316	39.2	(35.9, 42.6)	0.72 (0.58, 0.88)
I never watch TV, videos, or movies	300	22.5	(20.3, 24.7)	157	18.4	(15.8, 21.0)	162	20.7	(18.0, 23.7)	146	18.1	(15.6, 20.9)	-
$Has a cigarette company person or cigarette vendor ever offered you a free cigarette?													
Yes	214	16.6	(14.6, 18.6)	83	10	(8.0, 12.0)	57	7.3	(5.7, 9.3)	37	4.6	(3.4, 6.3)	0.54 (0.42, 0.70)
$Has a bidi company person or bidi vendor ever offered you a free bidi?													
Yes	203	16	(14.0, 18.0)	91	11	(8.9, 13.2)	56	7.1	(5.5, 9.2)	30	3.7	(2.6, 5.3)	0.47 (0.36, 0.61)
$Has a gutka or pan masala company person or vendor ever offered you a free sample?													
Yes	225	17.3	(15.2, 19.3)	106	12.6	(10.4, 14.9)	55	7	(5.4, 9.0)	25	3.1	(2.1, 4.6)	0.39 (0.30, 0.52)

Note: OR= Odds ratios, CI=Confidence interval, NA=not applicable. *year of survey was used as dependent variable with year 2000 coded as 0 & year 2015 was coded as 1 and adjusted for age and sex. +reference category used as sum of probably not, probably yes and definitely yes. $Totals may not add up due to inclusion of only valid cases.

Between 2000 and 2015 surveys (Table 2), decrease was also observed in the distribution of free cigarettes (OR=0.54) or bidis (OR=0.47) or gutka/pan masala (OR=0.39) by company persons/vendors. Among the students who watched TV or videos or movies, less students reported seeing actors smoking (OR=0.42) or chewing (OR=0.51) a lot on screen, in 2015 than in 2000. In contrast, students who attempted to buy cigarettes (OR=2.82), bidis or other smoking products (OR=3.03) who were not refused tobacco products because of their age has increased threefold between 2000 and 2015 surveys.

In 2015 (Supplementary Table 1), more boys than girls were aware about the ban on sale of gutka, ban on sale of flavored tobacco and ban on sale of pan masala or flavored areca nut. About 51% boys and 41% girls reported seeing shops selling tobacco products near their schools. Even though 41% boys and 32% girls observed boards prohibiting the sale of tobacco products to minors at tobacco shops, 49% boys and 35% girls have observed people of their age not only buying tobacco but 41% boys and 26% girls even selling tobacco products. About 36% boys and 27% girls observed pictorial health warnings only on cigarettes packets, 4.7% boys and 3% girls observed only on bidis packets, 6.9% boys and 7.2% girls only on chewing packets and 24.1% boys and 18.8% girls have observed pictorial warnings on both smoke and chewing tobacco packets. Interestingly, 10% boys and 13% girls who use tobacco reported never observing pictorial health warnings on tobacco products.

Supplementary Table 1. *COTPA Compliance among Youth, Maharashtra GYTS 2015*

Questions	Options	Year 2015					
		Boys (N=788)			Girls (N=816)		
		n	%	95% CI	n	%	95% CI
$Do you want to stop tobacco use now?	I do not use any tobacco now	25	16.7	(10.7, 22.6)	27	13.8	(9.0, 18.7)
	Yes	109	72.7	(65.5, 79.8)	160	82.1	(76.7, 87.4)
	No	16	10.7	(5.7, 15.6)	8	4.1	(1.3, 6.9)
Do you think the sale of gutka is banned in Maharashtra?	Yes	323	41.2	(37.8, 44.7)	250	30.9	(27.8, 34.1)
Do you think the sale of flavoured tobacco is banned in Maharashtra?	Yes	287	36.6	(33.3, 40.0)	230	28.4	(25.4, 31.6)
Do you think the sale of pan masala or flavoured areca nut (Supari) is banned in Maharashtra?	Yes	432	55.2	(51.7, 58.6)	382	47.3	(43.9, 50.7)
Have you seen any shop selling tobacco products near to your school?	Yes	405	51.7	(48.2, 55.2)	332	41.2	(37.8, 44.6)
$During the past 30 days (one month), did anyone ever refuse to sell you gutka/pan masala or any other smokeless product because of your age?	Yes, because of age	14	17.3	(9.0, 25.5)	25	27.8	(18.5, 37.0)
	Yes, because of smokeless tobacco is banned	24	29.6	(19.7, 39.6)	31	34.4	(24.6, 44.3)
	No	43	53.1	(42.2, 64.0)	34	37.8	(27.8, 47.8)
Have you seen any sign boards prohibiting the sale of tobacco products to minors at the tobacco selling shops?	Yes	319	40.7	(37.4, 44.2)	260	32.1	(29.0, 35.4)
Have you observed any one of your age selling tobacco products?	Yes	324	41.2	(37.8, 44.7)	206	25.5	(22.6, 28.6)
Have you observed any one of your age buying tobacco products?	Yes	381	48.5	(45.1, 52.0)	282	34.9	(31.7, 38.3)
$Do you think smoking is banned in public places like restaurants, buses, train stations, bus stations, schools, gyms and sports stadiums, etc.?	Yes, all places	464	59.2	(55.7, 62.6)	390	48.6	(45.1, 52.0)
	Yes, some of these places	183	23.3	(20.5, 26.4)	258	32.1	(29.0, 35.4)
	No	137	17.5	(14.9, 20.3)	155	19.3	(16.7, 22.2)
$Have you ever objected to anyone smoking in your presence in any public places?	Yes, always	293	37.4	(34.1, 40.9)	250	31.3	(28.2, 34.6)
	Yes, sometimes	273	34.9	(31.6, 38.3)	273	34.2	(31.0, 37.5)
	No, never	162	20.7	(0.18, 23.7)	170	21.3	(18.6, 24.3)
$Have you observed pictorial health warnings on tobacco products?	Yes, only on cigarettes packets	281	35.8	(32.6, 39.3)	216	26.9	(23.9, 23.0)
	Yes, only on bidi packets	37	4.7	(3.4, 6.4)	24	3.0	(2.0, 4.4)
	Yes, only on chewing tobacco packets	54	6.9	(5.3, 8.9)	58	7.2	(5.6, 9.2)
	Yes, both smoke and chewing tobacco packets	189	24.1	(21.3, 27.2)	151	18.8	(16.2, 21.6)
	No, I never observed as I do not use any form of tobacco	142	18.1	(15.6, 21.0)	253	31.5	(28.4, 34.8)
	No, even if I am a tobacco user	81	10.3	(8.4, 12.7)	102	12.7	(10.6, 15.2)

Note: CI=Confidence interval. $Totals may not add up due to inclusion of only valid cases.

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DISCUSSION

Smoking and chewing tobacco has severe influence on social,[11,13-24] economic[13,14,18,21-24] and health[11-17,19-24] components of individuals. Use of tobacco exposes, both the first hand users and second hand smokers, to many harmful carcinogenic chemicals and diseases.[12] Current study clearly shows, decline in overall tobacco use among boys and girls in survey conducted in 2000 (before COTPA implementation) and in 2015 (after COTPA implementation). However, as demonstrated in other states of India,[13] this study also observes narrowing of the prevalence differences of tobacco use among boys (11%) and girls (8.5%) but continues to remained high among boys than girls. Smoking by girls is culturally not accepted in Indian society,[10,14] and therefore; higher cigarette smoking gender differences were observed among students in most states, mainly in the northeastern states of India.[10] In this study, the proportion of girls reporting smoking was almost identical to that reported by boys in both the surveys. Findings from the GYTS from 132 WHO member states shows that around half of all the students surveyed worldwide were exposed to tobacco smoke at home (36.4% in India) and outside their home (48.7% in India).[15] In this study, between 2000 and 2015, decreased in the exposure to SHS 7 days prior to the survey was observed in their homes as well as outside their homes. One such study was conducted in Mumbai in 2010, found that students own tobacco use, their age, their parents tobacco use, their close friends tobacco use were associated with their exposure to second hand smoke at home and outside home.[16]

The family members and friends tobacco use was associated with adults[17] and children's[10,14,15,18,19] tobacco use. A population base survey of high school students in Mumbai[19] further delineated that both parents using tobacco increased the odds four times while any one parent using tobacco increased the odds two times that their children will also use tobacco. Even though the current study reported a decline in tobacco use by both parents but tobacco use by any one parent (father only) remained high between 2000 and 2015 surveys. Similarly, smoking acts like as a catalyst to make new friends and develop relationships.[20] In this study post COTPA implementation, boys and girls reporting any of their closest friends smoke, chew or apply tobacco decreased in 2015 than it was reported in 2000.

Pocket money increases the purchasing power among the youth and is therefore, an influential factor for initiation of tobacco products.[14,18] Pocket money was also associated with student's intention to use tobacco and ease to access tobacco products.[19] In this study, around two-fold increase observed in the proportion of students who receive pocket money between 2000 and 2015 surveys.

Tobacco control policies compliance decreased smoking prevalence.[19,21-23,26,28] A policy on tobacco sale to minor was associated with lowering youth smoking.[22] Although the laws clearly prohibit the sale of tobacco products to young people <18 of age in India,[8] the current study shows initiation of tobacco use continues to starts as early as 9 years or younger.

However, the current study observed shift in the distribution of age of initiation of tobacco use among both boys and girls from 11 years or younger (~70%) in 2000 survey to older than 11 years (~70%) in 2015 survey. Section 6 of COTPA prohibits the sale of tobacco products to minors and near educational institutions; however, students have reported seeing people of their age not only buying but selling tobacco products as well. Sale of tobacco and related products was also observed by students near their schools. Another study conducted in Mumbai in 2012[23] also found that tobacco vendors were present within 100 m of nearly all sampled schools and student's tobacco use was associated with the presence of tobacco vendors and advertisements within 100 to 500 m of schools.

Among all tobacco products, smokeless tobacco is the predominant form used by men (29.6%), women (12.8%), and youth of age 15-24 (10.8%); it exceed the prevalence of cigarette smoking and that of other smoked products (e.g., bidis).[24] Section 8 of COTPA emphasizes on specific health warnings through pictorial messages on all tobacco products, tobacco industry influence led to years of delays and dilutions.[25,26] The pictorial health warnings introduced in 2009 were weak, with one symbolic image that of a scorpion (which was unrelated to cancer) on 40% of the fount of the package. In December 2011, four graphic images of cancer of the mouth, jaw, or neck were replaced the scorpion image. While in 2013 three new graphics health warnings were implemented. All smokeless tobacco warnings since 2011 were accompanied by the text "Tobacco Kills"; however, size, location, the lack of rotation and freedom to tobacco industry to choose any one of the available warnings continues to dilute the impact. This study confirms low level of noticing and effectiveness reported in other Indian studies.[27,28] Additionally, change from the symbolic warnings (before COTPA) to graphics health warnings (after COTPA) did not lead to increase in noticing as well as effectiveness.

Students in this study have reported around 40% decrease in the distribution of free cigarettes, bidi's and gutka or pan masala by vendors or company persons between 2000 and 2015. Distribution of free cigarettes to minors was prohibited under COTPA but now making it a punishable offense under section 77 of the Juvenile Justice Act (Care and Protection of Children), 2015,[29] will further restrict the free sample distribution in future.

Even though sale of gutka is banned in Maharashtra under the Food Safety and Standards Act (FSSA), 2012,[30] similar to other studies,[31-33] the current study highlights continuation of poor knowledge among students in relation to existing laws. The lack of knowledge about existing laws and harmful effects of tobacco was correlated well in our study with reduction in discussion about harmful effects of tobacco at home as well as in schools. As observed in other studies,[15,34] academic teaching on harmful effects of tobacco, during the last school year had reduced in 2015 (59% boys *v/s* 56% girls) to that reported in 2000 (67% boys *v/s* 64% girls) Maharashtra GYTS. School tobacco control policies have shown success in delayed initiation of smoking and in reduction of smoking rates in the Western settings[35] and in India.[36] Thus health education and awareness with a comprehensive, evidence-based curriculum could be helpful to protect the youth from the risk of tobacco use.

STRENGTHS AND LIMITATIONS

There are several notable strengths and limitations to this study. First strength, data for this paper came from the GYTS surveillance system, which was followed globally with a standard study protocol and hence comparable within country and between countries. However, the results are generalizable mainly to school going children might be a potential limitation. This was cross-sectional survey; hence causality between exposure and outcome may not be established, might be another limitation. Also, the responses were self-reported without any biological validation of their tobacco use status and might subject to recall biases. However, such measures have been widely used and shown good validity and reliability when administered anonymously within classroom.[37] Additionally, we did not asked about number of other variables that might have contributed to the low knowledge and low awareness of COTPA, such as, while responding weather they were thinking of tobacco products that were available in a packages or container, illicit products, or homemade products.

CONCLUSION

Tobacco use has decreased after implementation of COTPA in school going children of Maharashtra. Shift in the distribution of age of initiation of tobacco use needs attention of researcher and policy makers. Established factors that influence children's tobacco use such as parents, close friends tobacco use, exposure to second hand smoke at home and outside home, pocket money received by students requires continuous monitoring. Increasing the awareness and knowledge at home and at school will further support the tobacco control efforts. Overall compliance with tobacco control laws in India needs further enforcement in order to decrease availability, accessibility, and affordability of tobacco products.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Original Research

Ovine Network between Fatteners and Breeders in Middle Atlas of Morocco: Where to Act to Prevent the Spread of Epidemics?

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ABSTRACT

Objective
This cartographic and analytical study, using the social network analysis method, aims to characterize sheep mobility from breeders producing lambs in the Middle Atlas Mountains to the fattening centers, passing through livestock markets in order to describe the exchange network, to identify the main mobility hubs and to secure the production of fattening lambs by improving the efficiency of the epidemiological surveillance system for "*peste des petits ruminants*" (PPR) in lambs producers of the Middle Atlas in Morocco.

Design
Descriptive cross-sectional study within the framework of active epidemiological surveillance. It was carried out at a regional level, for mapping the movements of sheep and to analyze the network of exchange relating to this species by the Social Network Analysis (SNA) method in the middle atlas massif and in the zone of sheep fatteners to guide epidemiological surveillance efforts. A total of 807 breeders producing lambs, sheep fatteners and traders were surveyed, including 54 fatteners in fattening workshops (sampling fraction of 48.6%), 150 breeders and traders in livestock markets of Middle Atlas and 603 producing lamb breeders of this massif (sampling fraction at the massif level of 6.64%).

Results
The study revealed a very strong commercial relationship between middle Atlas breeders and fattening zone breeders whose long-range outflows mainly converge towards urban consumption centers. The major strategic livestock markets of the middle Atlas proved to be key points in the articulation of the flows, as was the commune gathering the fatteners. Centrality indicators were used to identify the main trade hubs that contribute to the spread of diseases and to quantify their importance in the influence of sheep movement, while network cohesion parameters have shown that network is vulnerable to the spread of epidemics.

Conclusion
The results of this survey revealed the main commercial hubs at the Middle Atlas level and at the level of the province of fatteners, which represent a great risk of spreading sheep diseases over long distances rather quickly in event of an epizootic, but also representing the ability to control the spread through the control of the movement of animals at their level.

Keywords
Ovine network; Epizotic of (Peste des Petits Ruminants); Mapping; Social network analysis; Hub; Betweenness; Degree; Strong component; Cutpoint.

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INTRODUCTION

Morocco experienced in 2008 an epizootic of "*Peste des Petits Ruminants*" (PPR) caused by an invasive lineage IV virus, particularly in North Africa. It is a cross-border disease of sheep and goats that is widespread in Africa, the Middle East and Asia and can manifest as enzootic (diminished gravity but continuously present) when settles in a country as a result of insufficient control measures or manifest as epizootic (spectacular severity but transient in case of appropriate control measures) when the disease has just appeared. The assessment of the Moroccan epizootic for this disease reported 257 outbreaks scattered throughout the northern half of the country in just over three months[1] (Figure 1), despite the low morbidity (11, 93%) and mortality (5.53%) rates observed in small ruminants,[1] which triggered the role of sheep mobility in the spread of the disease.

Surveys conducted during the epizootic of 2008[2] showed that the disease was concentrated in three provinces of northern Morocco including the one hosting the fattening centers (Benslimane) and the two large sheep provinces of the Massif of Middle Atlas (Khenifra and Ifrane) which supplies in sheep the first province (Table 1).

Table 1. *Total Number of PPR Outbreaks During the Epizootic of 2008 in the Provinces of the Middle Atlas*[1]

Province	Number of Outbreaks
Khenifra	53
Ifrane	22
Benslimane	15
Sefrou	10
Boulemane	7
Taza	6
El Hajeb	4
Khemisset	4

The Middle Atlas is a mountain range extending over 350 km (Figure 1). It is home to a sheep-rearing system of the lambs producing type, mainly exploiting the Timahdite breed, which is coveted for the quality of its meat and which is consequently marketed in other parts of the country, mainly through fattening centers. The farming system is lamb producing type, extensive and based mainly on forests, grazing path and marginally on agricultural by-products. This mode of conduct promotes contacts between herds of different origins and is conducive to the transmission of diseases.

In Morocco, apart from areas with almost exclusively goats, which are confined to the high altitudes of the Rif and High Atlas Mountains (Figure 1), small ruminants live in mixed herds of sheep and goats (when these last exist) throughout the rest of the country. At the national level, the sheep population of 19,956,384 is more three times higher than the goat population of 6,235,861.[3] Based on the above and considering that sheep are much more mobile and more present in the field than goats, it has been decided to carry out mobility surveys in sheep.

For countries that do not have a sheep identification and traceability system, animal mobility surveys is an important epidemiological tool for studying commercial movements in this species and for identify key exchange hubs to guide monitoring efforts.

Thus, following the PPR epizootic that affected Morocco in 2008, it was decided to carry out sheep mobility surveys at the provinces with the largest number of outbreaks in order to map the existing flows and treat them by the Social Network Analysis (SNA) method.

The mapping of movements is based on the use of geographic information system (GIS) software to represent commercial flows, while the method of social networks analysis has been borrowed from sociology. It is based on the theory of graphs in mathematics and assumes that the contact network has a greater influence than the personal behavior on the spread of the disease.[4]

A social network consists of a set of individuals or groups that form nodes, connected by links that represent a certain type of relationship (friendship for example) or interaction (sexual for example), or also indicating the presence or the absence of a relationship, such as the fact that a male and a female have come closer, coupled or not.[5] The analysis of social networks allows an assessment of the influence that connections between individuals can have in the transmission of a given disease.[4]

Historically, the first example of the use of SNA in epidemiology dates back to sexually transmitted diseases. In 1985, Klovdahl used this method to describe a focus (outbreak), and thus provided arguments in favor of the theory that an infectious agent causing acquired immune deficiency syndrome (AIDS).[6] The method has shown in particular the importance in epidemiology of the diseases that make the link between the central individuals (who maintain the disease) and the individuals of the periphery (with a lower prevalence).[4] In recent years the method has also been used in animal epidemiology, particularly in the movements of the livestock.[7] Indeed, in Great Britain Webb used it in 2006 to determine the role of agricultural competitions in the transmission of ovine diseases.[8] Kao et al[9] studied cattle movements in the United Kingdom to estimate the risk of outbreak of fibromuscular dysplasia (FMD), and provided guidance for targeted surveillance and control, which would be effective in reducing this risk.[9]

The SNA method allows calculating a large quantity of indices and parameters, which will make it possible to qualify the network and to classify the nodes according to their importance.[10] There are different types of parameters that can be calculated from the network:

The first category brings together the cohesion parameters at the network level. They apply to all nodes and links, and allow for example to have an idea of how the structure of the network influences the spread of the disease. Examples of such parameters are size, density, coefficient of clustering (all gives information on global connectivity within the network).

Figure 1. *Geographical Distribution of PPR Outbreaks During the 2008 Epizootic in Morocco*

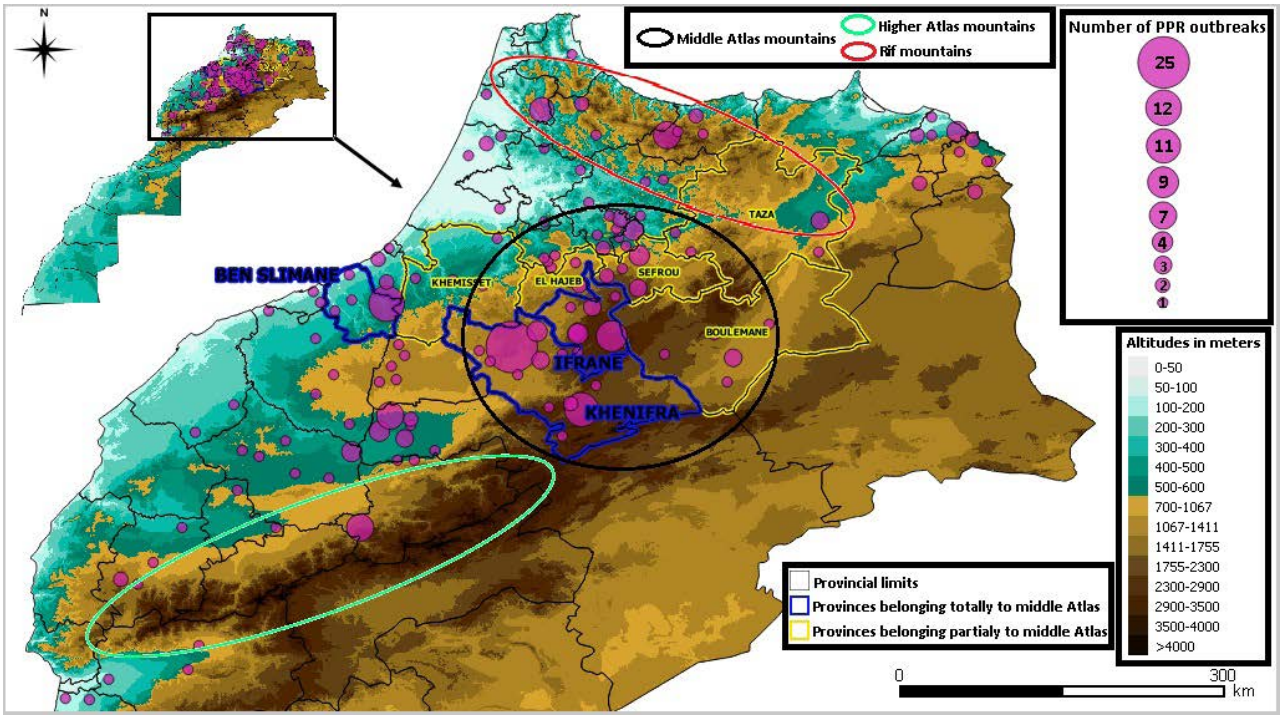
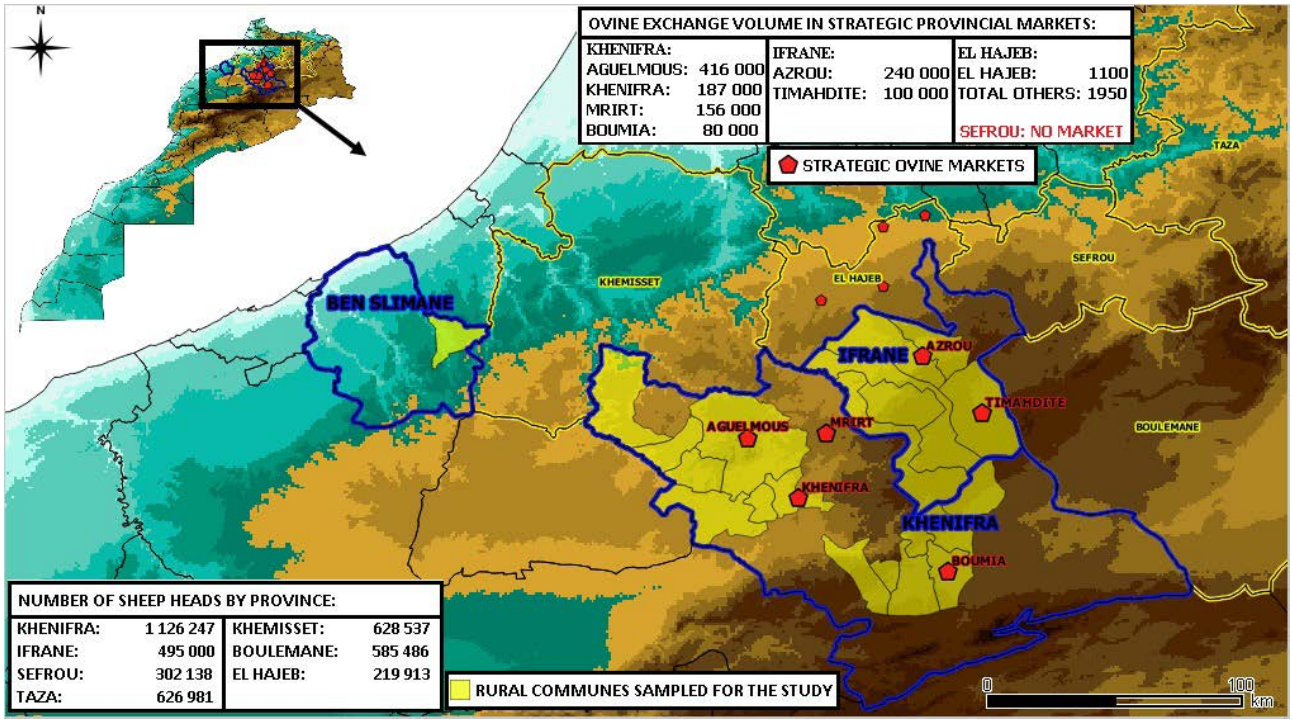


Figure 2. *Study Area Indicating Importance of Strategic Markets*[3] *and the Importance of Sheep by Province*[14]



The second category includes connection and connectivity settings. These are parameters that reflect the ability of one node to reach others by following network links. It tells us that the nodes are connected or not, regardless of the length of the path to take. A network is said to be connected if from each node it is possible to reach all the others. A "component" is a largest set of connected nodes.

The third category groups the centrality parameters.[11,12] These settings indicate whether a node is central and important in the network. It measures its structural importance. These parameters are widely used in epidemiology to detect individuals/nodes, or key actors. Example of parameters: The "degree" which informs about the infectivity of the node and which includes the "indegree" indicating the incoming movements and expressing the power of the node to self-infect as well as the "Outdegree" indicating the outward movements and expressing the infectivity to other nodes in contact with this node, the "betweenness" which represents the ability of the node to force flows to pass through it before reaching the other nodes and informs about the essential role of the node as a crossroads hub of mobility. The list of the types of parameters used is exhaustive, but those mentioned above are the most frequently encountered.

This study is the first conducted in Morocco using GIS mapping and the SNA tool in animal mobility, and was conducted to identify the main possible outbreak centers for sheep diseases to guide surveillance.

Choice of the Study Area

The middle Atlas occupies an area of 23,000 km², it is linked to seven provinces whose administrative boundaries of three of them (Khénifra, Ifrane and Sefrou) are an integral part of this mountain, while four other provinces contribute only by a part of their territory in the geography of this massif (Taza, Khémisset, Boulemane and El Hajeb) (Figure 1).[13]

Among Middle Atlas breeders, the provinces of Khenifra and Ifrane were selected for this study due to:

(i) Their significant sheep potential (Figure 2), (ii) the high incidence of PPR outbreaks during the 2008 epizootic (Table 1 and Figure 1), (iii) their significant geographic coverage (Figures 1 and 2). and (iv) the presence at their level of the largest sheep trading markets in the massif in terms of trade volume (Figure 2).

Among fatteners, the study area was represented by the commune of Sidi Bettache which groups together all fattening centers in the province of Benslimane (Figure 2) with a reported production of 65,000 head and a potential production capacity of 157,550 heads.

Choice of Method

In addition to using the Qgis software for flow mapping, the SNA method, which is the most appropriate for studying the relationships between different key players in a network, was adopted with a view to examine the different components of the sheep mobility network in the targeted area.

Type of Study and Period

This is an epidemiological study of the descriptive transversal type which aims to map the direction and the range of sheep flows and to study the mobility network by the social networks analysis method to describe its structure, its fragility to epidemics, to evaluate the influence that can have the connections between the various actors of the network in the transmission of a given disease but also to determine the main hubs of exchange to guide the surveillance.

The data collection work in the field lasted a total of 4-weeks from March 17 to April 11, 2014.

Population

Target population: In lamb's producers, the target population consists of the lamb's producer's breeders of the middle atlas.

For fatteners, the target population consists of fattening farmers in the province of Benslimane.

In traders, the target population concerns the traders of livestock markets of the middle atlas.

Source population: For lamb's producers, the source population is made up of lamb's producers belonging to the provinces that form an integral part of the middle Atlas Massif, which showed a high incidence of PPR outbreaks during the 2008 epizootic and which include a high sheep population and strategic cattle markets with a large volume of exchange, namely the provinces of Khenifra and Ifrane.

In fatteners, the source population consists of fattening farms in the commune of Sidi Bettache, which groups together all fatteners in the province of Benslimane.

Among traders, the source population concerns traders in strategic livestock markets in the middle atlas with the largest trade exchange volume in sheep.

Sample Size

A total of 807 breeders producing lambs, sheep fatteners and traders were surveyed, including 54 fatteners in fattening workshops (sampling fraction of 48.6%), 150 owners (breeders and traders) in livestock markets of Middle Atlas and 603 producing lamb breeders of the Massif (sampling fraction at the Massif level of 6.64%).

In fatteners: Sampling was carried out in fattening centers on the 111 fatteners identified by Office National de Sécurité Sanitaire des produits Alimentaires (ONSSA). A sample of 54 farms was randomly selected using the "OpenEpi, Version 2" software that represent a survey fraction of 48.6% in the municipality of Sidi

Bettache.

In livestock markets: The investigation in fattening zone allowed going up towards the markets of the middle atlas. In Ifrane province, the two existing strategic livestock markets of the province, Azrou and Timahdite were investigated (Figure 2). Due to the heavy rainfall that occurred on the day of the Azrou market, only 30 owners could be interviewed, while 60 owners were interviewed at the Timahdite market.

In the province of Khénifra, two strategic livestock markets out of four present were investigated, Mrirt and Boumia Markets (Figure 2). In order to optimize the time allocated to the survey, 30 owners were interviewed in each of these two markets since they have the same day of occurrence which is Thursday but they are distant geographically. The two other strategic markets in the province, Aguelmous and Khénifra, could not be investigated since their days of occurrence were during the last two days of the week. It should be noted that livestock markets in Morocco are weekly markets that take place only once a week.

In lamb's producer's farms: In lamb's producers zone a sampling was carried out on the lamb's producer's databases of the provinces of Khénifra and Ifrane. Four hundred and twenty-six farmers were sampled on a total of 8594 farmers (survey fraction of 4.95%) spread over 15 communes out of a total of 38 communes in the province of Khenifra (Figure 2) and 207 farmers were sampled on 3184 farmers presents (fraction of survey of 6.50%) spread over six municipalities out of a total of eight in the province of Ifrane (Figure 2). Sampling was done by SPSS software.

Data Collection

The questionnaire used: For this study a specific anonymous questionnaire of light character not exceeding one line per owner was used. It includes questions on the communes of origin and destination of the movements that are being made, on the type of origin and destination (producing lamb farms, livestock market, fattening workshop or slaughterhouse) and on the number of sheep in the transaction, including the percentage of females and the percentage of lambs under 6-months of age, then the questionnaire looks at the presence of the extensive component in the flocks surveyed (to get an idea of the possibility of collective contamination of herds in collective pastures), as well as information on the majority race (to identify the breeds that are most involved in this commercialization circuit).

Test of questionnaire: The questionnaire was tested in the field before starting in a market of Skhirate-Temara province on a dozen farmers.

Conduct of investigations: The epidemiological unit defined is the flock unit and the survey was conducted in the form of a face-to-face interview with the owner (trader, farmer). All questionnaires were administered by the same investigator in each farm and market.

Data Entry

The data has been entered on access tables and their processing was performed on the Quantum GIS (Qgis) 2.8.3 software for cartography and the (R) software for the SNA analysis.

Data Analysis Method

With the Qgis software, all the sheep flows surveyed were mapped to highlight the direction and range of the movements, while the R software made it possible to estimate the various indicators that allows to characterize the contact network, with general measures such as density, average distance and diameter. Other parameters were evaluated, including the centrality parameters with degrees (degree, indegree, outdegree) and betweenness to identify the most important key actors/nodes of the network. To complete these analyzes, measures such as "components" ("strong components" and "weak components") and "clustering coefficients" were calculated to determine the cohesion between the different municipalities, in addition to the "cutpoints" who have been identified.

RESULTS

Flow Analysis

Flow mapping: The communes of the middle atlas hosting the major strategic markets are key points in the articulation of flows, as is the municipality gathering the fatteners (Figures 3, 4 and 5). It can be seen that there are long-range trade of sheep flows converging mainly on urban consumption centers (Figures 4 and 5).

Type of movements: From the sheep mobility database, we retrieved information on the origins and destinations of the flows to create matrices of adjacencies. These represent movements with online origins and column destinations. A total of 133,539 sheep in circulation distributed among the different establishments was recorded corresponding to a total of 808 movements.

Within the network, flocks of producing lamb's breeders to markets are the most important. In fact, 58.58% of sheep movements take place from producing lamb farms to markets; movement between lamb's breeders does not exceed 11.9%, whereas movements from markets to fattening centers represent 10.63%. Percentage of the network's movements, the latter are supplied exceptionally at the level of markets, while market-to-market movements account for 8.6% of these trips (Table 2).

Table 2. *Movement of Sheep between Different Establishments*

From \ To	Slaughter Houses	Fattening Centers	Lamb's Breeders	Markets	Total
Fattening Centers	5490 (4,11%)	0	900 (0,67%)	1100 (0,82%)	7490 (5,6%)
Lamb's Breeders	1590 (1,19%)	0	15785 (11,82%)	78237 (58,58%)	95612 (71,59%)
Markets	610 (0,45%)	14200 (10,63%)	4190 (3,13%)	11437 (8,56%)	30437 (22,79%)
Total	7690 (5,75%)	14200 (10,63%)	20875 (15,63%)	90774 (67,97%)	133539 (100%)

Figure 3. *Graphic Representation of the Ovine Network*

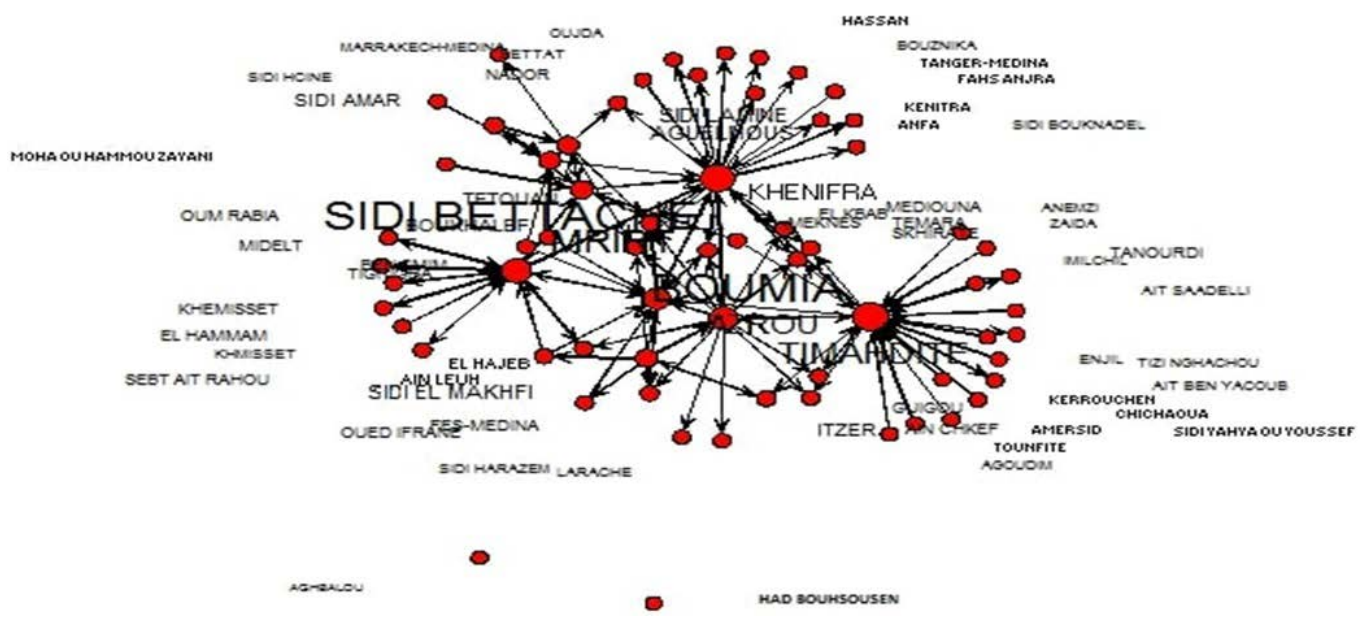
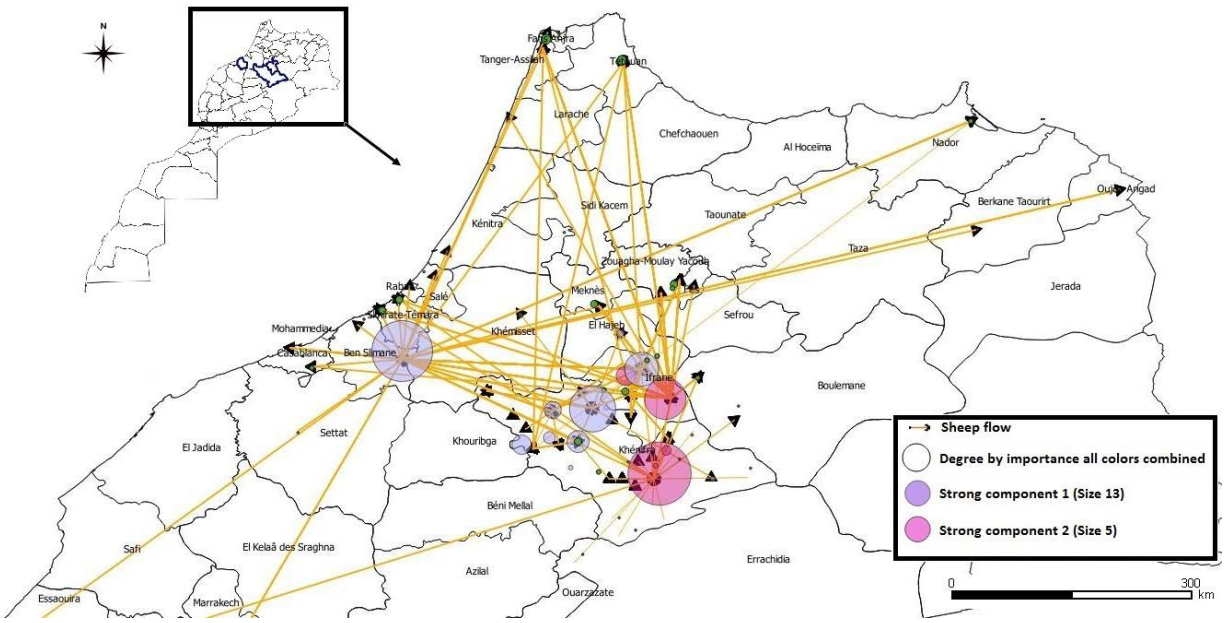


Figure 4. *Mapping of Sheep Flows, Degree and Strong Component*



Analysis of Network

Of the total of 808 movements found, 128 distinct flows were identified. Several movements between the same origin and destination as well as several animals in the same movement are considered as a single flow. In the presented network, the nodes represent the markets, the slaughterhouses, the lamb's producers or the fatteners.

Description of Network: The network has 65 nodes. On average one commune is connected to more than 3 times (3,477) (average degree) with other municipalities. The network has a low density of the order of 0.027. Two communes are on average separated (average distance) by almost 5 (4.83) animal movements (Table 3).

Figure 5. *Mapping of Sheep Flow and Betweenness*

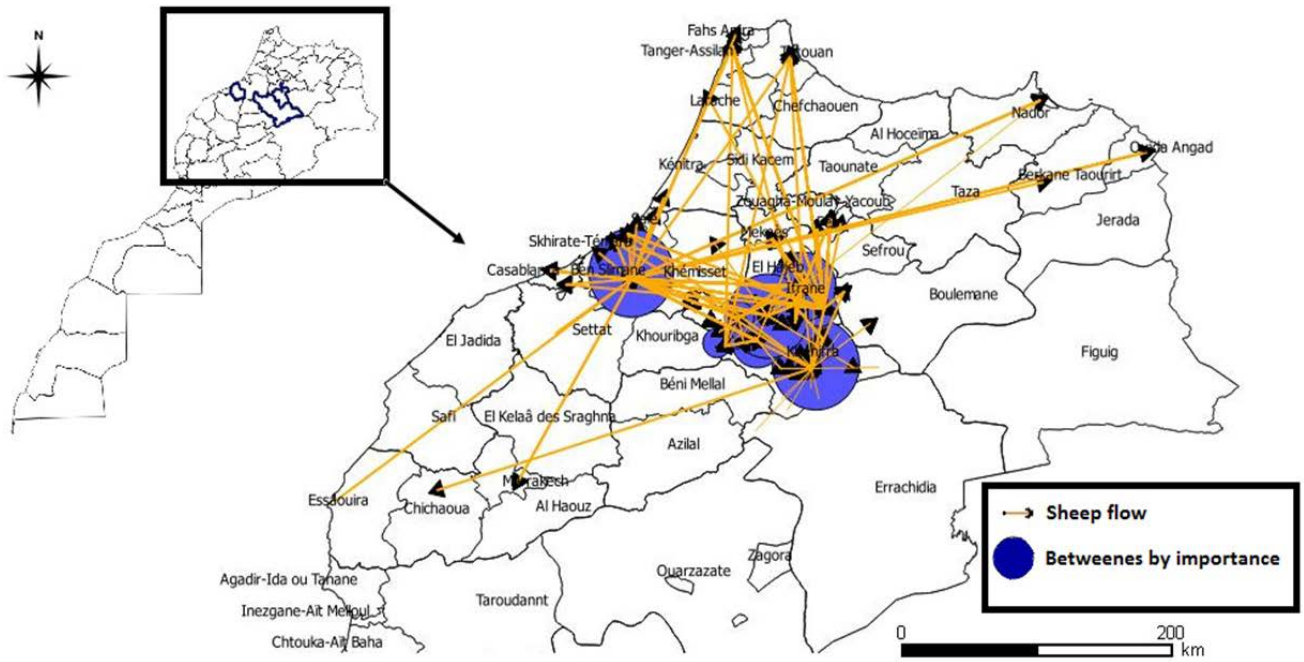


Table 3. *Network Description Settings*

SNA Parameters	Value
Number of nodes	65
Length	4.831953
Mean of Degree	3.477
Mean of Out degree	1.738
Mean of In degree	1.738
Mean of Betweenness	38.69
Diameter	6
Density	0.027
Number of (weak components)	3
Size of the largest component	13
Cutpoints	5

Graphic representation of the network: The graphical representation makes it possible to visually identify that the network is connected, if we are interested in a node we can then see its connection links and also appreciate the central position or not of this node represented graphically by a variable size. The networks were represented with different thicknesses of the links according to the importance of the route taken between two communes, and the direction of the arrow corresponds to the direction of the flow (here we have a directed network because we have the information of incoming and outgoing). The size of the wording indicates the importance of the locality. We can easily notice the central position of the communes of Sidi Bettache, Boumia, Mrirt, Azrou and Timahdite (Figure 3).

Indicators

Degree: The classification of communes according to the number of total movement (degree), of entries (in degree) and of outputs (out degree) was calculated. The average number of links is 3,477 (Table 3) with a maximum of 26 (Table 4). The average of the entries and exits was 1,738 (Table 3) with a maximum of 15 for the entries and 17 for the exits (Table 4). Given the large number of nodes studied, only the 10 with the highest centrality indicators were presented (Table 4).

Table 4. *Ranking of the Top Ten Localities According to their Number of Degree, In-Degree et Out-Degree*

Total Degree	In Degree	Out Degree
Boumia (26)	Boumia (15)	Sidi Bettache (17)
Sidi Bettache (25)	Mrirt (10)	Timahdite (15)
Mrirt (19)	Azrou (9)	Boumia (11)
Timahdite (17)	Sidi Bettache (8)	Mrirt (9)
Azrou (14)	Khenifra (5)	Sidi El makhfi (7)
Khenifra (9)	Aguelmous (5)	Sidi lamine (6)
Sidi lamine (8)	Timahdite (2)	Azrou (5)
Sidi El makhfi (8)	Sidi lamine (2)	Khenifra (4)
Aguelmous (7)	Sidi Amar (2)	Sidi Amar (3)
Sidi Amar (5)	Sidi El makhfi (1)	Aguelmous (2)

The number of degrees of a node indicates the number of incoming and outgoing motions on that node and therefore on its activity level. Here we see that for the nodes with the highest total degree are Boumia and Sidi Bettache, So these are the two localities with the strongest commercial activity (='hub'). Mrirt, Timahdite and Azrou are also important nodes of the network. Aguelmous and Khenifra, which have the largest markets in terms of trading volume, also appear among the top 10 despite the fact that their markets were not surveyed during the study (Table 3 and Figure 4). Some communes (5/65) are very connected (degree> 12) (Table 4) while the average of the communes is 3.47 (Table 3), which clearly allows to identify a network of "Scale free" type, suggesting a sensitive network for the spread of epidemics.

The "out degree" is the number of different localities to which the animals return. The ranking is more heterogeneous but we find Sidi Bettache, Timahdite, Boumia, Mrirt and Sidi el makhfi in the 5 most popular origins in ruminants. Note that a locality with a large number of slaughterhouses has a low outdegree. The "in degree" is the number of different localities from which a node receives the animals. We find Boumia, Mrirt, Azrou, Sidi Bettache, in addition to Khenifra and Aguelmous present in the most attractive destinations. Knots in the lamb producers zone such as the communes of Timahdite and Sidi El Makhfi have a high outgoing degree (respectively 15 and 7) and low values of degrees entering (Respectively 2 and 1), to a lesser degree the fatteners communes represented by Sidi Bettache has a little more than double of the outgoing degrees (17) compared to the incoming degrees (8). Conversely, more than a third of the network communes that are geographically distant from the lamb producers zone have positive values of the incoming degrees and zero values of the outgoing degrees.

Intermediarity or (Betweeness): This measure corresponds to the number of shortest paths between two vertices on which the studied nodes are located. She reports that 13 communes show a positive betweenness (Table 5 and Figure 5). The highest betweenness is Boumia, Sidi Bettache and Mrirt, followed by Azrou, Khenifra and Timahdite. It should be noted that Khenifra and Aguelmous, whose markets are the two most important in sheep exchange volume in the middle Atlas and whose markets could not be investigated, still show an important betweeness, ranking them among the first 7 communes surveyed. These are the most central localities because the actors must pass through this locality to reach other localities "intermediarity". They provide a coordinating role and control (Table 5).

Table 5. *Classification of Localities by Intermediary or (betweenness)*

Commune	Betweeness
Boumia	575, 38
Sidi Bettache	546,8
Mrirt	495,33
Azrou	329,25
Khenifra	205,81
Timahdite	92,16
Aguelmous	67,83
Sidi Lamine	61,28
Itzer	47
Sidi El Makhfi	44,96
Sidi Amar	34,5
Ain Leuh	9,16
El Hajeb	5,5

Subject to the sampling bias for Khenifra and Aguelmous whose markets could not be investigated, we can say that Boumia, Sidi Bettache and Mrirt are the three communes that have a very central place in the studied network, so they are crossroads that will promote the spread of diseases but which will also have the ability to control the movement of animals.

The (Components): Three "Weak Components" are identified within the network, providing information on the fragmentation of the network by the detection of isolated settlements; two are represented by the communes of Aghbalou and Had Bouhssousen, while the third is composed by the remaining communes of the network which are 63 (Table 3). Communes that are strongly connected can be grouped into subgroups. Within the network, there is a first sub-group of "strong components" made up of 13 communes which is the most important component of the network and a second subgroup composed of 5 communes (Table 3 and Figure 4).

The points of articulation or (cutpoints): These are the critical points whose if removed of the network can upset its entire structure. These points were five in the studied network. These are the communes of Boumia, Khenifra, Azrou, Mrirt and Itzer (Table 3).

Limitations of the Mobility Study

The limits of this mobility study are:

- The duration of the investigation that was "Instantaneous". Indeed, it would be necessary to repeat these surveys at different time steps to be able to show the existing fluctuations.

- All markets could not be visited for lack of time and remoteness, namely the two largest strategic markets with the largest volume of trade in the Middle Atlas which are Aguelmous and Khenifra, nevertheless, the particularity of the mobility survey to capture the links in contact with other nodes made it possible to capture data on these 2 markets.

DISCUSSION

The role of the lamb's producer's zone in the supply of sheep in the different provinces of the country has been highlighted. Indeed, most of the nodes with the outgoing links but with zero inbound link are in the zone of the lamb's producers. Conversely, most nodes with inbound but no outbound links are in other remote regions of the country that are sourcing from the Middle Atlas.

The majority of movements in the network are represented by movements from lamb's producer's farms to strategic markets in the Middle Atlas, which account for 58.58% of trips, while movements from the strategic markets of the middle atlas to the area of the fatteners represent 10, 63% of trips.

These two big movements are explained for the first by firstly the existence of large strategic markets in middle atlas whose exchange volume is very important (Between 80,000 head/month for the Boumia market and 416,000 for the Aguelmous market)[14] allowing the concentration of sheep products of the massifs at their level before being sold to the other provinces of the country, and secondly by the fact that most of the commercial exchanges in Morocco are done at the level of the markets[15] wich is in agreement with our find (58,58%), while a very small proportion is realized between farms for which Boulanouar et al[15] cited 5% of all sales which would relate to the average of 5,91% found in this study, who is the average of 11.82% exchange between lamb producers and 0% exchange between lamb producers and fatteners.

While for the second movements, this is perfectly in line with the results of the ONSSA 2008[16] survey conducted among fatteners, which reports that the majority supply area for these breeders was the Middle Atlas markets. These two major movements are behind other more extensive distribution movements from the Middle Atlas markets and the fatteners zone that could well explain the rapid spread of PPR in Morocco in 2008 given the extent of their reach found in the mapping of sheep flows from the present study, which seems to be in agreement with the major destinations identified by ONSSA in 2012.[14]

Within the network only a few municipalities are very linked, maximum for Boumia, Sidi Bettache, Mrirt, Timahdit and Azrou with respectively 26, 25, 19, 17 and 14 links. In fact, these communes correspond to the town of fatteners for the case of Sidi Bettache and the communes housing the major strategic markets of the Middle Atlas for the four others, while others have about 3 links (average degree=3.4). It should be noted that although the markets of the communes of Khenifra and Aguelmous which comprise the two largest markets of the massif in terms of volume of exchange could not be investigated, they still stand out with respectively 9 and 7 links, showing the importance of these communes in the exchange circuit. Such a distribution of links with a great heterogeneity of the number of links shows the properties of the network which is of the "scale free" type. Scale free networks are extremely vulnerable to epidemics; they have been described as networks capable of spreading and persisting infections irrespective of the pathogen,[17] thus showing that the virus has been able to spread quickly. An average diameter indicates that the number of generations for a disease to spread throughout the network is reduced.

The two "strong components" found confirm the very strong commercial relationship existing between the middle Atlas breeding farms and the markets of this massif on the one hand (size of 5 communes) and between the latter and the fattening farms on the other hand (size of 13 communes) which explains the concentration of the disease in these areas as noted by Diallo and Campo in 2008.[2] In this connection, it can be said that the predominantly extensive breeding method of the middle atlas has favored the contact between healthy animals and sick animals and made it possible for the disease to spread in this region whereas the strong commercial relationship with the fatteners has allowed to propagate it at the level of these establishments which contribute to disseminate it in their turn.

The points of articulation or "cutpoints" are of particular interest when we seeks to identify critical positions in the networks, if we removes them, we modifies the properties of connectivity of the graph.[18] In this context, these nodes can be considered as indicators of a high-risk of transmission of the pathogen. The main centers are the 5 communes of Boumia, Khenifra, Azrou, Mrirt and Itzer, corresponding to the communes hosting strategic sheep markets of the Middle Atlas for the first four in terms of centrality of the movements (degree and betweenness) but also of cohesion (clustering, cutpoints). These 5 nodes play an important role for the diffusion, because they are at the same time susceptible to infect and to transmit the infection, which shows their importance for the control of the disease.

CONCLUSION

The results of this study highlighted the role of the lamb's producer's zone as well as the role of the fatteners' zone in the sheep supply of the other regions of the country. The communes concerned in the area of the breeders producing lambs host the major markets of the Middle Atlas which serve to very great distances the various other communes of the country to which they are very related, it is the same for the commune of the fatteners whose supplies in these markets before serving even more remote areas. These key crossroads represent a great risk of spreading diseases over very long distances across the country in the event of epizootics, since the structure of the network is sensitive to the spread of diseases, but they will however have also the ability to control the spread through the control of the movement of animals and their health situation at their level.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Original Research

Epidemiological Description of a Protracted Cholera Outbreak in Hagadera Refugee Camp and the Surrounding Host Community within Fafi Sub County and Garissa County in Kenya during March-September 2019

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ABSTRACT

Background
On 27th March 2019, the Hagadera Refugee Camp reported an outbreak of acute watery diarrhea. An investigation was initiated to confirm the causative organism and define the epidemiology of the outbreak to support evidence-based control measures.

Method
A suspected case was a resident of Hagadera Refugee Camp or the surrounding community with a sudden onset of acute watery diarrhea and vomiting between March 27 and September 16, 2019. A probable case was defined as a suspected case with a positive rapid test for *Vibrio cholerae*; a confirmed case was a probable case with a positive stool culture for *V. cholerae*. We conducted a systematic case finding by visiting health facilities and villages. We reviewed patient records to identify suspected cholera case-patients. We conducted a descriptive epidemiologic study, examining the distribution of the cases. We computed the attack rates by age, sex, and residence. The case fatality rate was calculated as the ratio of the total number of suspected cholera death to the total number of cholera case-patients. We conducted targeted interventions including spraying, handwashing demonstration, distribution of soaps health education and promotion.

Results
We identified 667 suspected cholera cases between March and September 2019 of these, 38% (253/667) had a positive rapid diagnostic test for *V. cholerae*; 6% (43) were negative and 56%(371) rapid diagnostic test (RDT) were not conducted. Out of the 94 rectal swabs for culture, 71% (64/94) were confirmed to be *V. cholera* O1 serotype Inaba. The epidemic curve exhibited a continuous common-source outbreak with several peaks. The mean age of the case-patients was 15-years (range: 0.2-70-years). Both males and female had an attack rate of 9/10000 respectively. The highest attack rate was in ≥30-years (14 per 10,000).

Conclusion
This was a continuous common source cholera outbreak caused by *V. cholerae* 01 serotype Inaba. We recommended strengthening the surveillance system improving early detection and effective response.

Keywords
Cholera; Outbreak; Kenya; Garissa; Protracted; Hagadera; Epidemiological; Descriptive.

Abbreviations
RDT: Rapid diagnostic test; WHO: World Health Organization; CTC: Cholera Treatment Centre; AWD: Acute watery diarrhea; TCBS: Citrate bile salt sucrose; CFR: Case fatality rate; OCV: Oral cholera vaccine; IRC: International Rescue Committee; UNHCR: United Nations High Commissioner for Refugees

Epidemiol Open J. 2019; 4(1): 31-35. doi: 10.17140/EPOJ-4-116

BACKGROUND

Cholera is an intestinal infection caused by a bacteria *Vibrio cholerae*. It remains a global health problem with several hundreds of thousands of reported cases each year. Despite all the major advances in research, the condition still remains a challenge to the modern medical world.

The disease is often contracted from drinking unclean water. Each year, 1.3 million to 4 million people around the world suffer from cholera and 21,000 to 143,000 people die of the disease, the incidence is estimated to be greatest in children less than 5-years of age according to the World Health Organization (WHO).

Cholera is a high-risk in sub-Saharan Africa where clean water and sanitation are often lacking. The disease crops up in other parts of the world when conflict or natural disasters damage water systems and displace families. Though there has been a decrease in the endemicity and intensity of epidemics across the continent, the case fatality rates remain higher in Africa than elsewhere. Some of the risk factors that contribute to these outbreaks include; water contamination, heavy rainfall and flooding and population displacement. It has also been associated with poverty and closely linked to inadequate drinking water and poor sanitation.

In Dadaab Refugee Camp, a cholera outbreak has been common trend annually over the last seven years with the highest caseload reported between November 2015 and March 2016 outbreak, with around 1234 suspected cholera cases and 5 deaths.

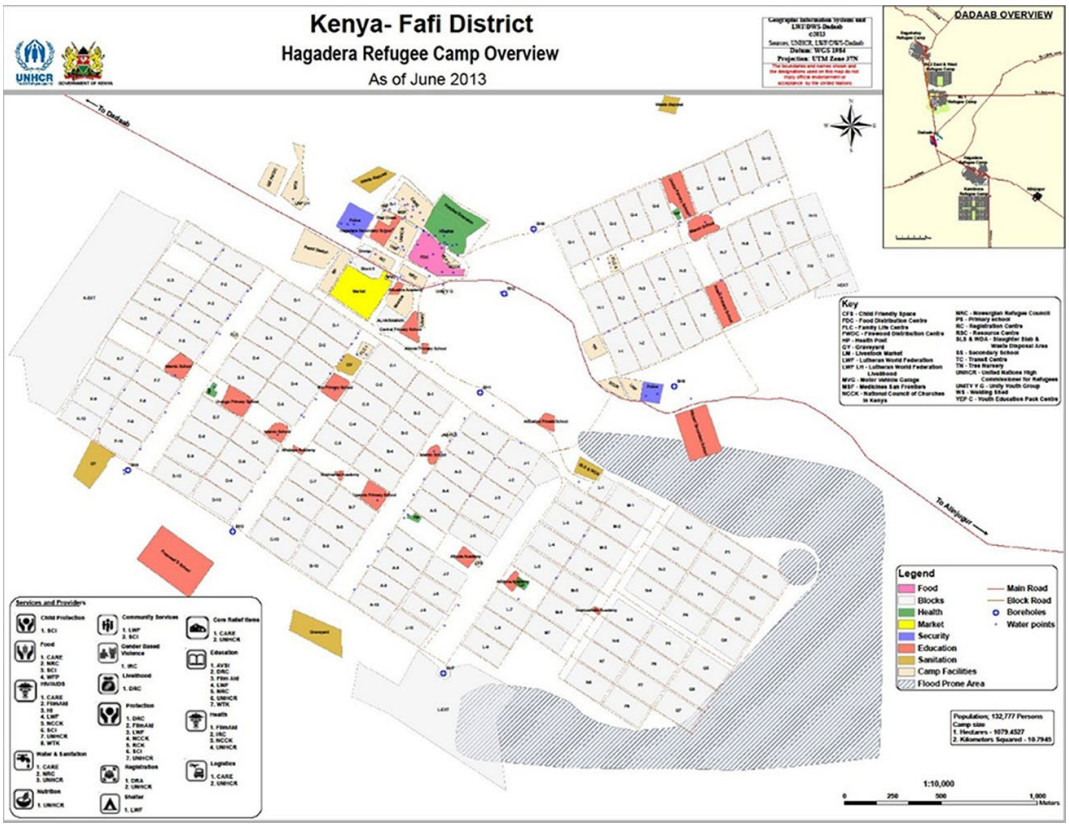
On 27th March 2019, The International Rescue Committee (IRC) (health implementing partner) in Dadaab Refugee Camp reported an outbreak of acute watery diarrhea. A 15-years-old had reported to the IRC main hospital with acute onset on watery diarrhea and vomiting, two days later on 29th March, two children aged 5 and 4-years respectively, reported with similar presentation and were admitted at the Cholera Treatment Centre (CTC). Preliminary assessment by the health team and Sub County Surveillance officer showed that these case-patients developed these symptoms a few hours after eating food. The report also highlighted that there were 8 suspected acute watery diarrhea (AWD) developed symptoms after having attended a wedding in the same community initial testing with cholera rapid diagnostic test showed the samples were positive. We conducted this investigation to confirm the causative organism and describe the epidemiology of the outbreak in order to support evidence-based control measures.

METHODS

Study Site

The outbreak occurred in Hagadera Refugee Camp and the surrounding host community within Fafi Sub County, Garissa County in Kenya. Dadaab Refugee Camp is located in the Northeastern part of Kenya in Garissa County, about 500 kilometres from Nairobi Kenya's Capital and 90 kilometres from the Kenya-Somalia border.

Figure 1. *Hagadera Camp Snapshot*



Dadaab is a complex with three Refugee Camps that have a combined population of 208,733[1] refugees. Hagadera Refugee Camp is one of the complex with a population of 117,000 of whom 4860 (4%) are children <1-year. (Figure 1) whereas the surrounding Fafi Sub County comprising of a population of approximately 11,700. The two regions reported 585 and 82 cases for Hagadera and the surrounding host community respectively. The population is nomadic and the main economic activity in the area is pastoralism

Case Definition

A suspected case was a resident of Hagadera Refugee Camp or its surrounding environs with sudden onset of acute watery diarrhea and vomiting between March and September 2019. A probable case was a suspected case with a positive rapid test for *V. cholerae*; a confirmed case was a probable case with a positive stool culture for *V. cholerae*. In an event of demise of a suspected, probable or confirmed cholera case – this was termed as cholera death.

Case Finding

A systemic case finding was carried out by paying a visit to the health facilities and households affected within the camp. During the outbreak period (27th March-15th September, 2019), the team reviewed the patients' records to identify suspected cases of cholera among the patients. Thereafter, verification was done by visiting infected households and health facilities. A checklist was administered to obtain demographic and epidemiological information for analysis and those fitting the criteria were referred to CTC for further care and management. The community health workers were instrumental in the referral of cases from the households to the health facility.

Descriptive Epidemiologic Analysis

To examine how the cases were distributed, a descriptive epidemiological study was conducted, the development of an epidemic over the period of time was represented by the use of an epidemic curve. The finding and the data from the analysis were used to compute the attack rate for both age and sex. The case fatality rate was calculated using the prescribed formula as a ratio of the total number of suspected cholera death to the total number of case patient.

Laboratory Analysis

In the health facility, stool specimens were collected before patients received antimicrobial treatment and tested using Onsite rapid diagnostic test kit. The results were read at 10 min and interpreted following the manufacturer's recommendation. The test was considered positive if the control line and either T2(O1) or T1 (O139) or both (O1 and O139) appeared; negative if the control line only appeared and invalid if the control line did not appear. The reported sensitivity for onsite rapid diagnostic kit (RDT) under field conditions was 96.7 and specificity was 94. All stool specimens meeting criteria proceed to laboratory for bacteriological culture analysis.

Stool Culture and Bacterial Identification

Stool specimens were inoculated in alkaline peptone water, incubated at 35-37 °C for 4 h, plated on thiosulfate citrate bile salts sucrose (TCBS) agar plates and incubated overnight at 35-37 °C. Culture plates were visually examined for medium-sized convex, smooth, yellow colonies and sub-cultured on nutrient agar plates overnight at 35-37 °C. Colonies from the nutrient agar were screened using oxidase discs (Becton, Dickinson and company, USA) and oxidase positive isolates serotyped using polyvalent O1 specific antiserum (Becton, Dickinson and company, USA) and monovalent Inaba and Ogawa antisera (Becton, Dickinson and company, USA).

Case Management

Cholera treatment center were set up to support in the isolation and management of cholera case-patients. Upon confirmation of a cholera case, the patient was referred to the cholera treatment unit for further management including oral and intravenous rehydration.

RESULTS

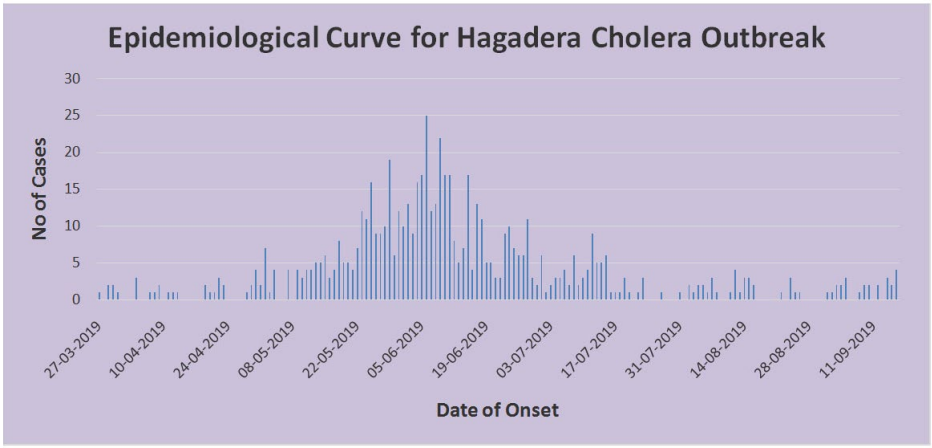
During the outbreak that occurred in Hagadera Refugee Camp and the surrounding host community between March and September 2019, 40% (264/667) of the suspected cholera cases had stool samples collected and tested using RDTs. Of those tested at the different treatment centers, 84% (221/264) were positive with RDT. Out of the 264 stool samples taken to the Laboratory, 30% (78/264) were confirmed to be *V. cholerae* O1 serotype Inaba. The representation in the epidemic curve displayed that the outbreak was from a common source with several peaks and waves (Table 1).

The index case that was admitted on March 27, 2019, showed symptoms of cholera after attending a wedding celebration from block K9 in Hagadera Refugee Camp. The time lag between the index case and notification was due to surveillance challenges. The highest number of cases had onset on 28th May, 2019 in epidemiological week 22, the first case from the surround-

Table 1. *Demographical Breakdown of the Cholera Cases*

Age	0-5		6-10		11-15		16-20		21-25		26-30		31-35		>35	
Gender	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	153	136	33	35	25	25	26	32	17	19	26	25	11	12	35	55
CFR	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Figure 2. *Epidemiological Curve for Cholera Outbreak in Hagadera*



ing host community was reported in epidemiological week 19. The case fatality rate (CFR) was 0.0% (0/667). The mean age of the case-patients was 15-years (Range: 0.2-75 years) (Figure 2).

DISCUSSION

Our investigation findings uncovered a continuous common source outbreak of *V. cholerae* 01 serotype Inaba occurred in Hagadera Refugee Camp and surrounding host community, in Garissa County of Kenya with a CFR of 0.0%. The highest attack rate was in age ≥30 years; there was no difference in gender.

V. cholerae O1, serotype Inaba has been associated with outbreaks of high morbidity and mortality. Epidemics occur mostly in crises situation where water and food supplies become contaminated with *V. cholerae* in areas with crowded living conditions and poor sanitation. Its ability to cause death within hours of onset especially in undeveloped countries has become a major public health challenge. It is therefore important that awareness about cholera outbreaks is created to stimulate better understanding of the disease and lead to development of practical preventive and therapeutic measures. Death of cholera case-patients usually results from profuse secretory diarrhea which leads to severe dehydration, metabolic acidosis, electrolyte imbalance and circulatory collapse.

The continuous common source outbreak that occurred in Hagadera, between March and September 2019 was attributed by several factors; poor sanitation and inadequate water supply, congestion and overcrowding, inadequate availability of pit latrines and limited knowledge by camp residents on hygiene, whereas for the surrounding host community, inadequate water supply, inadequate surveillance system and insecurity that hampered the movement of the response team in controlling the outbreak topped the list of contributing factors. The incapacity of the team to efficaciously single out the source of the outbreak prolonged the exposure beyond one incubation period. Several studies have demonstrated that cholera outbreaks can persist in the community if not adequately investigated.[1,2]

The CFR has been known to be a measure of the adequacy of the health care system in cholera outbreak response.[3] The health team in Hagadera and larger Garissa County managed to maintain the CFR at 0.0% a figure that is well below *t* the recommended WHO standard of ≤1%. Such low CFR suggests adequate preparedness, surveillance, case management, community targeted health promotion.[4] In the year 2010, a cholera outbreak response in northern Nigeria, recorded a higher CFR (3.75%) which was quite higher compared to our study. Another study done in South Sudan showed a CFR as high as 11% in some counties.[5]

Our study found the highest attack rate to be among age ≥30 years, with no significant difference in gender. This could have been due to the fact that this age group was involved in the care and transportation of the cholera case-patients from the villages to the CTCs with subsequent contamination. This contrasts a study done in Kasese District, Western Uganda which showed the highest attack rate among 5-14 years at 4.2%, though there was also no difference in gender. The WHO position paper highlights that young children living in endemic areas are the most affected by the disease but any age group may suffer.[6]

Oral cholera vaccines (OCVs) have been recommended in cholera-endemic settings and pre-emptively during outbreaks and complex emergencies.[7]

This study reveals the challenges and gaps experienced in countries with insecurity regarding, prevention, early detection and effective response to public health threats. However, it also has limitations: Owing to surveillance challenges and insecurity in the area, several cases could have been missed at the beginning of the outbreak, which limits the reliability of our descriptive epidemiology. Also, due to nomadic nature of the pastoralist community surrounding the Hagadera Refugee Camp, there were population movements from one area to another affecting the insurgency to mobile areas within the Refugee Camp.

CONCLUSION

This was a continuous common source cholera outbreak caused

by *V. cholerae* serotype Inaba. We recommended strengthening of surveillance system to improve detection and response.

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AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are not publicly available due to ethical issues but are available from the IRC Kenya Program upon reasonable request or from the Director of Preventive and Promotive Health, Ministry of Health, Garissa County, Kenya.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Verbal informed consent was obtained from the cholera case-patients involved in the study or, caretakers (if the interviewee/patient was a minor) before the start of the interview. We sought verbal consent because this study was conducted as part of an outbreak investigation whose primary purpose was to inform disease control efforts rather than outright human subjects' research. Non-etheless, the purpose of the investigation was explained to the patients. Also patients were informed that their involvement was entirely voluntary and their refusal to respond to any or all of the questions would not result into any penalty. Participants' confidentiality involving personal information were de-identified during data analysis.

CONTRIBUTIONS

KN designed study, analyzed, interpreted data, and drafted the initial manuscript. KN reviewed the initial manuscript for intellectual content. WK, SM conducted a laboratory examination of the samples and wrote the laboratory analysis section. All co-authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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