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UPDATE STATUS OF TOXOPLASMOSIS AND BRUCELLOSIS IN SMALL RUMINANTS AND HUMAN IN SHARKIA PROVINCE

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SUMMARY

Toxoplasmosis and brucellosis are widely distributed, zoonotic diseases affecting a wide spectrum of animals and humans. Serum samples were collected from 182 sheep, 294 goats and 60 human from different localities of Sharkia province (Zagazig - Abo-Hammad - Fakous and Abo-Kabeer) in the period between March - July 2005 and serologically tested for presence of specific antibodies against toxoplasmosis and brucella species. Out of 182 sheep, 48 (26.4%) were sero-positive for toxoplasmosis. By using of enzyme linked immunosorbent assay (ELISA), 17 (9.3%) were IgG positive and 6 (3.3 %) were IgM positive. Among goats, 38 (12.93%) were positive by IHAT; and by ELISA, 12 (4.08%) were positive for presence of IgG as well as 9 (3.06%) were positive for presence of IgM. Rose Bengal Plate Antigen (RBPA), Buffered Acidified Plate Antigen (BAPA), Rivanol test (Riv.T) and serum agglutination Test (SAT) were applied to identify brucellosis. Out of 182 sheep sera samples, 21 (11.54%) were sero-positive; Meanwhile out of 294 goat sera, 12 (4.08%) showed sero-positivity to brucellosis. *Brucella melitensis* biovar - 3 was isolated from milk samples and tissue specimens (especially lymph nodes) of the reactor animals and from 17 ewes sero-positive to brucellosis there were 8 positive for isolation and identification of *Brucella melitensis*, whereas there were 3/15 sero-positive goats (20%) so that control measures of toxoplasmosis and brucellosis were suggested.

Concerning to human the incidence of toxoplasmosis and brucellosis were 28.3% and 8.3 % respectively.

INTRODUCTION

Toxoplasmosis is a zoonotic protozoal disease caused by the intracellular protozoan parasite, Toxoplasma gondii, affecting man and a wide spectrum of livestock. Abortion, stillbirth and neonatal mortalities in sheep and goats were record-

ed (Dubey and Welcome, 1988; Dubey and Kirkbride, 1990 and Szeredi and Bacsadi, 2002). Moreover, undercooked meat of sheep and goats containing tissue cysts of *T. gondii* represent a potential source of human toxoplasmosis (Dubey and Beattie, 1988).

Toxoplasmosis in man may be sub clinical, congenital (retinochorditis, hydrocephaly, convulsions and intracerebral calcification), acquired (lymphatic from which may be febrile) and inducing abortion (Acha and Szyfres, 1991).

From this point of view, screening of sheep and goats for toxoplasmosis in Sharkia province was investigated using enzyme linked immunosorbent assay (ELISA) and the indirect haemagglutination test (IHAT) to stand on the update status of the infection among this species of hosts.

Brucellosis is a zoon tic disease caused by a facultative intracellular bacteria of genus Brucella, which infect humans and animals especially in the developing countries (Thakur et al., 2002; Dornand et al., 2004 and Navarro et al., 2004).

Brucellosis in man begins with undulant fever then localized intra-cellular in reticulo-endothelial system (Acha and Szyfres, 1991).

The disease transmits by spraying during contamination by fetal membrane and fluid (Guihot et al. 2004). Diagnosis of brucellosis requires isolation of the bacteria and confirmation through serological tests. A combination of serological tests including (RBPA), (BAPA), (Riv.T), (SAT) and dot enzyme linked immunosorbent assay (dot-ELISA) were employed for screening of brucellosis (Anon, 1984; Nelson, 1989 and Thakur and Thaplyial, 2002).

From the available literatures, there is a great need to study the update epidemiological data of toxoplasmosis and brucellosis in human and animals, so this investigation was carried out to study the occurrence of both diseases among sheep, goats and human in different localities in Sharkia province (Zagazig - Abo-Hammad - Fakous and Abo-Kabeer) by different serological tests. In turn, the follow up data of both diseases will help in monitoring the control of these important zoonoses.

MATERIAL AND METHODS

1- Samples:

A- Serum samples:

Serum samples were collected from 182 sheep (Ewes) and 294 goats from different localities in Sharkia province (Zagazig - Abo-Hammad - Fakous and Abo-Kabeer). All tested animals were not vaccinated against brucellosis one year ago. Moreover, 40 human sera samples were collected from the general Zagazig Hospital [Women with history of abortion (20 samples) and feverish (20 samples)], as well as, control sera (20 samples) from Blood Bank, Zagazig.

B- Milk samples:

They were collected from lactating reactors for bacteriological isolation and identification.

C- Tissue specimens: Supramamary lymph nodes and udder tissues were taken from all reac-

tors at abattoir, these samples were exposed to Brucella isolation, identification and sero-typing according to the methods recommended by Morgan and Makinon (1979).

2- Screening of the sera for toxoplasmosis :

Toxoplasma gondii IgG and IgM ELISA kits (Fumoz-France) and Toxoplasma IHA Kit (ABC-diagnostics, Egypt) were purchased from local handlers and detection methods were carried out following the manufacturerís instruction in the used kits.

3- Screening and Identification of Brucellosis:

A- Serological tests:

RBPA, BAPAT, Riv.T and TAT were applied to detect brucellosis among the collected serum samples (Morgan and Makinon, 1979; Anon, 1984 and Nelson, 1989)

B- Antigens:

I - Antigen for Buffered Acidified Plate test (BAPAT) and antigen for tube agglutination test (TAT) were obtained from Veterinary Serum and Vaccines Research Institute, Abbasia, Cairo, Egypt.

II - Antigen for Rose Bengal Plate Test (RBPT) was obtained from Bio-Meriuex Laboratories for Reagent and Products, France.

III - Rivanol Antigen and Solutions: were obtained from National Veterinary Services Laboratories, Ames., Iowa, U.S.A.

C- Anti-sera:

I - Monospecific anti-Brucella abortus and anti-Brucella melitensis sera were obtained from Central Veterinary Laboratory, NewHaw, Weybridge, England.

II - Brucella abortus control negative serum was obtained from Bio-Merieux Institute, France.

III - Brucella abortus control positive sera with a variety of positive against Brucella antigen were kindly supplied by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

D- Bacteriological media:

I - Brucella agar medium was used for isolation, identification and sero-typing of Brucella. It was obtained from Difeco Laboratories, Detroit, Michigan, U.S.A.

II - Brucella albimi agar containing antibiotics and ethyl violet dye was prepared according to Kuzdas and Morse (1953).

III - Brucella agar with thionin at a final concentration of 1:25000 and 1:100.000 was prepared according to Alton and Jones (1967).

IV - Brucella agar with basic fuchsin at concentration of 1:50.000 and 1:100.000 was prepared according to Alton et al., (1975).

E- Brucella Phage:

Standard Tibilisi (Tb) Brucella phage strain (Central Veterinary Laboratory, Weybridge, England) was used for typing of Brucella isolates Kadry et al., (2004).

F - Laboratory animals : Brucellosis free guinea pigs of about 250 - 350 gms

body weight were used for isolation and identification of brucella species from highly infected animals.

RESULTS

Table (1): Results of Sero-survey for toxoplasmosis and brucellosis in small ruminants in Sharkia province.

Animal Species	Total Ex. animal No	Toxoplasmosis			Brucellosis		
		N.R.	R.	R. %	N.R.	R.	R. %
Sheep	182	142	40	21.98	161	21	11.54
Goats	294	256	38	12.93	282	12	4.08
Total	476	398	78	16.39	443	33	6.93

N.R. = Non Reactors R. = Reactors R. % = Reactor Percentages

Table (2) : Prevalence of toxoplasmosis and brucellosis among sheep and goats in different localities of Sharkia province.

Animal Species	No.of Ex.	Disease		Zagazig	Abo-Hammad	Fakous	Abo-Kabeer
Sheep	182	Toxoplasmosis	R.%	26.79	20	27.9	12.5
			N.R.	41	28	31	42
			R.	15	7	12	6
		Brucellosis	R.%	14.3	8.6	11.6	10.42
			N.R.	48	32	38	43
			R.	8	3	5	5
Goats	294	Toxoplasmosis	R.%	15.1	11.1	15.4	9.9
			N.R.	73	64	55	64
			R.	13	8	10	7
		Brucellosis	R.%	3.57	2.9	4.35	5.48
			N.R.	81	66	66	69
			R.	3	2	3	4

N.R. = Non Reactors R. = Reactors R. % = Reactor Percentages

Table (3): Sero-prevalence of Toxoplasmosis in sheep and goats tested by ELISA .

Serological test	Sheep			Goats		
	N.R.	R.	R. %	N.R.	R.	R. %
ELISA (IgM)	176	6	3.3 %	285	9	3.06 %
ELISA (IgG)	165	17	9.3 %	282	12	4.08 %

Table (4) : Results of bacteriological Examination for Brucella species isolation from sheep and goats in Sharkia Province.

Animal Species	No. of Ex. Animal Specimen	No. of Positive	Positive %
Sheep	17	8	47.06 %
Goats	15	3	20.00 %
Total	32	11	34.38 %

Table (5) : Bio-typing of Brucella isolates recovered from sheep and goats in Sharkia Province according to Kadry et al., (2004).

Animal Species	No. of bact. +ve animals	Co_2 reqirements	H_2S production	Urease activity (hours)	Catalase test	Growth on media containing dyes		Agglutination with monospecific sera		Lysis by phage Tiblisi		Species
						Thionin 20 ug/ml	Basic fuchsin 20 ug/ml	A*	M*	RTD	10^4 X RTD	
Sheep	8	-	-	+ve	+ve	+	+	+	+	-	-	B.melitensis Serotype
Goats	3	-	-	+ve	+ve	+	+	+	+	-	-	

Reference strains of B. abortus, B. melitensis and B. suis were used in the identification.
A* = mono-specific anti-brucella abortus serum.
M* = mono-specific anti-brucella melitensis serum.
RTD = Routine test dilution.

Table (6): Incidence of Brucella among sheep and goats in 4 different localities in different localities of Sharkia province by using 4 serological tests.

Locality	No. of Ex. Animals	RBPT			BAPA			TAT							Riv.T						
								Titer				Total			Titer					Total	
		+	-	%	+	-	%	1/40	1/80	1/160	1/320	+	-	%	1/25	1/80	1/150	1/100	1/200	+	-
Zagazig	56*	9	47	16.1	9	47	16.1	1	2	4	2	8	48	14.3	2	1	0	3	1	8	48
	84 **	4	80	4.8	6	78	7.14	1	0	1	1	3	81	3.57	0	0	2	1	0	3	81
Abo-Hammad	85*	4	31	11.4	5	32	13.5	1	0	1	1	3	32	8.6	1	1	0	1	0	3	32
	68 **	3	65	4.4	4	64	5.9	0	1	0	1	2	66	2.9	1	0	1	0	0	2	66
Fakous	43*	6	37	14	7	36	16.28	2	1	2	0	5	38	11.6	1	0	2	1	1	5	38
	69 **	5	64	7.25	5	64	7.25	0	2	1	0	3	66	4.35	2	0	1	0	0	3	66
Abo-Kabeer	48*	5	43	10.4	6	42	14.2	1	2	1	1	5	43	10.4	0	2	1	0	1	5	43
	73 **	5	68	6.85	5	68	12.5	0	2	2	0	4	69	5.48	1	0	1	2	0	4	69

* = No. of Sheep **= No. of Goats

Table (7): Sero-prevalence of toxoplasmosis among human in Sharkia province.

State of Ex. People	No. of Ex. cases	Sero-positive for Toxoplasmosis		Sero-positive for Brucellosis	
		No.	%	No.	%
Apparently healthy	20	4	20	0	0
Previously aborted women	20	9	45	2	10
Feverish human	20	4	20	3	15
Total	60	17	28.3	5	8.3

DISCUSSION

The present study report the update prevalence of toxoplasmosis and brucellosis among sheep, goats and human by using ELISA and IHAT for toxoplasmosis and RBAT, BAPAT, Riv.T and TAT for brucellosis .

Although ELISA is more sensitive and specific than IHAT, the later is simple to perform, not species specific and used extensively to test animals for toxoplasmosis (Marriana Wilson et al. 1990; McLoad and Remington, 1996 and Van der Puije et al., 2000). Generally, the findings (as shown in table 1) show that the overall sero-positivity of sheep and goats to toxoplasmosis was 21.98 % and 12.93 %, respectively. The results are nearly in agreement with the findings of Hashemi-Fesharki (1996) in Iran (24,6% and 18,5% in sheep and goats, respectively by using IHAT), Esmat (1997) in Sharkia province, Egypt [27,1 % (latex agglutination) and 24,3% (IHAT) of sheep as well as 23,1% (LAT) and 21.5% (IHAT) of goats showed sero-positive for toxoplasmosis, respectively], Abou-Zeid (2002) in Sharkia, Egypt, who detected antibodies against Toxoplasma gondii in 20,36 % of sheep and 26,26 % of goats by Latex agglutination test), Sawadogo et al., (2005) in Marrakech, Morocco (27.6% sheep were positive for IgG by ELISA). Moreover, Mohamed and Eissa (2004) detected antibodies of Toxoplasma in sheep (22.9%) and goats (21.0%) from Dakhlye and Sharkia using latex agglutination test. These data conclude that toxoplasmosis still an endemic disease and world wide. On the other hand, higher and lower prevalence of *T. gondii* in sheep and goats were stated elsewhere, for example, 63.31% of goats were sero-positive for *T. gondii* in Grand Canary Island, Spain (Rodrìguez-Ponce et al., 1995) and 3.6% of sheep in north America were positive to toxoplasmosis (Dubey and Foreyt, 2000). The differences in sero-positive of sheep and goats to toxoplasmosis may vary strongly in the same country from one locality to another (Feldman and Miller, 1956) and this may be attributed to the serological tests used, the breeding system, husbandry precaution and accessibility of cats to the animals.

Comparing the prevalence of toxoplasmosis in both sheep and goats by using ELISA and IHAT in Sharkia province (table 3) lower prevalence was detected by ELISA. No doubt that the specificity and sensitivity of ELISA is higher than IHAT, but detection of both IgM and IgG at the same time by IHAT may be the cause. At the level of immunoglobulin classes, anti-Toxoplasma IgM and IgG antibodies by ELISA were founded (3.3% and 9.3% in sheep and 3.06% and 4.08% in goats respectively). In the present task, lower percentage of animals proved to be positive to anti-Toxoplasma IgM than to anti-toxoplasma IgG. Similar results were stated by Abdel-Moneim and Sosa (1996) and coincide with the fact that IgG response to toxoplasmosis begins later and can be longer detected than that of IgM antibodies which increase mostly in recent infection and within one week after abortion (Trees et al. 1989; Marriana Wilson et al., 1990; Lunden, 1995; Vitor, et al., 1999 and Conde et al., 2001).

Since toxoplasmosis represent a great risk factor

for man and animal health and as reported by Dubey and Beattie (1988), where sheep and goats represent a potential source for human toxoplasmosis; on the other hand approximately 500 million people assumed to be infected with this disease. In Egypt, Hussein et al., (2001) stated that sero-positive to specific ELISA anti-Toxoplasma IgG antibodies was observed in 57.9%, 58.1% and 44.7% of randomly tested individuals, full term and aborted women at Qualyobia governorate respectively; also in the same locality El-Fakahany et al., (2002) detected antibodies against T. gondii by using PCR in 20%, 50% and 60% of 55 women with abortion, premature deliveries and deliveries of babies with congenital anomalies in Qualyobia, respectively, so that Attention should be directed to control the disease.

Brucellosis is still one of the serious problems due to its zoonotic and economic importance.

The control of the disease requires efficient diagnostic procedures for diagnosis of all infected animals; the efficacy of procedures depends on the quality of serological tests in sensitivity and specificity (Tizard, 1982 and Nelson, 1989).

Tables (1) and (2) shows that the infection percentages of sheep with toxoplasmosis and brucellosis is higher than recorded in goats at the same area, similar results achieved by El-Gamal (2004) and this reflect the potenial role of sheep and goats for transmission of brucellosis to cattle, buffaloes, other healthy sheep, goats and humans.

From obtained results in table (2) serological prevalence of brucellosis among 182 tested sheep by four different serological tests (RBPT, BA-PAT, Riv. T and TAT) in different localities in Sharkia province (Zagazig ñ Abo-Hammad ñ Fakous and Abo-Kabeer) were (14.3 %, 8.6 %, 11.6 % and 10.4 %) respectively. While goats were (3.57%, 2.9%, 4.35% and 5.48 %) respectively.

This results are lower than that recorded by Kamel and Abdel-Fattah (1961), Abdulla (1966), Abdel-Aal (1985), Zaghloul and Kamel (1985), El-Sheery (1987) and Mohamed and Eissa (2004), this lower incidence of brucellosis may be attributed to vaccination, periodical examination of animals and place of sampling.

Also, these results were higher than those reported by Ammar (2000) and Kadry et al., (2004) who recorded that the incidence of brucellosis among sheep and goats in Sharkia province was 2.2 % and 0.7 % respectively.

Table (4) shows that the bacteriological examination revealed 8 ewes positive for isolation and identification of brucella species from 17 sero-positive (47.06%) while from 15 sero-positive goats there were 3 positive (20%) these two percentages are higher than those reported by Kadry et al., (2004) who proved that the percentages of isolation and identification of brucella species were 12.5% and 0 %.

Table (5) revealed that brucella melitensis biovar-3 was isolated from milk samples and tissue specimens (especially lymph nodes) of the reactor sheep and goats in Sharkia governorate this

agrees with Kadry et al., (2004).

From obtained results in table (6) it cleared that RBPT and BAPT could be regarded as screening tests for detection of positive reactors to brucellosis as they showed the higher percentages of positive reactors in sheep (16.1%, 11.4%, 14% and 10.4 %) and (16.1%, 13.5%,16.28 and 14.2%) if compared with TAT and Riv. test (14.3%, 8.6%, 11.6% and 10.4%) for (Zagazig, Abo-Hammad, Fakous and Abo-Kabeer) localities respectively. Whereas in goats, RBPT and BAPT showed (4.8%, 4.4%, 7.25 and 6.85) and (7.14%, 5.9%, 7.25% and 12.5%) but TAT and Rivanol test showed (3.57%, 2.9%, 4.35% and 5.48%) for (Zagazig, Abo-Hammad, Fakous and Abo-Kabeer) localities respectively. This indicates the higher sensitivity of RBPT and BAPT as reported by Davies (1971) and El-Bouomy (1989) and the acidic PH (3.65 in RBPT and 4.o in BAPT) of the antigen used in these tests inhibit activity of non specific immunoglobulins to a certain extent. RBPT is more efficient in the detecting of early and chronic infection (Montaser, 1995) but Riv. test is specific due to most of positive reactors were in chronic stage in which the IgG antibodies.

IgM appears earlier than other immunoglobulins after short time of infection and persists shorter than other immunoglobulins. So RBPAT indicates positive reaction sooner than TAT, moreover the acidity of BAPAT inhibits the non specific immunoglobulins leaving specific agglutinin (Alton et al., 1988).

Table (7) shows the seo-prevalence of toxoplasmosis and brucellosis among human in Sharkia governorate. The overall incidence in examined human sera is 28.3 % and 8.3% respectively. Concerning brucellosis the obtained results is higher than that previously founded by Mohamad and Eissa (2004) whose results ranged from 3.2% to 4.8 % this results may reflect the increasing risk of brucellosis in Sharkia. Regarding toxoplasmosis the revealed results (21.98 %and 12.93%) for (sheep and goats) are higher than that found by Mohamad and Eissa (2004). The higher incidence recorded in aborted women agree with that found by Haggag (2000).

The update study of both toxoplasmosis and brucellosis reveal that they still prevails with a potential risk and need further control and prevention programs. This is may be achieved through public health education, test and slaughter of reservoir hosts and focal hygiene protocol with special reference to hazard analysis and critical control point programs (HACCP).

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