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# ABSTRACT

Data for the prevalence of brucellosis in ruminants and humans were scarce in Egypt. Recent studies suggested that, the disease was endemic in all ruminant species, with a high prevalence. Since 1981, the national brucellosis control programme involved testing all female ruminants older than six months and slaughtering serological positives, with the voluntary vaccination of calves and kids. The aim of present study was to assess the impacts and costs of the current control programme on the temporal pattern of brucellosis among ruminants and humans using data from the active surveillance programme between the years 1990 and 2012. Frequency tables were used to calculate the proportions of animals tested and proportions of seropositive for *Brucella* spp. To compare between seropositive proportions in different years, univariate binary logistic regression model, with seropositive as the response variable and year 1990 as a reference was used. The annual proportion of positive animals ranged from 0.66% to 4.01% in the study period. There was a significant decrease of the positive proportion of ruminants between 1990 and 2003 (P< 0.001), followed by an increase from 2004 to 2010 then a significant decrease in 2011 and 2012 (P< 0.001). The incidence of brucellosis in humans was also decreasing. These results indicated that brucellosis is endemic in ruminants and humans in the study area. Further studies are required for determining the socio-economic impacts of brucellosis and its control measures.

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# INTRODUCTION

Brucellosis is a zoonotic disease of public health and economic significance in most developing countries. Although the disease has been controlled in many countries, it is still endemic in others and the rate of incidence is increasing in regions such as the Middle East and central Asia, where new foci of brucellosis are emerging (Pappas and Memish 2007). Brucellosis is caused by Gram negative coccobacilli of the genus Brucella within the genus Brucella there are six major species responsible for the infection of animals and humans; B. melitensis, B. abortus, B. suis, B. neotomae, B. ovis and B. canis (Alton et al., 1988; Glynn and Lynn, 2008). Brucellosis not only affects animals but also humans. Brucellosis can be transmitted to humans via direct contact with livestock (occupational disease for farmers, veterinarians and abattoir personnel) or more often via the ingestion of unpasteurized milk or milk products (Godfroid et al., 2005). Brucella abortus was found in uterus, milk, mammary glands and associated lymph nodes from aborted heifers. It was also found in apparently healthy calves born from experimentally infected heifers (Xavier et al., 2009). It is not likely that Brucella can be transmitted to humans via consumption of meat however unsafe butchering and consumption of undercooked meat can be a potential route for human infection (Glynn and Lynn, 2008). About 500 human brucellosis cases are reported annually worldwide. However the estimated number of unreported cases due to the unspecific clinical symptoms of the disease is supposed to be 10 times higher (Pappas et al., 2006). In endemic countries prevalence rates often exceed 10 cases per 100,000 population (Pappas et al., 2006). Brucellosis has been successfully controlled and eradicated in developed countries by the implementation of a vaccination programme followed by the testing and slaughtering of the infected animals (Marcotty et al., 2009). However in developing countries due to the lack of data about brucellosis, scarcity of financial resources as well as the perceptions and attitudes of communities the implementation of such programmes is not possible and other innovative programmes should be thought of (Marcotty et al., 2009).

In Egypt, brucellosis was reported for the first time in 1939, since then the disease has been endemic in the country with a high prevalence. Since 1981, the national control programme for brucellosis in ruminants involves testing all females older than six months and slaughtering serological positives, with voluntary vaccination of calves using *Brucella abortus* S19 vaccine also lambs and kids by Rev1 vaccine (Hassanain and Ahmed, 2012; Refai, 2002). Data for the

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prevalence of brucellosis in ruminants in Egypt were scarce, recent studies suggested the disease is endemic in all ruminant species, with a high prevalence. Few studies have assessed the seroprevalence of brucellosis in ruminants in different geographical regions of the country. In Monofia Governorate (Egypt), 5.36%, 3.33% and 3.17% of cattle, buffaloes and goats were seropositive for *Brucella* spp. by Rivanol test and 7.14%, 4.26%, 2.47% and 6.35% of cattle, buffaloes, sheep and goats were seropositive by the buffered acidified plate antigen, respectively (Samaha et al., 2008). In another study, samples were collected from 126 herds from all over Egypt, 26.66%, 18.88% and 17.22% of sheep flocks, goat flocks and cattle herds were seropositive for *Brucella* spp., respectively (Kaoud et al., 2010). In Kafrelsheikh Governorate (Egypt), the seroprevalence in cattle, buffaloes, sheep and goats was at 12.2%, 12%, 12.2% and 11.3%, respectively (Hegazy et al., 2011). In one of the Nile Delta villages, the individual and household seroprevalence of *Brucella* spp. in cattle and buffaloes was 11.0% and 15.5%, respectively (Holt et al., 2011). Recently, the seroprevalence of *Brucella* reported 18.09% in blood samples from sheep and goat flocks from five Governorates in the Nile Delta (Mahboub et al., 2013).

In Egypt, accurate estimates of the prevalence and/or incidence of human brucellosis were sparse. In a hospital based survey, out of 270 patients with Acute Febrile Illness (AFI) admitted to two hospitals in Cairo, 5% were positive for *Brucella* spp. (Montasser et al., 1991). Between March 1999 and October 2003, blood samples were collected from 10,130 AFI patients from 13 out of 109 infectious disease hospitals all over Egypt, 3% and 11% were positive for *Brucella* spp. by culture and serology, respectively (Afifi et al., 2007). These hospital based surveys are limited because not all patients seek medical care from the public hospitals; consequently estimates from such studies do not reflect the actual incidence and/or prevalence of the disease in the population. In 2007 blood samples were collected from 100 human subjects that had a history of contact with animals at dairy farms and/or abattoirs, 5% to 8% were seropositive for *Brucella* spp. and there were no significant differences between seasons of the year (Samaha et al., 2009). High numbers of seropositive results from the last study reflected the fact that samples were collected from groups with a high risk of occupational exposure to *Brucella* spp. via direct and/or indirect contact with infected animals. The annual incidence of human infections is thought to have increased from 0.5/100,000 in 1994 to 1.9/100,000 population in 1998 (Refai, 2002). In year 2000, it was estimated to be 18/100,000 in Bilbeis district and recently it has been estimated to have been 64/100,000 in 2002 and 70/100,000 population in 2003 in Fayoum Governorate (Egypt), the median age of cases was 26 years and 70% of them were males (Crump et al., 2003; Jennings et al., 2007).

In summary, brucellosis was endemic in ruminants in Egypt with a high prevalence and the incidence of the disease in humans was increasing. The aim of present study was to assess the impacts and costs of the current control programme on the temporal pattern of brucellosis among ruminants and humans in El-Beheira Governorate, Egypt using data from the active surveillance programme between 1990 and 2012.

## MATERIALS AND METHODS

## Study area

El-Beheira Governorate is located in the west of the Nile Delta region, Egypt (30,61° N, 30,43° E). It is bordered in the north by the Mediterranean sea, in the east by Rosetta branch, in the west by Alexandria and Matrouh, and in the south by Giza and El-Menofia Governorates. The total area is about 10,130 km<sup>2</sup> and composed of 13 administrative districts. The human population according to the most recent national survey is about 4,737,129, February 2014.

#### **Data sources**

The data for this study was collected from the active surveillance programme for brucellosis control conducted by the General Organisation of Veterinary Services (GOVS), El-Behira Directorate of Veterinary Medicine, Egypt. The available data were for years 1990 to 2012 for the total number of ruminants tested. Detailed data per each ruminant species were available only for the years 2007 to 2012.

#### Costs of brucellosis eradication programme

Surveillance and testing of animals: According to the programme, GOVS' veterinarians are responsible for collecting blood samples from animals all over the country. The Egyptian ministry of agriculture is responsible for logistics and for supplying all material needed such as cars, needles, blood tubes, and ice boxes. The cost of sampling an individual animal is 10 (Egyptian pound) LE (Current exchange rate; 1 LE = 0.13 US \$, Central Bank of Egypt) for labour, and five LE for consumables (needles, tubes, syringes and ear tags) and administration (planning, implementing and transporting samples from collection sites to the laboratory). The cost of serological tests, Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) is 20 LE, that included reagents, consumables and labour. These costs were based on the actual prices and expert consultation from GOVS and the Department of Brucellosis Research, Animal Health Research Institute (AHRI) Cairo, Egypt.

## Compensation

According to the programme, the slaughtering of seropositive animals by both RBPT and CFT under the supervision of GOVS is compulsory and livestock owners are compensated. The amount of compensation varied according to the species, age and breed of seropositive animals (Table 1). As there were no detailed data for the species,

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breed and age of compensated cattle and buffalo an average of 3,876 LE was used to calculate the compensation of a cattle or buffalo in the economic model.

Species/breeds	Age	Compensation (LE)*
Cattle and buffalo	Less than 6 months	1,680
	From 6 months to 2 years	3,200
Foreign breeds cattle, buffalo and bulls	From 2 to 5 years	7,200
	More than 5 years	5,040
	From 6 months to 2 years	2,400
Mixed breeds	From 2 to 5 years	6,000
	More than 5 years	3,640
	From 6 months to 2 years	2,000
Baladi cattle and buffalo (native breeds)	From 2 to 5 years	4,800
,	More than 5 years	2,800
Sheep	All ages	840
Goat	All ages	600

**Table 1.** Compensation for animals slaughtered by the brucellosis control programme in Egypt from 1990 to 2012 (source: GOVS)

\*LE (Egyptian pound); 1 LE = 0.13 US \$, Central Bank of Egypt

### Vaccination

Vaccination against brucellosis is voluntary. The cost of a single dose of vaccine is 5.5 LE, which is administered by a GOVS' veterinarians. The cost of transporting, cold chain, syringes, needles, ear tags and labour for a single animal is 5 LE. By law, animals must be tested before being vaccinated, therefore the cost of sampling and testing (as detailed before) was added to the cost of vaccination. The total number of animals vaccinated annually was only available for the years 2007 to 2012.

## Data for human brucellosis

Data for human cases were obtained from inpatient logbooks and hospital annual records for the years 1997 to 2012 from Damanhour fever hospital, El-Behira Governorate. Data collection and characteristics of patients have been described in detail previously (El-Ghitany et al., 2014).

# Data management and analysis

Data were stored in a Microsoft Office Excel 2007. Frequency tables were used to calculate the proportions of tested and the proportions of seropositive animals for *Brucella* spp. per year for each ruminant species. Statistical analyses were conducted to allow comparison between different years and ruminant species using IBM SPSS Statistics for Windows, (Version 20.0. Armonk, NY: IBM Corp). To compare between seropositive proportions for different years for each species, a univariate binary logistic regression model, with seropositive as the response variable and year 1990 was used as a reference for the period 1990 to 2012 and year 2007 for the period 2007 to 2012.

# RESULTS

# Temporal pattern of brucellosis in ruminants

The results showed that, the proportion of seropositive ruminants in El-Behira Governorate during the study period ranged from 0.66% to 4.01%, (Table 2). The proportion of seropositive ruminates fluctuated up and down. However the general trend according to the available data was decreasing, (Figure 1). The lowest seropositive proportion (0.66%) was in 2011. Results for each species, (Table 3), showed that, the proportion of seropositive cattle was significantly increased from 2007 (1.08%) to 2009 (1.81%, OR=1.69, p<0.001), then decreased and then increased again. Results for buffalo were relatively similar to that of cattle. The proportion of seropositive sheep decreased from 2007 (7.64%) to 2009 (0.67%, OR=0.08, p<0.001) followed by an increase in 2010 then decreasing again. For goats, there was a significant decrease of the seropositive proportion from 2007 (12.59%) to 2010 (1.40%, OR = 0.10, p<0.001) then increasing from 2011 to 2012.

# Cost of brucellosis control programme

The average annual costs for surveillance and compensation was 1,493,380.33 LE. The results showed the highest proportion (71.66%) of brucellosis control programme was for cattle, followed by buffalo (15.32%), sheep (11.67%) and finally goats (1.35%). The highest costs were in 2009, (Figure 3). The average annual costs including vaccination were 2,080,359.50 LE. Vaccination costs represented about 28% of the total costs. Also the highest total costs were in 2009, (Figure 4).

#### **Brucellosis in humans**

The incidence (number of cases per 100,000) of brucellosis in humans, (Figure 5), fluctuated but generally increasing from 19 to 102 cases in 2002. The incidence of brucellosis cases in humans was decreasing in 2003 and 2004 but increased again in 2005 then decreasing after that to the lowest incidence at 2012, (32 cases). The average length of illness in human cases of brucellosis was 11 days and the average period of hospital stay was five days.

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Table 2. Proportion of seropositive ruminants in El-Behira Governorate, Egypt from 1990 to 2012

Year	<b>Tested animals</b>	Seropositive (%)	OR	95% CI	P value
1990	8000	321 (4.01)	Reference	-	-
1991	9000	340 (3.78)	0.92	0.80-1.10	0.43
1992	11000	399 (3.63)	0.90	0.78-1.05	0.17
1993	13000	411(3.16)	0.78	0.67-0.91	0.001
994	13800	500 (3.62)	0.90	0.78-1.04	0.15
1995	15000	532 (3.55)	0.88	0.76-1.01	0.08
996	18000	598 (3.32)	0.82	0.72-0.94	0.005
.997	25000	670 (2.68)	0.66	0.58-0.75	0.001
.998	24536	628 (2.56)	0.63	0.55-0.72	0.001
.999	20000	540 (2.70)	0.66	0.58-0.76	0.001
2000	32102	449 (1.40)	0.34	0.29-0.39	0.001
2001	30587	482 (1.58)	0.38	0.33-0.44	0.001
2002	31004	540 (1.74)	0.42	0.37-0.49	0.001
2003	38234	319 (0.83)	0.20	0.17-0.24	0.001
2004	28369	384 (1.35)	0.33	0.28-0.38	0.001
2005	25004	492 (1.97)	0.48	0.42-0.55	0.001
2006	23525	483 (2.05)	0.50	0.43-0.58	0.001
2007	20681	471 (2.28)	0.56	0.48-0.64	0.001
2008	20875	279 (1.34)	0.32	0.28-0.38	0.001
2009	21731	324 (1.49)	0.36	0.31-0.42	0.001
010	20827	341(1.64)	0.40	0.34-0.47	0.001
011	21889	145 (0.66)	0.16	0.13-0.19	0.001
2012	20396	166 (0.81)	0.20	0.16-0.24	0.001

OR=Odds Ratio, CI=Confidence Interval

**Table 3.** Proportion of seropositive ruminants in El-Behera Governorate, Egypt from 2007 to 2012

Animal spp.	Year	Tested animals	Seropositive (%)	OR	95% CI	P value
	2007	13944	151(1.08)	Ref	-	-
	2008	14680	160 (1.09)	1.01	0.81-1.26	0.95
Cattle	2009	12572	228 (1.81)	1.69	1.37-2.08	0.001
Ca	2010	14566	99 (0.68)	0.63	0.49-0.81	0.001
-	2011	17572	100 (0.57)	0.52	0.41-0.67	0.001
	2012	15325	118 (0.77)	0.71	0.56-0.90	0.005
	2007	3209	37 (1.15)	Ref	-	-
0	2008	3626	28 (0.77)	0.67	0.41-1.09	0.11
Buffalo	2009	3838	42 (1.09)	0.95	0.61-1.48	0.82
gut	2010	3359	14 (0.42)	0.36	0.19-0.67	0.001
<b>H</b>	2011	3161	26 (0.82)	0.71	0.43-1.18	0.18
	2012	3634	19 (0.52)	0.45	0.26-0.79	0.005
	2007	3258	249 (7.64)	Ref	-	-
	2008	2383	79 (3.32)	0.41	0.32-0.54	< 0.001
Sheep	2009	4471	30 (0.67)	0.08	0.06-0.12	< 0.001
She	2010	2616	224 (8.56)	1.13	0.94-1.37	0.19
•1	2011	874	14 (1.60)	0.20	0.11-0.34	< 0.001
	2012	1380	25 (1.81)	0.22	0.15-0.34	< 0.001
	2007	270	34 (12.59)	Ref	-	-
	2008	189	12 (6.35)	0.47	0.24-0.94	0.03
at	2009	850	24 (2.82)	0.20	0.12-0.35	< 0.001
Goat	2010	286	4 (1.40)	0.10	0.03-0.28	< 0.001
-	2011	282	5 (1.77)	0.13	0.05-0.33	< 0.001
	2012	157	4 (2.55)	0.18	0.06-0.52	0.002
<u> </u>	2007	20681	471(2.28)	Ref	-	-
All ruminant	2008	20878	279 (1.34)	0.58	0.50-0.68	< 0.001
ii	2009	21731	324(1.49)	0.65	0.56-0.75	< 0.001
12	2010	20827	341(1.64)	0.71	0.62-0.82	< 0.001
- F	2011	21889	145(0.66)	0.29	0.24-0.35	< 0.001
A	2012	20496	166(0.81)	0.35	0.29-0.42	< 0.001

OR=Odds Ratio, CI=Confidence Interval

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**Table 4.** Duration of illness and hospitalization of human brucellosis at Damanhur fever hospital, Damanhur, Egypt from 1997 to 2012

Duration/day	Illness	Hospitalization	
Minimum	1.00	1.00	
Maximum	120	31	
Mean (SD)	11±9.52	$5.00 \pm 2.44$	

SD=Standard Deviation

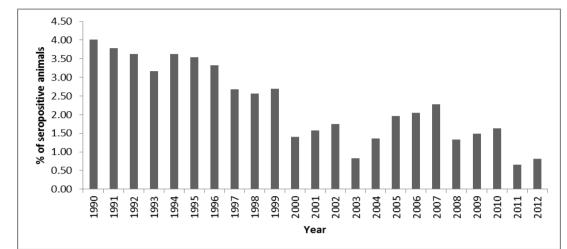


Figure 1. Temporal distribution of brucellosis in ruminants (cattle, buffalo, sheep and goat) in El-Behira Governorate, Egypt from 1990 to 2012

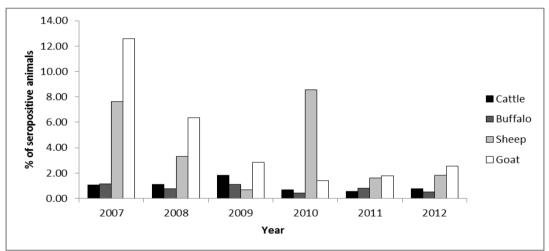


Figure 2. Temporal distribution of brucellosis in ruminants (cattle, buffalo, sheep and goat) in El-Behira Governorate, Egypt from 2007 to 2012

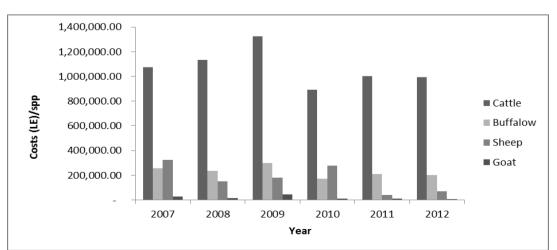


Figure 3. The annual costs (LE) for sampling, testing and compensation for brucellosis control programme El-Behira Governorate, Egypt from 2007 to 2012

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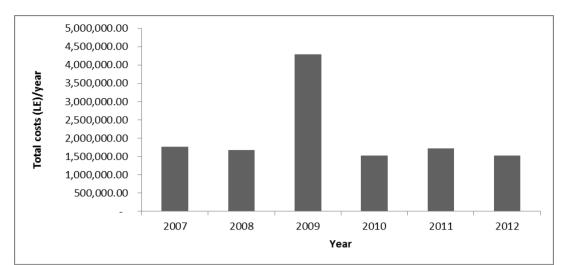


Figure 4. Total annual costs (LE) for the brucellosis eradication programme in El-Behera Governorate, Egypt from 2007 to 2012

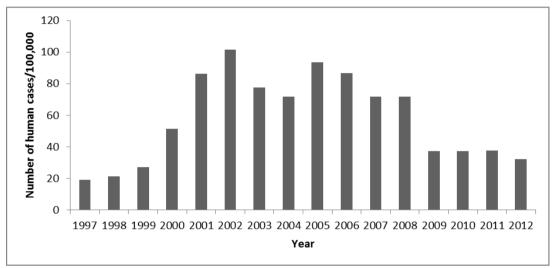


Figure 5. Incidences of human brucellosis in El-Behira Governorate, Egypt from 1997 to 2012

# DISCUSSION

The aim of present study was to assess the impact and costs of the national brucellosis control programme on the temporal pattern of brucellosis in ruminants and humans in El-Behira Governorates, Egypt. The proportion of seropositive ruminants in El-Behira Governorate during the study period ranged from 0.66% to 4.01%. However the actual prevalence and/or incidence of brucellosis cannot be estimated from the available data. As the animal censuses were not available and the method of sampling was not clear, the proportion of animals that have been tested may not represent the animal population. Moreover, the number of tested animals varied from year to another which may be due to the available budget. This proportion of seropositive animals was quite low compared with results from other studies in the same Governorate. The proportion of brucellosis seropositive camels in contact with farm animals was about 10% (Moghney, 2004). Also, the proportion of seropositive cattle, buffalo, sheep and goats for brucellosis in El-Behira Governorate was found to be 5.86%, 5.83%, 7.20% and 11.33%, respectively (Sayour and Azzam, 2014). Generally, the proportion of seropositive ruminates fluctuated up and down. A significant increase in the proportion of seropositive animals after a significant reduction might be related to the variation in the number of tested animals for each year. In our opinion if there was an actual reduction in the prevalence of the diseases due to the control programme, the proportion of seropositive animals would not have increased again unless there were other factors. The results also showed that, the main focus of the control programme was on cattle, as the highest numbers of tested animals were cattle which caused about 70% of costs.

Regarding the costs of the control programme, the results showed that, there was a considerable proportion of costs (about 40%) due to surveillance and testing of samples. However other diagnostic methods could be cheaper, more practical, more sensitive and specific for the diagnosis of brucellosis. Other methods such as molecular diagnosis

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including both Polymerase Chain Reaction (PCR) and Dot Blot Hybridization (DBH) assay were found to be more sensitive with maintaining reliable specificity compared with conventional techniques (Ibrahim et al., 2012). Also testing milk using milk tanks and/or individual milk samples could be another possibility for which further investigation is required for assessing the accuracy and reliability. The annual costs for vaccination were found to be less than 30% of the total costs. However in countries like Egypt with endemic brucellosis, starting with vaccination followed by test and slaughter would be more appropriate for controlling the disease. Until now vaccination of animals against brucellosis has been optional. Therefore, the effectiveness of different combinations of control strategies should be investigated.

The results showed the incidence of human brucellosis was from 19 to 102 cases per 100,000 during the study periods. This incidence was consistent with that reported by (Jennings et al., 2007) but higher values reported by (Refai, 2002) and (Crump et al., 2003). However, it should be taken into account these estimates were hospital based that would underestimate the actual incidence. There are many reasons for the underestimation of the incidence of brucellosis in humans such as misdiagnosis, absence of a reliable notification system, reluctance or financial disability of patients to seek medical help and the habits of self-medication or purchasing medication from pharmacies without prescription (Dean et al., 2012; El-Ghitany et al., 2014; Jennings et al., 2007; Meky et al., 2007). The incidence of brucellosis in humans in the study period was fluctuating, however there was not enough evidence to link these changes with the changes in brucellosis in ruminants. Not only the incidence of brucellosis in humans was underestimated but also there were no available data for the healthcare resources used for the treatment of cases. The recorded length of illness and hospital stay seems to be underestimated. It was recorded that brucellosis patients tended to wait between 1 to 120 days (average 11) before looking for medical assistance (El-Ghitany et al., 2014). Therefore it was not possible to estimate the costs associated with human infections with brucellosis in the present study. The costs of comorbidities and complications were not possible to be estimated too.

# CONCLUSION

True prevalence and/or incidence for brucellosis in ruminants and humans cannot be estimated from the current national programme for brucellosis or the hospital survey, respectively. There were no evidences to link the fluctuations of brucellosis incidence in humans with that in ruminants in El-Behira Governorate, Egypt. One of the limitations of this study was the lack of data to estimate the socio-economic impacts of brucellosis and the current control programme. To our knowledge this had been the first attempt to evaluate the impact of the current brucellosis control programme on the temporal aspect of brucellosis in ruminants and the associated costs. Further studies are required to evaluate the efficacy and the socio-economic impact of brucellosis control programme in Egypt.

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