

Occupational Health Hazard of Egyptian Employees in Contact with Wastage Nourished SwineAshraf, M. Barakat^{*1}; Hassan, A. El Fadaly¹; Raafat, M. Shaapan¹ and Fathia, A.M. Khalil²¹Zoonotic Diseases Department, National Research Center, Giza, Egypt²Parasitology and Disease Department, National Research Center, Giza, Egyptashrafbarakat2@hotmail.com*

Abstract: Egyptian swine still they are free nourished on wastages in small herds without veterinary health measures. Because of their omnivore's behavior, pigs are naturally exposed to zoonotic agents in their setting with subsequent direct human occupational hazards. Brucellosis, Leptospirosis and Toxoplasmosis are the major diseases link human exposure for natives in contact with swine. So, updating the sero-prevalence of these pathogens among contact employees reflect to how extent the human bio-hazards are due to direct contact with swine or their contaminant subset. Therefore, sera of 230 free wastage nourished pigs were collected at Cairo, Egypt. Also, 127 serum samples were collected from racing occupational workers. Human and swine sera were serologically analyzed for antibodies against *Brucella*, *Leptospira* and *Toxoplasma* by using commercial kits. Antibodies against *Brucella* were detected in 29/ 230 (12.61 %) of swine sera, and 11/127 (8.66 %) of workers sera by using Rose Bengal plate test. Antibodies against *Leptospira* serovars were detected in 53/230 (23.04%) of swine sera using the microscopic agglutination test (MAT) at a titer of 1:200. The highest seroprevalence was recorded for *L. pomona* (45.28%), followed by *L. grippityphosa* (33.96%) and *L. icterohaemorrhagiae* (20.75%). The seropositive human sera were 25.9% with the highest incidence corresponding to *L. pomona* serovar (11%). Results of the indirect fluorescent antibody test showed that anti-*Toxoplasma* antibodies were detected in 74.78% (172/230) and 37.79% (48/127) of swine and contact employees respectively. It can be concluded that serological assays concerning brucellosis, leptospirosis and toxoplasmosis verify direct occupational exposure for high risk group's manipulating employees through carrier animals or their pollutant conditions.

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1. Introduction:

Pigs are omnivores, feed on wastage that grasping insects, rodents, plus different bio-hazards residuals. The swine living situation shear habitats with dogs, cats, rodents and wild birds that maximize their exposure to be reservoirs for various zoonoses. The local Egyptian wastage feeding swine are scattered in small herds without rigid veterinary health measures that signify spots for spreading epidemics. Brucellosis, leptospirosis and toxoplasmosis characterize the most swine's occupational zoonoses that induce swine's abortion and fetus depletion (Samaha et al., 2008).

Swine brucellosis is a worldwide zoonosis symbolize an important source for human brucellosis and is mainly caused by *Brucella suis*, while *Brucella abortus* and *Brucella melitensis* can infect pigs but are only mildly pathogenic (Shimshony, 2009). The zoonotic impacts of those pathogens were designated as select biological agents of Category B by the Centers for Disease Control in Atlanta, USA (Corbell, 2006). Brucellosis causes more than 500 000 human infections per year worldwide (Corbell, 2006). According to WHO (1998), brucellosis is endemic in Egypt, *Brucella melitensis* biovar 3 is the

most commonly isolated species from Egyptian animals (Refai, 2002). Brucellosis in animals causes tremendous economic losses due to abortion, premature birth, decreased milk production, and reduced reproduction rate (WHO, 2009). Human brucellosis is mainly an occupational risk for farmers, veterinarians, abattoir workers, laboratory personnel, and others who work in contact with animals or their raw products (CDC, 2007).

Leptospirosis has been recognized as an emerging global public health problem because of its increasing incidence in both developing and developed countries (Slack et al., 2008). A number of leptospirosis outbreaks have occurred in the past few years in various places such as Nicaragua, Brazil and India (Health et al., 1965). It is a direct zoonotic disease caused by spirochetes belonging to different pathogenic species of the genus *Leptospira*. Human infection results from accidental contact with carrier animals or contaminated environment. The primary source of leptospirae is the excretory animal, from whose renal tubules leptospirae are excreted into the environment with the animal urine (Mckiel et al., 1961). The majority of the leptospiral infections is either sub clinical or result in very mild illness and

recover without any complications. Illness develops and progresses rapidly, leading to organ failure and often death if not treated and the case fatality ratio could be about 40% or more. Abortion, occurring 2-4 week before terms is the most common manifestation of swine leptospirosis. Because of the variable manifestations of leptospirosis, it is often misdiagnosed and under-reported (Slack et al., 2008).

Toxoplasma gondii is an obligate intracellular tissue cyst-forming coccidian protozoan with zoonotic impact. The course of disease is generally benign, but during unfit host immune condition through virulent strain, the protozoan stimulates serious affection with significant morbidity and mortality including humans with Acquired Immunodeficiency Syndrome (AIDS) or submitted to corticosteroids and cancer chemotherapy (Jones et al., 2001). The protozoan transmitted vertically via placenta to the fetus by acute stage tachyzoites, while horizontal transmission may involve either ingesting of the environmentally sporulated oocysts that eliminated un-sporulated only via shedder cats or via ingesting the dormant chronic tissue cysts stage in meat of food animals. Diffusion may also occur via tachyzoites through blood transfusion, tissue transplants, un-pasteurised milk or aborted fetal fluids and membrane (Tenter et al., 2000). Humans become infected mainly postnatal by eating raw or inadequately cooked meat containing tissue cysts (Jones et al., 2001). Ingestion of pork tissue cyst signifies one of the most prevalent sources of human toxoplasmosis, because they can remain viable at 52°C for 9.5 minutes (Aspinall, 2002). There is no possible mode of transmission to human via lively animals in contact including pigs, but manipulating pork confirm occupational risk due to bradyzoites diffusion through skin abrasions (Cook et al., 2000).

Detection of the sero-prevalence of occupational brucellosis, leptospirosis and toxoplasmosis in working personnel in contact with free nourished swine is of ecological impact, reflecting to how extent the human bio-hazards are due to occupational activities. Also, this study confirms the necessitate of public health worry by Egyptian veterinary authorities' toward wastage feeding swine on such unhygienic situation that believed to be spots for expand epidemics.

2. Material and methods:

A. Sample collection:

Blood samples were collected from both 230 pigs and 127 racing occupational workers at Cairo in Egypt. Human and swine sera were separated and stored at -20 until analyzed.

B. Serological tests:

1. Indirect fluorescent antibody test (IFAT) for diagnosis of toxoplasmosis:

The formalized whole tachyzoites antigen slides for the IFAT was prepared as described by Goldman (1957) and the technique was adopted according to the procedures mentioned by Shaapan et al. (2008) and at a dilution of 1:200 of human and swine sera.

2. Microscopic agglutination test (MAT) for diagnosis of leptospirosis:

Leptospira interrogans serovars *pomona*, *icterohaemorrhagiae*, *grippityphosa* were used for MAT. They were grown in EMJH liquid and semisolid media (Difco, USA) at 29-30 °C and the growth was assessed by dark field microscopy regularly. These reference leptospiral strains were kindly obtained from C. Sulzer, C.D.C., and Atlanta, U.S.A. The gold standard serodiagnostic test for leptospirosis is MAT, which was performed as per the method of Galton et al. (1965) and its modification (Cole et al., 1973). Briefly, the sera from the swine were serially diluted from 1:100 to 1:3200 in phosphate buffered saline (PBS), pH 7.2 and allowed to react with live antigen suspensions of the reference leptospiral serovars. After 2 hours incubation at 37 °C, the serum-antigen mixtures were examined by dark field microscopy for the presence of agglutination/ clearance of the organisms and the titers were determined. Reciprocal agglutination titers of greater than or equal to 200 were considered as positive reactions.

3. Rose Bengal plate Test (RBPT) for diagnosis of brucellosis:

The Rose Bengal stained brucella antigen is used for the early detection of brucella agglutinins (*Brucella Suis*) according to (Alton et al., 1988). For RBT, 1 drop (30 ml) of test serum was added to Rose Bengal antigen on a white porcelain plate and mixed thoroughly with a stick. The plate was rocked slowly for 4 minutes and observed.

3: Results:

Swine and in contact human sera which assayed serologically, illustrated sero-positive results with different percents (74.78 & 37.79), (12.61 & 8.66) and (23.1 & 25.98) corresponding to the *Toxoplasma gondii*, *Brucella suis* and *Leptospira spp* respectively. The used testes were Indirect Fluorescent Antibody Test, Rose Bengal plate test and Microscopic Agglutination Test consequence to the three zoonoses respectively (Table 1).

Swine and human sera demonstrating wide-ranging results of *T. gondii* antibodies by IFAT titers range from 1/16 to 1/1024. The higher swine

percent (18.60) was established at titer of (1/512), while the higher human percent (22.92) was recognized at titer of (1/128) (Table 2).

Varied percents were detected in swine and human sera (45.3 & 11), (20.8 & 7) and (33.9 & 7.9) corresponding to the *L. pomona*,

L.icterohaemorrhagiae and *L. gipotyphosa* respectively. Also, swine and human sera confirming various results of Leptospiral Serovars antibodies by MAT titers range from 1/200 to 1/3200 (Table 3).

Table 1: *Toxoplasma gondii*, *Brucella suis* and *Leptospira spp* Comparative sero-positive results in humans and swine sera.

	No. of tested swine sera	No. of sero-positive & (%)	No. of tested Human sera	No. of sero-positive & (%)	Used test
<i>Toxoplasma gondii</i>	230	172(74.78)	127	48(37.79)	IFAT
<i>Brucella suis.</i>	230	29(12.61)	127	11(8.66)	RBT
<i>Leptospira spp.</i>	230	53(23.1)	127	33(25.98)	MAT

IFAT: Indirect Fluorescent Antibody Test MAT: Microscopic Agglutination Test
RBT: Rose Bengal plate test.

Table 2: Human and swine sera comparative results of *T. gondii* detected by IFAT titers.

	IFAT Titers (total immunoglobulin) No of positive cases						
	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
No. of seropositive swine 172 /230 (%)	17 9.89	18 10.46	19 11.05	29 16.86	29 16.86	32 18.60	28 16.28
No. of seropositive human 48 /127 (%)	2 4.16	4 8.32	7 14.58	11 22.92	9 18.75	7 14.58	8 16.66

Table 3: Human and swine sera comparative results of Leptospiral Serovars detected by MAT titers.

serovars		≥200 MAT Titers (total immunoglobulin)				
		1/200	1/400	1/800	1/1600	1/3200
	53/230 Seropositive swine & (%)					
<i>L. pomona</i>	24(45.3)	3	4	6	6	5
<i>L.icterohaemorrhagiae</i>	11(20.8)	1	2	3	2	3
<i>L. gipotyphosa</i>	18(33.9)	2	3	4	6	3
Total	53(23.1)					
	33/127 Seropositive persons & (%)					
<i>L. pomona</i>	14(11)	2	3	3	5	1
<i>L.icterohaemorrhagiae</i>	9(7)	3	3	2	1	0
<i>L. gipotyphosa</i>	10(7.9)	4	2	1	3	0
Total	33(25.9)					

4. Discussion:

Infected pregnant sows may be aborted consequence to infection by *Brucella*, *Leptospira* or *Toxoplasma*, their foeti, fetal fluid, and membranes are harboring the causative pathogen. So, the aborted swine constitute direct occupational health hazard for manipulating employees or animals that licked or eaten the infected fluids or tissues. Accordingly, the three pathogens could maximize their persistence in infected swine's herds. Apparent healthy seropositive swine may be chronic shedders for *Brucella* and *Leptospira* via urine and other body fluids, while

T. gondii sero-positive swine may harbor tissue cysts which don't usually constitute occupational bio-hazard to human, except through manipulating or feeding under cooked pork.

In this study, 12.61% and 8.66% of the examined swine and contact persons sera had *Brucella* antibodies, the compatibility between swine's and human percentages are due to the fact that *Brucella* is of highly contagious characters. Where humans in contact mainly contracted infection from shedder swine, and usually predisposes farmers, shepherds, butchers, laboratory workers,

veterinarians and slaughterhouse workers. Also, indicate that swine's behavior in clay water pools may play vital role for swine's and human communication via droplet infection and through intact or abraded skin. The practical confirmation was done when three occupational groups exposed to brucellosis hazard were investigated in pork in 1967, using three standard tests. of 80 veterinarians, 92.5% had serological evidence of past or present brucella infection (Pappas et al., 2006). On the other hand, lower results of brucella antibodies in human were recorded by Omer et al. (2002) who found that the prevalence of brucellosis among high risk occupational groups using Rose Bengal test is among occupational personnel (4.5%). Mudaliar et al. (2003) recorded prevalence of brucellosis of 5.33% in animal handlers and advised that the clinician should keep in mind the possibility of an occupational or environmental exposure in cases of fever of unknown origin. The countries with the highest incidence of human brucellosis are Iran (29.8/100,000), Saudi Arabia (32.8/100,000), Syria (21.0/100,000), Jordan (20.4/100,000), Palestine (21.5/100,000) and Oman (16.6/100,000). Bahrain and Cyprus have reported zero incidences. In the rest of the countries, the incidence varies from 0.8/100,000 in Egypt to 9.0/100,000 in Tunisia (Smits and Culter, 2004).

Concerning leptospirosis, in this study, the percent of infected swine and contact humans are compatible 23.04 and 25.98, respectively. The higher incidence in workers may be clarified due to the fast changeability in swine's herd individuals, in contrast to long term stability with the same occupational employees. Nei and Kumar (2000) investigated the sera from 1215 meat inspectors and 1248 meat workers for the presence of agglutinating titers of 1:24 or greater to the serovar μ known to be endemic in New Zealand. Although 10 percent of meat inspectors and 6.2 percent of meat workers were seropositive, only 9.5 percent of meat inspectors and 4.1 percent of meat workers had titers compatible with occupational exposure to domestic stock, although the results of this survey demonstrate that leptospirosis is a definite occupational hazard in the meat industry, the risk is threefold less than for dairy farm workers and pig farmers (Slack et al., 2008). Infection is usually due to contact with the pig's or other wildlife urine. Rodents play a significant role in disease maintenance within confinement operations and other swine facilities (Levett, 2001). Venereal transmission from carrier boars and sows may play a role in maintenance of the disease (Bharti et al., 2003).

In the present investigation, high percent (74.78) of the examined swine sera are carried *T.*

gondii antibodies and could be regarded as high risk animal groups for both public and animals' health, connected to the pattern of wastage raising swine's on oocysts dirty unsanitary condition. The prevalence usually higher in sows that suffers toxoplasmic abortions due to placental transmission or postnatal infection via licking aborted foeti and amniotic fluids containing tachyzoites, plus congenital transmission may occur during pregnancies (Dubey, 2002). Swine feed from the ground; consequently the high seropositive percent in free range swine is accepted as significant bio-indicator evaluate the degree of *T. gondii* oocysts environmental pollution, and reflect the fragile measures opposite to stray cats in the locality of swine subset (Howe et al., 1997). Hassanain et al., (2008) confirm high oocyst Egyptian environmental pollution through high incidence of naturally infected kittens (70.6 %) with consequence shedding oocyst. Also, ELfadaly, (2007) confirm 61.4 % sero-positive Egyptian sheep fed on the same unhygienic condition, The difference between swine and sheep species may be related to the omnivorous behavior of swine that usually feed on rodent, meat or poultry residuals containing tissue cysts, this in contrast to herbivorous sheep.

Inadequate rodent control is considered to play a role in swine toxoplasmosis. Three organic pig farms with known rodent infestation were included in study conducted by Fuentes et al. (2001). On these farms, presence of *T. gondii* in trapped rodents was evaluated by real-time PCR. All rodent species and shrews investigated had *T. gondii* DNA in brain or heart tissue. Prevalence was 10.3% in *Rattus norvegicus*, 6.5% in *Mus musculus*, 14.3% in *Apodemus sylvaticus* and 13.6% in *Crocidura russula*. Initial *T. gondii* seroprevalence in the slaughter pigs was dropped on the three farms from 17% to 8% after rodent control.

Pigs are considered to be the most important meat source of *Toxoplasma gondii* for humans in the United States (Grigg and Boothroyd, 2001). Antibodies to *T. gondii* were found in 16.97% (141/831) with slaughter pigs having the highest rate (22.28%), followed by breeding sows (16.59%) (Ajzenberg et al., 2002). During the hunting seasons 2002-2008 from wild boar in France, Antibodies to *T. gondii* were found in 26 (17.6%) of 148 wild boars using the modified agglutination test (MAT, positivity threshold: 1:24). Seroprevalence was 45.9 % (Cook et al., 2000). Prevalence of *T. gondii* in market pigs appears to have declined in the US with the advent of improved sanitation in large production facilities (Davies et al., 1998). However, a serological survey of pigs of variable age from 85 New England farms showed an overall prevalence of 47%, with 91% of the herds having at least one

seropositive pig, and within-herd prevalence varied between 4 and 100% (Gamble et al., 1999).

In the present work, swine's that carrying *T. gondii* antibodies were 74.78 %, while 37.79% of occupational humans in contact. The difference in percent between swine and working persons was referred to that *T. gondii* is not mainly transmitted via occupational mode, unless through skin abrasions during handling aborted foeti harboring tachyzoites or pork containing bradyzoites.

Egyptian swine's included in this study found to be apparent health when blood samples were taken, although they are still free feed on wastages; perhaps confirming the belief that, in pigs, equilibrium exists between zoonotic agents and swine species. It can be concluded that serological assays concerning brucellosis, leptospirosis and toxoplasmosis verify direct occupational exposure for high risk group's manipulating employees through carrier swine's or their pollutant conditions. Also, this study reflect the need of public health worry by Egyptian veterinary authorities' toward wastage nourished swine's on such condition that costumes spots for spread out epidemics .

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