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Brucella sero-prevalence and associated risk factors among farm animals in El-Gharbia Governorate - Egypt

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Abstract

A cross sectional study was carried out on different districts of El-Gahrbia Governorate, Egypt to evaluate the potential major risk factors, mal- biosecurity practices and their role in the spread of the brucella infection among farm animals. The results pointed out that, prevalence of brucella sero-positive cases among cattle, buffaloes, sheep and goats were (5.09%), (2.67%), (3.5%) and (3%) respectively. A total of 255 newly diagnosed human cases were recorded during a year of the study period (from June 2015 to June 2016) all over the governorate. Concerning the distribution of high-risk activities among the cases, 163 (63.92%) patients were livestock keepers and persons in direct contact with animals, 51 (20%) were veterinarians, 35 (13.73%) were Para veterinary medicals and 5 cases (1.96%) recorded in persons proved not to deal with animals in addition to one child (0.39%). A structured questionnaire was used to collect information on the animal herds/flocks health and management. It was concluded that some major risk factors play a very important role in the spreading of the disease among farm animals. Addition of new animals and mixed farming were important risk factors for animals brucella sero-positivity. On the other hand, presence of good sanitary measures in farms, the proper use of disinfectants and the presence of adequate veterinary services were identified as protective factors.

Keywords*: Brucella, El-Gharbia governorate, Egypt, risk factors, Ruminant, human.*

Introduction

Brucellosis is a bacterial zoonotic infection, caused by members of the *Brucella* genus. Currently there are nine known species in terrestrial animals and 2 in marine animals. The species in terrestrial animals include *B. abortus, B. melitensis, B. suis, B. neotomae, B. canis, B. ovis, B. microti, B. inopinata* and *B. papionis* **(Scholz *et al.,* 2008; Scholz *et al.,* 2010** and **Whatmore *et al.,* 2014).** The species in marine mammals include *B. ceti* and *B. pinnipedialis* **(Foster *et al,* 2007).** The most common domestic animals affected by brucellosis are cattle, buffaloes, goats, sheep, pigs and camels **(WHO, 2006).**

Brucellosis is still endemic in most parts of the world in both humans and animals except a few countries like Australia, Canada, Cyprus, Finland, Denmark, The Netherlands, United Kingdom, Norway, Sweden and New Zealand, where bovine brucellosis has been eradicated (**Seleem *et al,* 2010)** It is primarily a reproductive disease, characterized by abortion, retained foetal membranes and impaired fertility. The disease is important because of its widespread distribution, multiplicity of hosts and the public health hazard that it causes **(Refai, 2002).** Humans are infected with *B. abortus, B. melitensis, B. suis, B. canis,* and marine mammal *Brucella* species. The disease is mostly caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products **(Elkhansaa and Abd Rahman, 2014**). In Egypt, it is largely an occupational disease involving veterinarians, dairymen, livestock producers, butchers and lab workers. (**Holt et al,**

2011).
The most effective way of reducing incidence in humans, is by controlling brucellosis in livestock **(Jelastopulu *et al,* 2008).** Brucellosis was first reported in Egypt in 1939 and is now considered endemic in most parts of the country **(Refai, 2002).** Despite its economic and public health importance, in recent years, the official Egyptian brucellosis control programme does not appear to be fully implemented **(Samaha *et al,* 2009).** Furthermore, little has been done to control brucellosis in small ruminants, which has led to the transmission of *B. melitensis* into cattle and buffalo populations of Egypt. *B. melitensis* biovar 3 is currently the most common isolate of Brucella in Egypt **(Refai, 2002),** while isolates of *Brucella abortus* biovar 1 and *Brucella suis* biovar 1 are frequently isolated **(Menshawy *et al,* 2014).** *Brucella melitensis* poses the higher public health threat due to its high pathogenicity and infectiousness **(Afifi *et al,* 2005).**
The aim of the current study was to investigate the epidemiology of brucellosis in El-Gharbia governorate. The study objectives were i) to estimate seroprevalence of brucellosis in farm animals (cattle , buffaloes, sheep and goats) at both individual animal and household levels ii) study patterns of occurrence of brucellosis in dairy animals, veterinarians and animal handler iii) to identify risk factors for the spread of brucellosis in studied seropositive animals.

Materials and Methods

The study was carried out from June 2015 to June 2016 on different districts in El-Gharbia Governorate. The study covers 1450 adult animals belonging to different species (550 cattle, 300 buffaloes, 400 sheep and 200 goats) from sixty nine farms. The sample size from each district was determined based on the density of animals in each district. Farms were randomly selected using the records of the general organization for veterinary services. The techniques used in collection of samples were simple random and\or systemic sampling techniques according to **Thrusfield (1995).** Sampled animals from each herd were randomly selected using a table of random digits for only female cows older than 6 months of age.

Blood sample collection

A sample of approximately 10 ml of blood was collected from the jugular vein of each selected animals, using plain vacutainer tubes. The samples were left at room temperature to allow clotting for serum separation. The serum was collected and stored at –20°C until performing serological testing.

Serological analysis

All serum samples were screened using Rose Bengal Plate test (RBPT). All sera which tested positive to the RBPT were further tested using complement fixation test (CFT) for confirmation according to the procedures described by **Alton *et al,* (1988)** and **OIE, (2009).**

Questionnaires

1- A Herd/flock questionnaire was fulfilled by the farm managers to collect information on management and several herd's health and sanitary items to identify the possible independent variables associated with the presence of seropositive animals on the farm. The questionnaire cover in addition to the basic herd/ flock information, the following items:
a- The management information including multiple raising of different animal species, presence of separate parturition pens and addition of newly purchased animals.
b- Common sanitary measures including regularity of disinfection program, disposal of aborted foeti and foetal membranes and quality of veterinary services.
c- The clinical data including abortion, stillbirth and retained foetal membranes in addition to brucellosis vaccination history.
2- Questionnaire for human patients at the fever hospital in El-Gharbia governorate include the following data:
Information about age, sex. job and address.
History of contact with animals.
Severity of infection and clinical history

Statistical analysis

A univariate analysis of the different studied variants with *Brucella* antibody status was con-

ducted initially using chi-square tests. Chi-square is significant at $p < 0.05$ with confidence interval of 95%, Pearson's chi-square test of independency (χ^2) measures whether there is an association or relationship between two categorical variants. *Phi* coefficient values of ≤ 0.2, 0.3-0.6 and ≥ 0.7 correspond to that could be described as small, medium and large effects respectively **(Cohen, 1988)**.

Results

Table (1). Prevalence of Brucellosis *in El*-Gharbia Governorate among different animals species using RBPT and CFT

	RBPT		CFT	
	Positive		Positive	
	No.	%	No.	%
Cattle (550)	30	5.45	28	5.09
Buffaloes (300)	9	3	8	2.67
Sheep (400)	15	3.75	15	3.75
Goats (200)	7	3.5	6	3

Table (2). Distribution of Brucella positive animals through different districts of El-Gharbia governorate and the number of positive human cases

District	Samnod	Mahla-kobra	Kotor	Basion	Zyfta	Santa	Tanta	Kfrzyat	Total
Cattle (550)	0/50	5/80	6/70	1/40	1/74	1/30	5/68	9/138	28/550
	(0.00)	(6.25)	(8.57)	(2.50)	(1.35)	(3.33)	(7.35)	(6.52)	(5.09)
Buffa-loes (300)	1/22	2/70	1/50	0/40	1/28	1/38	1/30	1/22	8/300
	(4.55)	(2.86)	(2.00)	(0.00)	(3.57)	(2.63)	(3.33)	(4.55)	(2.67)
Sheep (400)	1/26	3/43	1/40	1/30	1/42	1/29	3/55	4/135	15/400
	(3.85)	(6.98)	(2.50)	(3.33)	(2.38)	(3.45)	(5.45)	(2.96)	(3.75)
Goats (200)	1/20	2/30	1/18	0/15	0/10	0/12	1/30	1/65	6/200
	(5.00)	(6.67)	(5.56)	(0.00)	(0.00)	(0.00)	(3.33)	(1.54)	(3.00)
Total (1450)	3/118	12/223	9/178	2/125	3/154	3/109	10/183	15/360	57/1450
	(2.54)	(5.38)	(5.06)	(1.60)	(1.95)	(2.75)	(5.46)	(4.17)	(3.86)
human cases	9	63	42	10	36	15	48	32	255

NB; number up the slant is positive cases, under the slant is number of samples collected from this district, number between the brackets is the percent of positive cases in the district.

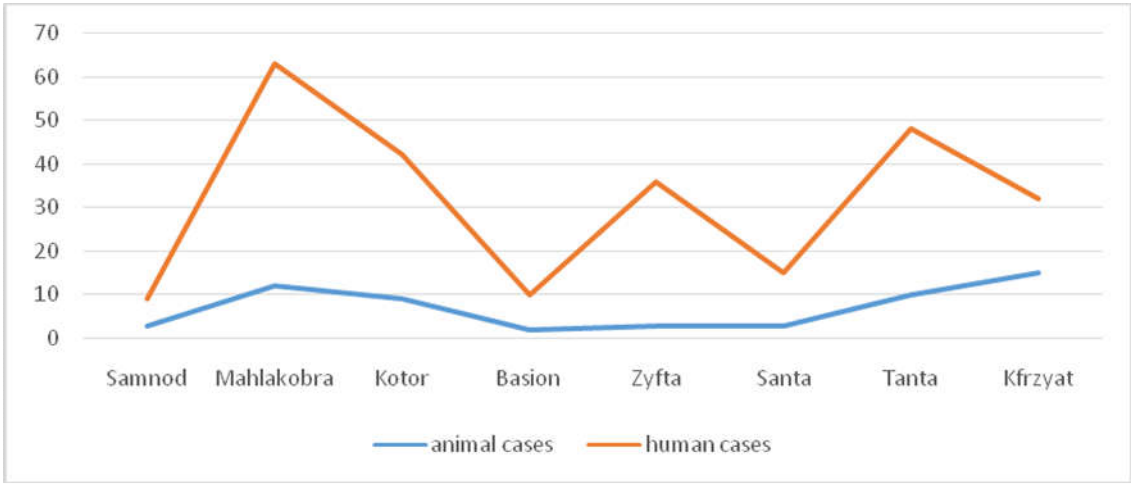

Figure (1). Distribution of positive animals cases through districts of Gharbia Governorate in relation to positive human cases.

Table (3). Distribution of the *Brucella* seropositive and seronegative herds/flocks of farm animals and the relevance with their different investigated variables

Variable	Cate-gory	Number (%)	Brucella result			
			Positive		Negative	
			No.	(%)	No.	(%)
Awareness about brucellosis	Yes.	21 (30.43%)	3	14.29	18	85.71
	No.	48 (69.57)	22	45.83	26	54.17
Isolation of newly bought animals	Yes.	21 (30.43%)	6	28.57	15	71.43
	No.	48 (69.57%)	8	16.67	40	83.33
No Mixed farming*	Yes.	12 (17.39%)	7	58.33	5	41.67
	No.	57 (82.61%)	9	15.8	48	84.2
Regular cleaning of barn and Using of disin-fectants	Yes.	18 (26.09%)	5	27.78	13	72.22
	No.	51 (73.91%)	25	49.02	26	50.98
Proper Veterinary service	Yes.	27 (39.13%)	2	7.4	25	92.59
	No.	42 (60.87%)	31	73.8	11	26.19
Occurrence of abortion	Yes.	8 (11.59%)	8	100	0	0
	No.	61 (88.41%)	16	26.23	45	73.77
Presence of parturition pens	Yes.	14 (20.29%)	3	21.43	11	78.57
	No.	55 (79.71%)	22	40.00	33	60.00
Proper dispose of placentas and aborted fetuses	Yes.	23 (33.33%)	5	21.73	18	78.26
	No.	46 (66.67%)	13	28.26	33	71.74
Vaccination against Brucella spp.	Yes.	22 (31.88%)	3	13.63	19	86.36
	No.	47 (68.12%)	22	46.8	25	53.2

NB: No. of studied herds is 69. *Mixed farming: raising sheep and/or goats along with cattle. Yes: means the presence of the factor. No: means absence of the factor

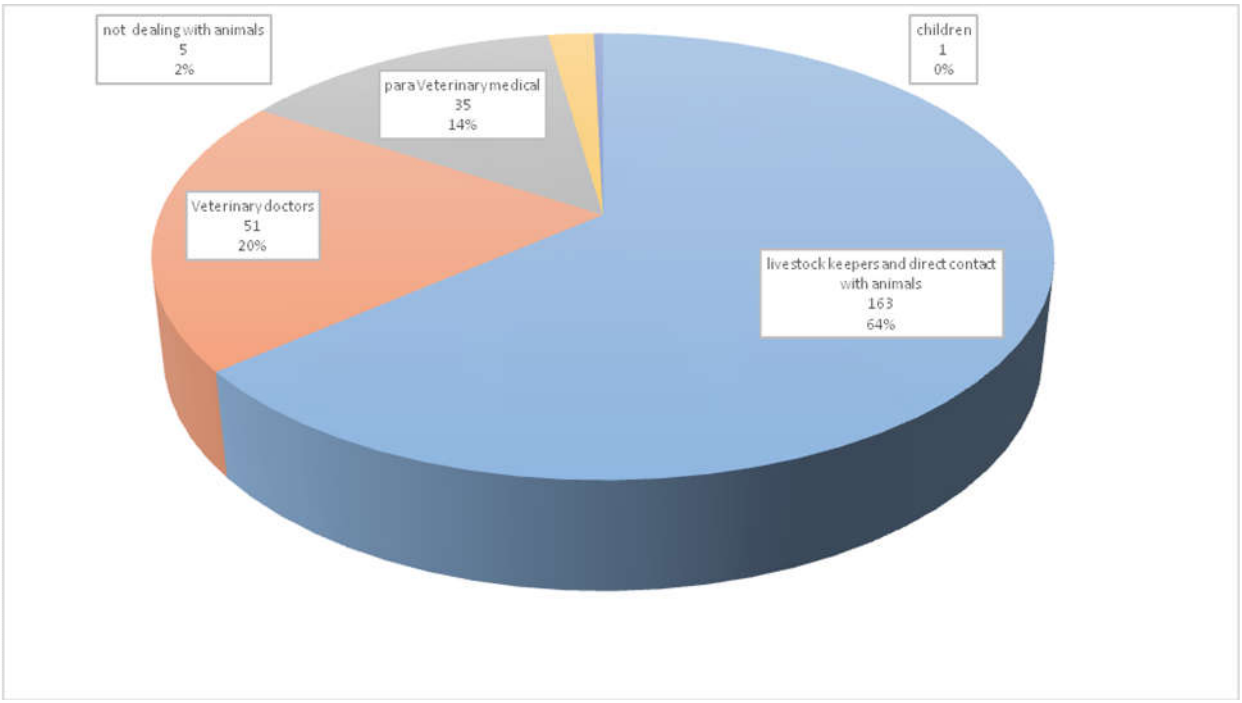

Figure (2). Classification of human positive cases according to their dealing with animals.

Table (4). Association and effect size between different risk factors and seropositive reactors of brucellosis in El-Gharbia Governorate.

Risk factors	Phi coefficient values	* Sig. (*p* value)	Effect size
Absence of knowledge about brucellosis	0.321	*0.000000	medium
Addition of new animal without isolation	0.94	*0.000000	large
Rearing of multiple-species	0.984	*0.000000	large
Inadequate cleaning and lack of disinfectants using	0.957	*0.000000	large
Shortage of veterinary service	0.181	*0.000337	small
Late stage abortion	0.877	*0.000000	large
No separate parturition pens	0.057	0.988	-------
No hygienic disposal of retained foetal membranes	0.842	*0.000000	large
Vaccination against Brucella spp.	0.312	*0.000000	medium

*Chi-square is significant at $p < 0.05$ with confidence level of 95%.

Table (5). Association and effect size between different risk factors and human positive cases in El-Gharbia Governorate

Risk factors	Phi coefficient values	* Sig. (p value)	Effect size
Infected animals kept in farm/flock	0.302	*0.000000	medium
Handling aborted foeti	0.183	* 0.000261	small
Previous knowledge about brucellosis	0.156	* 0.001961	small

*Chi-square is significant at $p < 0.05$ with confidence level of 95%.

Discussion

Brucellosis is a worldwide zoonotic disease that is recognized as a major cause of heavy economic losses to the livestock industry and poses serious human health hazard, so early detection, elimination of reactor animals is considered the corner stone of control and eradication of brucellosis **(McDermott and Grace, 2012).**

This study was conducted with the aim of identifying sero-prevalence and potential risk factors associated with brucellosis infection in ruminants and human in El-Gharbia Governorate. This information is required in order to outline the control measures of brucellosis and minimize the risk of human brucellosis **(Lithg-Pereira, 2001)**. In this investigation, we used two serological tests: the RBPT and CFT. RBPT is known to have high analytical sensitivity and lower specificity when compared to other serological methods **(Alton *et al,* 1988).** To overcome the low specificity of the RBPT, we used CFT as a superior confirmatory test.

Prevalence of brucellosis positive cases among different species of farm animals as shown in tables (1) revealed that cattle showed the highest percentage of brucella infection (5.09%) followed by sheep (3.75%) then by goat (3%) and buffaloes (2.67%). Several authors recorded the prevalence of brucellosis among different species of farm animals in Egypt.

Soliman (1998) reported 7.2% prevalence in cattle, 3.7% in buffaloes, 7.6% in sheep and 8.5% in goats. **Sharkia Veterinary Directorate (2004)** reported Brucella prevalence as 0.59% in cattle, 0.37% in buffaloes, 1.72% in sheep and 0.85% in goats. **Samaha *et al.* (2008)** reported this prevalence to be 5.44% in cattle, 4.11% in buffaloes, 3.52% in sheep and 2.19% in goats. The Sero-prevalence of brucellosis in sheep, goats, buffaloes and cattle were

widely but unevenly distributed throughout flocks and provinces **(Jackson *et al,* 2004; Al-Ani *et al,* 2004** and **Muma *et al,* 2006).**

The variations between prevalence of brucella infection obtained in this study and those obtained by other authors in different localities in Egypt may attributed to various factors. These factors include the time of studies performing, the areas from which animals were examined as well as the extend of population movement and the evolutionary change in animals husbandry which affect the rate of exposure.

The prevalence of animal brucellosis in Tanta, Mahlakobra, kotor and kfrzyat were higher than those reported in the other districts (Table 2 and Figure 1). Those four districts are the largest in animal population and this affect the quality of veterinary services provided to both small ruminant farmers and dairy cattle farmers. Proper animal movement control and vaccination of small ruminants are necessary to bring the prevalence of brucellosis in animals down to the levels that seen in the other districts.

In this study, we used pre-prepared questionnaires to collect information about management and sanitary behaviors, which may affect the spread of brucellosis. The questionnaire was of unquestionable value as a tool that assisted the attainment of qualitative and quantitative data. However, it would not be sufficient for the attainment of precise data because, as in all forms of interviews, it depends on the memory of the informer for the declaration of the information and to the impartiality of the answers, which cannot be guaranteed. The list of investigated management and sanitary practice items in this study include, awareness about brucellosis, isolation of newly bought animals, mixed farming, regular cleaning of barns and using of disinfectants, proper veterinary service supervision, occurrence of abortion, presence of parturition pens, proper disposal of placentas and aborted fetuses and history of previous vaccination against Brucella species (Table, 3).

Out of 69 interviewer, 57 (82.61%) practice mixing farming, 55 (79.71%) has no isolated parturition bens, 51 (73.91%) did not use regularly disinfectants, 48 (69.57%) has a limited awareness about the diseases and did not isolated the newly introduced animals to their flocks/herds, 46 (66.67%) did not hygienically dispose the placentae and aborted foetei. In the other hand, 27 (39.13%) of the interviewers has adequate veterinary supervision in their farms, 22 (31.88%) using Brucella vaccines and only 8 (11.59%) noticed the occurrence of unusual cases of abortion among their animals. The highest percentage of infected flocks/herds associated with the above mentioned items are occurrence of unusual cases of abortion among their animals (100%), absence of adequate veterinary supervision (73.8%), practicing of mixing faming (58.33%), no regular use of disinfectants (49.02%) and for those who did not use Brucella vaccine (46.8%). **Asfaw (1998)** and **Gebretsadik (2005)** have documented similar findings**,** they suggested that little attention has been given to prevent brucellosis and that, in turn, contributes in the spread and transmission of the infection in the area.

The statistical analysis (Table, 4) showed that inadequate cleaning and lack of disinfectants using, rearing of multiple-species; late stage abortion and the lack of hygienic disposal of retained foetal membranes, addition of new animal without isolation, have the highest risk impact in the spread of the diseases among farm animals. Absence of knowledge about brucellosis and absence of vaccination program against brucellosis constitute a medium risk affect on the presence of brucellosis seroreactor animals, while, shortage of veterinary supervision was of small risk impact.

Taddele, (2004) identified the absences of hygienic practices as a risk factors associated with seropositivity to Brucella antigens. Addition of new animal and mixed farming especially raising sheep and/or goats along with

cattle was reported by many researchers to be a risk factors for Brucella transmission between different animal species **(Omer *et al,* 2000, Abbas and Agab, 2002, Al-Majali *et al,* 2007)**. The use of disinfectants and the presence of adequate veterinary services supervision were identified as the factors that protect against bovine brucellosis (tables 3 & 4). Similar observations were reported for sheep, goats and camels **(Al-Talafhah *et al,* 2003** and **Al-Majali, 2005)**. Proper disposal of aborted materials and highly hygienic procedures are extremely important steps in any successful Brucella control program. It is well known that delivering adequate animal health services results in a low incidence of diseases, and especially those diseases that have an infectious nature. In addition, controlling brucellosis in small ruminants (mainly by Rev-1 vaccination) will indirectly reduce the prevalence of this disease in small ruminants and cattle. Poor veterinary service has been identified as a risk factor for brucellosis (**Samartino, 2002)**.

According to the records of **Ministry of health in El-Gharbia governorate (2014)**, about **255** human cases proved to be infected with brucellosis all over the governorate. (Table, 2 & Figure, 1) The highest number of human cases were recorded in Mahlakobra, Tanata, Kotor, Zyfta and Kfrzyat which are mostly the districts showed the highest prevalence of animal brucellosis in this investigation except for Zyfta. Concerning the distribution of high-risk activities among the cases, 163 (63.92%) patients were livestock keepers and persons in direct contact with animals, 51 (20%) were veterinarians and 35 (13.73%) were Para veterinary medicals. Five cases (1.96%) recorded in persons proved not to deal with animals and one child (0.39%) was infected with brucellosis (Figure 2). This finding substantiate the concept that brucellosis in El-Gharbia Governorate are mostly of occupational pattern.

McDermott *et al.* (2013) found high seroprevalence (an average of 11%) among livestock keepers/abattoir workers, and 7% among suspect hospital patients. **Mantur *et al,* (2006**) found that 2% of patients in the general hospital tested positive for brucellosis. In most countries, brucellosis is a notifiable disease. Ingestion, direct contact and airborne infection (laboratories and abattoirs) are the main routes of disease transmission to human. The disease primarily affecting consumers of raw milk and its derivatives, farmers, butchers, veterinarians and laboratory workers (**Pappas and Memish, 2007)**.

Avdikou *et al,* (2005) identified a potential role for sheep and goats in the transmission of Brucella species to large ruminants and went onto investigate at how human behavior may influence the spread of the disease both between animals and from animals to humans. Children are probably infected when consuming unheated milk and milk products. It is also possible that children accompany their parents to the farms, so they could also be infected by direct contact with animal **(Issa and Jamal, 2000).**

Statistically, in this study, the presence of infected animals kept in farm/flock constitute a medium effect risk factor for presence of human cases, while, handling aborted foeti and absence of knowledge about brucellosis were of small risk impact (table, 5). As in other regions, the main risks for people are occupational (contact with livestock). In countries where eradication in animals is not feasible, prevention of human infection is primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety **(Luna-Martínez and Mejía-Terán, 2002)**. Predominance of smallholdings that favor close contacts between humans and animals, presence of mixed populations of animals and consumption of unpasteurized milk and dairy products are among the main major risk factors for *Brucella* infection in Egypt (**Refai, 2002; Holt *et al.* 2011** and **Hegazy *et al.* 2011**).

In conclusion

this study documented the importance of animal brucellosis in El-Gharbia Governorate. The study shed more light on the lake of the principle information concerning the nature of disease in animals and the shortage of veterinary extension services to guide and aware animal owners about the epidemiological pattern of the disease. More attention should be paid towards implementing a proper control program for animal brucellosis and more efforts should be directed towards improving the animal health system in this Governorate that with large animal population and share borders with other Governorates.

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