International Journal of Veterinary Science and Medicine

ISSN: (Print) 2314-4599 (Online) Journal homepage: https://www.tandfonline.com/loi/tvsm20

Epidemiological studies on Johne's disease in ruminants and Crohn's disease in humans in Egypt

A. Fawzy, A. Prince, A.A. Hassan, A. Fayed, M. Zschöck, M. Naga, M. Omar, M. Salem & A. El-Sayed

To cite this article: A. Fawzy, A. Prince, A.A. Hassan, A. Fayed, M. Zschöck, M. Naga, M. Omar, M. Salem & A. El-Sayed (2013) Epidemiological studies on Johne's disease in ruminants and Crohn's disease in humans in Egypt, International Journal of Veterinary Science and Medicine, 1:2, 79-86, DOI: 10.1016/j.ijvsm.2013.09.004

To link to this article: https://doi.org/10.1016/j.ijvsm.2013.09.004

International Journal of Veterinary Science and Medicine (2013) 1, 79–86

Cairo University

International Journal of Veterinary Science and Medicine

www.vet.cu.edu.eg
www.sciencedirect.com

Full Length Article

Epidemiological studies on Johne's disease in ruminants and Crohn's disease in humans in Egypt

A. Fawzy [a,b], A. Prince [a,c], A.A. Hassan [d], A. Fayed [a,b], M. Zschöck [e], M. Naga [f], M. Omar [g,h], M. Salem [a,b,*,1], A. El-Sayed [a,e,1]

[a] Laboratory of Molecular Epidemiology, Faculty of Veterinary Medicine, Cairo University, Egypt
[b] Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt
[c] Agricultural Research Service, USDA, Beltsville, MD, USA
[d] Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq
[e] Landesbetrieb Hessisches Landeslabor, Giessen, Germany
[f] Endoscopy Unit, Department of Internal Medicine, Faculty of Medicine Kasralainy, Cairo University, Egypt
[g] Department of Microbiology and Immunology, Faculty of Medicine, King Abdul Aziz University, Saudi Arabia
[h] Department of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, Egypt

Received 17 May 2013; revised 28 September 2013; accepted 29 September 2013
Available online 5 November 2013

KEYWORDS

ASCA;
Crohn's disease;
Egypt;
Johne's disease;
Mycobacterium avium paratuberculosis;
Epidemiology

Abstract The correlation between Johne's disease (JD) and Crohn's disease (CD) in Egypt was investigated. A total of 371 human and 435 animal sera were collected from the same Egyptian governorates that had a known history of paratuberculosis infection and were subjected to screening for paratuberculosis using ELISA to assess the human/animal risk at a single time point. Five CD patients and five JD clinically infected dairy cattle were also included. Out of 435 animal serum samples, 196 (45.2%) were MAP-ELISA positive. Twenty three (6.1%) out of 371 human serum samples were MAP-ELISA positive, while 37 (9.9%) were positive for anti-*Saccharomyces cerevisiae* antibodies (ASCA) ELISAs. There was a very poor agreement between human MAP and ASCA ELISAs (0.036 by kappa statistics). The prevalence of MAP antibodies among humans is clearly lower than in animals. In conclusion there is an increase in Johne's disease incidence in animals and a very weak relationship between MAP and Crohn's disease in humans in Egypt.

* Corresponding author. Address: Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Giza, Egypt. Tel.: +20 (02) 35716841; fax: +20 (02) 35725240.
E-mail address: mohamedasalem@hotmail.com (M. Salem).
[1] The last two authors contributed to this work.

Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

ELSEVIER Production and hosting by Elsevier

1. Introduction

In the last few years a great attention was given for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) as its real threat is clearly underestimated for both cattle and human. This worry is justified as MAP could be detected in environmental samples, survives up to one year in contaminated pastures, can remain viable after milk pasteurization and resists the ripening process of cheese [1]. Although MAP was firstly isolated in Egypt in 2004 [2], yet no data are available about the

2314-4599 © 2013 Production and hosting by Elsevier B.V. on behalf of Faculty of Veterinary Medicine, Cairo University.
http://dx.doi.org/10.1016/j.ijvsm.2013.09.004

distribution, economic losses, prevalence in different species and the epidemiology of the disease in Egypt. The pilot study of Salem, et al. 2005 proved the widespread of MAP in Egyptian farms. The high resistance of MAP to environmental factors and its difficult high cost diagnosis hindered the performance of efficient control programs in Egypt. On the other hand, the role of MAP in public health as the causative agent of Crohn's disease (CD) has long been debated. Although an association between MAP and CD was reported since 1913 [3], there is no evidence till now for neither dependent causation nor independence of MAP in CD. Despite almost 100 years of investigation, conflicting data still occur and the responsibility of MAP as the etiological agent of CD were proven in some reports [4–6] and denied by others [7,8]. Meanwhile, some researchers incriminated certain but not all MAP genotypes to induce the disease [1,9]. Others detected MAP as a co-factor with other pathogens such as *Pseudomonas*, *Campylobacter, Listeria, Brucella* and *Yersinia* or other factors such as fragments of dead bacteria, immune dysregulation, genetic predisposing factors, smoking and even certain elements of food in the induction of CD [10–14].

This study was conducted to identify the areas at risk in Nile delta and the level of that risk and to investigate both incidence and correlation of MAP and CD among attendants on epidemiological, serological and molecular biological basis.

2. Materials and methods

2.1. Samples, human patients and controls

Serum samples were collected from 435 apparently healthy animals (3–8 years old) during the period from June 2009 till July 2010 to be screened for the presence of MAP (Table 1). These samples represent multiple ruminant species that were raised in some Egyptian governorates with previous history of MAP infection [2]. In the same period and from same governorates, sera of 366 humans were collected from individuals that do or do not have contact with farm animals to be screened for the presence of *M. avium* subsp. *paratuberculosis* and anti-*Saccharomyces cerevisiae* antibodies.

Five patients undergoing a routine ileocolonoscopy as a normal part of their clinical care were also enrolled in the study. Serum and mucosal biopsies were obtained from all patients. Informed consent was obtained from each individual. The case definition of CD was established on the basis of standard clinical, radiological, endoscopic, and histopathological criteria. Paired biopsy specimens were obtained from the terminal ileum and colon particularly from inflamed oedematous mucosa and not from the floor of the ulcers, as it may be fibrotic [15]. Each mucosal sample was taken from the biopsy forceps with a sterile needle and transferred to a 1.5-ml sterile screw-cap reaction tube containing 0.5 ml of sterile 0.85% saline. In addition, tissue samples were collected from five Holstein cows suffering from chronic digestive disorders in a private farm with a current paratuberculosis problem. MAP was isolated and characterized from fecal samples collected from these cows in an early report [16] and hence were selected to be included. These cows were brought to the regional slaughter house for culling purpose. Paired samples were obtained (approximately same size as human biopsies) from inflamed oedematous mucosa of the terminal ileum and colon by sterile scissor and forceps and were transferred in 1.5-ml sterile screw-cap reaction tubes containing 0.5 ml of sterile 0.85% saline.

All tissue samples were not frozen at any time, they were immediately taken to the laboratory to be processed. Serum samples were separated into aliquots of 20–1000 μl and stored at −20 °C for short-term storage (< 6 months) or at −80°C for long-term storage (> 6 months). Frozen specimens were mixed well after thawing and prior to testing.

2.2. ELISA for animal and human serum antibodies to *M. avium subsp. paratuberculosis*

The presence of MAP antibodies was detected in animal serum samples using the ID Screen® Paratuberculosis Indirect ELISA kit (ID VET diagnostics, Montpellier, France). Results were interpreted according to manufacturer's instructions. The same kit was adapted for human use. *M. avium* subsp. *paratuberculosis* antigen-coated 96-well microtiter plates and *Mycobacterium phlei*-containing serum diluent (used to absorb cross-reactive antibodies) supplied with the kit were used. A horseradish peroxidase-labeled protein G conjugate (Sigma, Germany) (diluted 1:100,000 according to manufacturer's instructions) was used instead of conjugate supplied with the kit, to detect binding of human antibodies to the solid-phase *M. avium* subsp. *paratuberculosis* antigens. On each ELISA plate positive and negative bovine and human control sera were tested in duplicate along with bovine control sera provided with the kit.

A commercial pool of serum from normal healthy blood donors (El Rouda laboratory, Cairo, Egypt) was used as the negative human control. The human serum samples were diluted 1:20 in kit serum diluent (identical to the kit protocol for testing bovine sera) and incubated for 30 min at room temperature prior to transfer to the microtiter plate. One hundred microliters of each diluted control serum and serum sample was dispensed into duplicate wells and incubated for 30 min at room temperature then were removed by washing three times with phosphate buffered saline. After removal of residual wash fluid, 100 μl of conjugate was added and the plate was incubated another 30 min. After washing the wells as before, 100 μl of TMB substrate solution was added to each well and incubated for 15 min. The enzymatic reaction was stopped after 15 min by addition of 100 μl of stop solution provided with the kit (diluted H_2SO_4). The absorbance for each well was measured spectrophometrically at 650 nm (ELx808 Absorbance Microplate Reader; Biotech Instruments, Inc. Winooski, VT.). Mean ELISA absorbance values were transformed to sample-to-positive (s/p) ratio as done for the cattle assay; however, absorbance values for the human sera were used as controls in the calculation s/p = absorbance of sample − absorbance of negative control/absorbance of positive control − absorbance of negative control. Three hundred and seventy one human serum samples were tested from different Egyptian governorates (Nile delta, Egypt.) for human serum antibodies to MAP were used to establish the s/p cut-off for a positive test.

2.3. ELISA for human serum anti-*S. cerevisiae* antibodies (ASCA)

Enzyme-linked immunosorbent assays for the semi-quantitative detection of anti-*S. cerevisiae* antibodies (ASCA) of the

Table 1 Seroprevalence of MAP in investigated animal species.

Species	Farm code	origin	Breed	Import origin	Nr. of samples	Nr. of MAP ELISA positive	Positive (%)
Cattle	1-F	Fayyum	Holstein	USA	32	11	
	2-F	Fayyum	Holstein	USA	4	4	42
	1-I	Ismailia	Holstein	USA	60	52	
	2-I	Ismailia	Holstein	USA	9	2	61
	3-I	Ismailia	Holstein	USA	33	8	
	1-M	Mansoura	Holstein	USA	8	2	25
	SA	Giza	*Bos taurus*	Egypt	3	1	66
	1-Mo	Monfia	Holstein	Egypt	35	19	54
	1-G	Gharbia	*Bos taurus*	Egypt	21	1	5
	1-S	Sharkia	Frisian	USA	28	8	29
					233	108	46.4
Buffaloes	SA	Giza	*Bubalus bubalis*	Egypt	39	18	
	SA	Giza	*Bubalus bubalis*	Egypt	1	0	
	SA	Fayyum	*Bubalus bubalis*	Egypt	18	8	
					58	26	44.8
Sheep	1-A	Cairo (El-Obour)	Rahmani	Egypt	25	17	
	SA	Giza (Dairie)	Osseimi	Egypt	22	18	
	SA	Giza	Osseimi	Egypt	41	6	
					88	41	46.6
Goat	SA	Giza	Nubian	Egypt	56	21	37.5
Total					435	196	45

IgA and the IgG classes were applied. All human serum samples were tested by both the QUANTA Lite™ ASCA (*S. cerevisiae*) IgG and IgA ELISAs as recommended by the manufacturer (Medical Technology Promedt consulting GmbH, Germany). In both the IgG and IgA assays, partially purified and disrupted *S. cerevisiae* antigen was bound to the wells of polystyrene microwell plates under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera were added to separate wells, allowing any ASCA IgG or IgA antibodies present to bind to the immobilized antigen. Unbound samples were washed away and an enzyme labeled anti-human IgG or IgA conjugate was added to each well. A second incubation was performed allowing the enzyme labeled anti-human IgG or IgA to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity was measured by adding a chromogenic substrate and measuring the intensity of the color that develops. Both assays were evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

2.4. DNA extraction for tissue samples

One tissue mucosal sample from each pair was processed for direct IS*900*-specific PCR testing following the method described by Bull and colleagues 2003 [15]. Briefly, tubes containing the biopsy specimens were centrifuged at 10,000×*g* for 3 min. The supernatant was removed and the pellet was resuspended in 600 μl of mycobacterial lysis buffer (2 mM sodium EDTA, 400 mM NaCl, 10 mM Tris HCl [pH 8.0], 0.6% sodium dodecyl sulfate and 33 μg of proteinase K (Sigma, Germany) made up in nuclease-free water and transferred to a Lysing Matrix B Ribolyser tube (Qbiogene, Noltingham, United Kingdom).

The Ribolyser tube was shaked at 37 °C for 2 h. The tubes were chilled on ice for 5 min and mechanically disrupted in the FastPrep Ribolyser (Qbiogene, Noltingham, United Kingdom) and then immediately chilled again on ice for 15 min. A volume of 600 μl of phenol (Sigma, Germany) was added and the mixture was vortexed and then centrifuged at 10,000×*g* for 1 min. The aqueous layer was transferred to a new screw-cap reaction tube containing an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) (Sigma Germany), and the mixture was saturated in 1× TE and centrifuged (10,000*g* for 1 min). The aqueous layer was again transferred to a new tube containing chloroform-isoamyl alcohol (24:1), vortexed and centrifuged (10,000*g* for 1 min). The final aqueous layer was transferred to a new tube containing 90 μl of 10 M ammonium acetate and mixed thoroughly. One milliliter of ice-cold 100% ethanol was added to enable DNA precipitation at room temperature for 1 h. Samples were then centrifuged (10,000*g* for 20 min) and the pellets were washed in ethanol. The pellets were dried at RT for 30 min, resuspended in 50 μl of 1× TE.

2.5. Culture of biopsy specimens

The second of each pair of biopsy specimens from each tissue sample was decontaminated when fresh and subsequently cultured in separate HEYM tubes at 37 °C for up to 20 months. Briefly, 0.5 L of NaOH–*N*-acetyl-L-cysteine (BBL Mycoprep; Becton Dickinson) was added to each sample in 0.5 ml of saline, and the mixture was incubated at RT for 20 min. The samples were then centrifuged (10,000*g* for 10 min). The pellet was resuspended in 0.5 ml of TEN buffer (2 mM sodium EDTA, 400 mM NaCl, 10 mM Tris HCl [pH 8.0]; Sigma) and

transferred to 4 HEYM slants supplied with mycobactin J (Becton Dickinson). After incubation at 37 °C for between 14 and 50 weeks, cultures with visible growth were removed aseptically for testing by the IS*900*-specific nested PCR as described below. Acid-fast colonies were subcultured onto HEYM tubes with and without mycobactin J.

2.6. IS900 nested PCR assay

The primer set used in this PCR was the primer pair TJ1 (5′ GCT GAT CGC CTT GCT CAT 3′), TJ2 (5′ CGG GAG TTT GGT AGC CAG TA 3′) in the first-round PCR and primer pair TJ3 (5′ CAG CGG CTG CTT TAT ATT CC 3′), TJ4 (5′ GGC ACG GCT CTT GTT GTA GT 3′) in the nested PCR [15]. In the first-round PCR the primers were used in a final concentration each of 2 μM in a 50 μl PCR reaction master mix which also included 1.25 U *Taq* polymerase, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP mix and 5 μl of isolated DNA. The thermal cycler parameters used for amplification included an initial 95 °C incubation step for 10 min for *Taq* polymerase activation followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 2 min with a final extension step at 72 °C for 10 min. The amplification products (357 bp) were visualized on a UV transilluminator after electrophoresis at 125 V for 45 min in 1.5% agarose gels pre-stained with ethidium bromide.

For the nested PCR, 5 μl of the first-round reaction mixture were transferred into a 45-μl reaction mixture that had the same ingredients as the primary reaction mixture but with 2 μM (each) primers from the primer pair TJ3 and TJ4. Cycling conditions were 1 cycle of 95 °C for 5 min and then 40 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min followed by 1 cycle of 72 °C for 10 min. Amplicons of the expected size (294 bp) were visualized with ethidium bromide on 1.5% agarose gels. In each PCR run, negative controls were tested in triplicate containing 5 μl of the buffer used to elute the DNA, 5 μl sterile bi-distilled water and 5 μl DNA from a coded sample that has shown to be IS*900*-specific PCR negative. While positive control tubes ($n = 2$) included 5 μl DNA from Map the 19698 strain and 5 μl DNA from a coded sample that has shown to be IS*900*-specific PCR positive.

2.7. Statistical analysis

Chi-squared test was used for assessment of risk of paratuberculosis seropositivity in sheep associated with breed susceptibility.

Kappa statistics were used to determine agreement between modified MAP and ASCA ELISAs as diagnostic tools for Crohn's disease in humans.

3. Results and discussion

MAP represents a serious problem since its first report in different governorates of Egypt [17]. The result obtained in this study highlights the increased incidence of MAP infection in Egypt that might lead to severe economic losses to ruminant industry and might also be a future threat to public health. All human and animal sera included in the present work were collected in the period from June 2009 till July 2010 at a single time-point from apparently healthy individuals. Tested animals and human individuals originated from the same Egyptian governorates that had a known history of paratuberculosis infection and were subjected to screening of MAP using ELISA in order to assess the human/animal risk at a single time point. Out of 435 animal serum samples, 45.2% were MAP ELISA positive. On the species level, 109 out of 233 dairy cattle were ELISA positive with a percentage of 46.6%.

This percentage is higher than previous reports [18–20]. The revealed high percentages may be due to bias by investigating paratuberculosis-suspected farms. Twenty six out of 58 buffalo serum samples were ELISA positive with a percentage of 44.8% that was higher than that reported by Singh et al. [6] (28.6%) for buffaloes in Northern India. Forty one out of 88 sheep serum samples were ELISA positive with a percentage of 46.5% that was significantly higher than that reported by Anna et al. [21] (6.29%) for sheep belonging to two provinces in central Italy. Twenty one out of 56 goat serum samples were ELISA positive with a percentage of 37.5% that was higher than that reported by Lee et al. [22] (15.3%) for black goats in some regions of Korea and Mercier et al. [23] (2.9%) for dairy goats in France. The detection of MAP antibodies in investigated apparently healthy animals emphasizes the role of subclinical animals in the spread of the disease.

Paratuberculosis was detected in water buffalo (*Bubalus bubalis*) in Indian subcontinent by different diagnostic techniques including ELISA [6], PCR [24], and culture [25]. The Egyptian water buffaloes (*Bubalus bubalis*) included in this study showed serological evidence of paratuberculosis infection.

For sheep samples, the seroprevalence is the highest in Dairie locality, Giza, Egypt (82%) and this might be attributed to the fact that sheep reared in this locality has replaced pigs in the elimination of organic wastes that include fecal matters of different animals, which will subject these animals to different pathogens specially those caused by robust microorganisms like MAP. The seroprevalence in El-Obour locality, Cairo, Egypt is 68% and this may be due to close contact of sheep in this locality with imported cattle. With the regard to governorate-based and breed-based seropositivity, it was found that seropositivity was higher in Cairo (68%, 17 out of 25 Rahmani sheep) than in Giza (38%, 24 out of 63 Osseimi sheep). Assessment of breed as a probable risk factor of paratuberculosis seropositivity revealed a chi-squared value (χ^2) of 6.43 (Table 2).

The corresponding *p*-value (probability value) was determined to be 0.01 in the chi-squared table, when the value of degrees of freedom is one. The obtained *p*-value is less than the significant cutoff value (0.05); consequently sheep breed is a probable statistically significant risk factor of paratuberculosis

Table 2 Chi-squared test for assessment of sheep breed as being risk factor of seropositivity for paratuberculosis.

Total	ELISA negative	ELISA positive	Sheep breed
25 (a + b)	8 (b)	17 (a)	Rahmani
63 (c + d)	39 (d)	24 (c)	Osseimi
88 (N)	47 (b + d)	41 (a + c)	Total

(a) ELISA positive Rahmani sheep, (b) ELISA negative Rahmani sheep, (c) ELISA positive Osseimi sheep, (d) ELISA negative Osseimi sheep. (N) Total number of sheep.

Table 3 Classification of human samples and corresponding ASCA and MAP antibodies status.

Origin	Number		Contact with farm animals	Clinical complains	Endoscope	MAP ELISA +ve[c]		ASCA ELISA IgA +ve		ASCA ELISA IgG +ve		ASCA +ve for both IgA +IgG	
	F[a]	M[b]				F	M	F	M	F	M	F	M
Cairo	60	62	No	No	No	1	4	3	11	1	6	1	
Giza	80		Yes	No	No	7		8		4		2	
		60	No	No	No		1		3		2		
		14	Yes	No	No								
		5[d]	Yes	Yes	Positive		2				2		1
Nile delta	30	60	Yes	No	No	3	5			1			
Total	170	201				11	12	11	14	6	10	3	1

[a] F: Female.
[b] M: male.
[c] +ve: positive.
[d] CD patients.

seropositivity; however, other factors may contribute to this highly significant difference provided that the two different breeds were reared under different environmental conditions and probably different exposure rates.

The seroprevalence of MAP among foreign breeds of cattle seems to be higher than others. Animals reared in Ariaf Imbaba and reared in sporadic form have the lowest prevalence. This may be attributed to the nature of this disease as an exotic disease that was recently introduced to Egypt [17]. In addition, most of the examined cattle (reared in large farms in Egypt) are imported from USA, Germany, Canada or other countries facing a problem with MAP [26].

With regard to governorate-based seropositivity, the highest percentage of cattle seropositivity to paratuberculosis was detected in Ismailia governorate (66%), followed by Monofia (54%) while seropositivity in other tested governorates (Fayoum, Giza, Sharkeya, Mansoura and Gharbia) ranged from 42% to 5% (Table 1). These relatively high positive percentages of the ELISA in investigated localities may be attributed to bias resulting from inclusion of a few numbers of dairy cattle herds previously suspected of having paratuberculosis infection.

In the last decade, there was a growing debate about the possible link between Crohn's disease in human and MAP infection. In the present work, we investigated the correlation between JD and CD based on three bases; epidemiological, serological and molecular biological. From the serological point of view, ASCA are antibodies primarily directed against a 200 kD a phosphopeptidomannan cell wall component of the common baker's or brewer's yeast *Saccharomyces* (*S*. cerevisiae). ASCA reactivity could be the result of cross-reacting antibodies to antigens found in a non-yeast organism that has not yet been identified. Mannose is not only found in yeast but also in mycobacteria and other microorganisms [27]. According to the international incidence rate, ASCA are found in 39–69% of CD patients [28]. Interestingly, the sensitivity of ASCA IgA was reported to be lower in Japanese and Chinese CD patients when compared to Caucasian CD patients suggesting that the ASCA response may be influenced by several distinct genetic determinants and/or environmental risk factors [27].

In Egypt, according to our data (Tables 3–5), the number of ASCA positive cases is above the detected MAP seropositive human cases in the population. Twenty three out of 371 human serum samples were positive when investigated for the presence of MAP antibodies using indirect modified ELISA with a positive percentage of 6.1% which was lower than that reported by Bernstein et al. [29] (35%).

A positive human serum was defined based on human sera collected from different Egyptian governorates as the mean plus 2 standard deviations, which by definition gave the assay 94% specificity. Whereas 37 out of 371 human serum samples were positive for either ASCA IgG or ASCA IgA ELISAs or for both, with a positive percentage of 9.9%. Agreement between modified MAP and ASCA ELISAs was calculated to be 0.036 by kappa statistics (Table 6), which indicates poor agreement between both tests and supposes either a weak or no relationship between Crohn's disease in humans and MAP infection based on serological status and may indicate that Crohn's is a multifactorial disease in which MAP plays a minor rule or has no role.

Although the diagnosis of CD depends on ASCA ELISA in combination with colonoscopy, yet only two patients out of 5

Table 4 Human serum samples: number of ASCA positive, number of MAP ELISA positive and number of both MAP and ASCA positive samples.

Origin	Total Number	ASCA positive	MAP positive	MAP and ASCA positive
Cairo	122	20	5	1
Giza	94	10	7	2
	60	4	1	0
	5	2	2	1
Nile delta	90	1	8	0
Total	371	37	23	4

Table 5 Human samples according to sex and to animal contact.

	Females	Males	Human – no contact with farm animals	Human – in contact with farm animals
MAP ELISA positive	11/170 (6.4%)	12/201 (5.9%)	6/182 (3.2%)	17/189 (8.9)
ASCA ELISA positive	14/170 (8.2%)	23/201 (11.9%)	24/182 (13.1%)	13/189 (6.8%)

Table 6 Agreement between MAP and ASCA ELISAs on human sera.

Total	ASCA ELISA Neg.[^]	ASCA ELISA Pos.[*]	
27	23	4	MAP ELISA Pos.
344	307	37	MAP ELISA Neg.
$n = 371$	330	41	Total

[*] Positive for either ASCA IgG or IgA or both.
[^] Negative for both ASCA IgG and IgA.

Table 7 Bacteriological and molecular biological detection of MAP in examined cattle tissue samples and human intestinal biopsies.

Tissue origin	IS*900* nested PCR	Culture	Culture-ZN stain	Culture IS*900* PCR
Human ($n = 5$)	0/5	0/5	ND	ND
Cattle ($n = 5$)	5/5	5/5	5/5	5/5

ZN: Ziehl–Neelsen, ND: not done.

CD confirmed cases were ASCA positive (40%). This indicates the low detection level of ASCA and confirms that CD may have different causes and cannot be detected with a single test. Moreover, the prevalence of MAP antibodies among humans is clearly lower than in animals. This may be either due to the recent introduction of MAP to Egypt (first reported in 2005 while it is a well-known disease in other countries exporting cattle to Egypt), or the low susceptibility of Humans to MAP. It is also of great importance to mention that most of the infected cattle were either imported from MAP endemic countries or were born in Egypt in contaminated farms [26].

Some literature suppose the susceptibility of human to certain but not all strains of MAP, which may be the cause of this lower seroprevalence in comparison to animals [9]. The obtained data showed also that the prevalence of MAP in human without contact with animals was 3.2% compared to 8.9% in those with contact with animals. Additionally, there was no big difference in susceptibility between females and males 6.4% compared to 5.9% and this might reveal the susceptibility of human to MAP direct infection from animals.

In this work, we could not detect MAP by traditional bacteriological and molecular biological methods in Crohn's sufferers surgically dissected intestinal sections. Contradictory it was very easy to detect the bacteria in all clinical cases of JD when processed using the same protocols (Table 7). One of five human patients previously diagnosed as Crohn's sufferers tested positive with both ASCA IgG and MAP ELISAs. Nevertheless, no MAP or MAP DNA could be detected in biopsies obtained from these five patients by IS900 nested PCR and/or culture method. On the other hand, all five tissue samples collected from clinically affected cattle showed positive IS900 PCR results and colonies growth of typical morphological characteristics of MAP including greyish white color and pinpoint half-balled shape.

These colonies were further characterized based on Ziel Nielsen stain, mycobactin J dependency and IS900 PCR [26].

This may indicate, either that there is no relationship between CD and JD or that CD is a multi-factorial disease and can be induced by various causes including MAP [30]. Such low detection sensitivity of culture of human biopsies might be attributed to the low detection sensitivity of the standard culture technique and other culture protocols and/or media like the MGIT or the Bactec system might be needed for the successful isolation of MAP from surgically dissected human biopsies. Additionally, some human isolates may take up to six years to grow even under standard culture and decontamination conditions [26]. On the contrary, some laboratories reported the successful isolation of MAP not only from Crohn's sufferers intestine, but also from human patients blood and breast milk samples [31].

4. Conclusion

Although different techniques were used, the comparison of the obtained data in the current study with that from our previous report in 2005 indicates the increase in disease incidence in animals [2]. So an effective control program is needed in order to prevent the spread of MAP infection on both local and national level in Egypt. The detection of MAP antibodies in investigated apparently healthy animals emphasizes the role of subclinical animals in the spread of the disease. There was a serological evidence of paratuberculosis infection among different species of livestock included in this study necessitating a more comprehensive monitoring of the disease among different animal species in Egypt. Sheep breed was found to be a probable statistically significant risk factor of seropositivity to paratuberculosis with Rahmani breed being more susceptible than Osseimi one, taking into consideration that the two different breeds were reared under different conditions. Egyptian water buffaloes showed seropositivity to MAP antigen; however the epidemiological role of buffaloes should be further investigated. A very weak relationship between MAP and Crohn's disease in humans was revealed in the current work.

Conflict of interest

None.

Acknowledgement

This work was financed by the Science and Technology Development Foundation (STDF), Egypt.

References

[1] Grant I. Zoonotic potential of *Mycobacterium avium* sp. *paratuberculosis*: the current position. Appl Microbiol 2005;98:1282–93.
[2] Salem MA, Zeid AA, Hassan D, El-Sayed A, Zschoeck M. Studies on Johne's disease in Egyptian cattle. Vet Med 2005;52:134–7.
[3] Herthnek D. Detection and confirmation of *Mycobacterium avium* subsp. *paratuberculosis* in clinical samples (Thesis). Uppsala, Sweden: Swedish University of Agricultural Sciences; 2006.
[4] Sechi L, Scanu AM, Molicotti P, Cannas S, Mura M, Dettori G, Fadda G, et al. Detection and isolation of *Mycobacterium avium* subspecies *paratuberculosis* from intestinal mucosal biopsies of patients with and without Crohn's disease in Sardinia. Am J Gastroenterol 2005;100:1529–36.
[5] Suárez-Fernández G. Paratuberculosis enteritis and Crohn's disease. Ann R Acad Med (Madrid) 2007;124:221–34.
[6] Singh SV, Singh AV, Singh R, Sharma S, Shukla N, Misra S, et al. Sero-prevalence of bovine Johne's disease in buffaloes and cattle population of North India using indigenous ELISA kit based on native *Mycobacterium avium* subspecies *paratuberculosis* 'Bison type' genotype of goat origin. Ind J Comp Microbiol Immunol Infect Dis 2008;31:419–33.
[7] Freeman H, Noble M. Lack of evidence for *Mycobacterium avium* subsp. *paratuberculosis* in Crohn's disease. Inflamm Bowel Dis 2005;11:782–3.
[8] Romero C, Hamdi A, Naser S. Evaluation of surgical tissue from patients with Crohn's disease for the presence of *Mycobacterium avium* subspecies *paratuberculosis* DNA by in situ hybridization and nested polymerase chain reaction. Inflamm Bowel Dis 2005;11:116–25.
[9] Ghadiali AH, Strother M, Naser SA, Manning EJ, Sreevasten S. *Mycobacterium avium* subsp. *paratuberculosis* strains isolated from Crohn's disease patients and animal species exhibit similar polymorphic locus patterns. Clin Microbiol 2004;42:5345–8.
[10] Nakase H, Nishio A, Tamaki H, Matsuura M, Asada M, Chiba T, et al. Specific antibodies against recombinant protein of insertion element 900 of *Mycobacterium avium* subsp. *paratuberculosis* in Japanese patients with Crohn's disease. Inflamm Bowel Dis 2006;12:62–9.
[11] Chamberlin W, Naser S. Integrating theories of the aetiology of Crohn's disease. On the aetiology of Crohn's disease: questioning the hypotheses. Med Sci Monit 2006;12:27–33.
[12] Uzoigwe J, Khaitsa M, Gibbs P. Epidemiological evidence for *Mycobacterium avium* subspecies *paratuberculosis* as a cause of Crohn's disease. Epidemiol Infect 2007;135:1057–68.
[13] Thibault VC, Grayon M, Boschiroli ML, Hubbans C, Overduin P, Stevenson K, et al. New variable-number tandem-repeat markers for typing *Mycobacterium avium* subsp. *paratuberculosis* and *M. avium* strains: comparison with IS900 and IS1245 restriction fragment length polymorphism typing. Clin Microbiol 2007;45:2404–10.
[14] Abubakar I, Myhill D, Aliyu SH, Hunter PR. Detection of *Mycobacterium avium* subsp. *paratuberculosis* from patients with Crohn's disease using nucleic acid-based techniques: a systematic review and meta-analysis. Inflamm Bowel Dis 2008;4:401–10.
[15] Bull TJ, McMinn EJ, Sidi-Boumedine K, Skull A, Durkin D, Neild P, et al. Detection and verification of *Mycobacterium avium* subsp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. Clin Microbiol 2003;41:2915–23.
[16] Fawzy AM, Epidemiological studies on paratuberculosis (Johne's disease) in livestock (Master's Thesis). Giza, Egypt: Cairo University; 2012.
[17] Salem MA, Studies on Johne's disease in cattle (Master's Thesis). Giza, Egypt: Cairo University; 2004.
[18] Hendrick S, Duffield T, Leslie K, Lissemore K, Archambault M, Kelton D. The prevalence of milk and serum antibodies to *Mycobacterium avium* subsp. *paratuberculosis* in dairy herds in Ontario. Can Vet J 2005;46:1126–9.
[19] Dreier S, Khol Jl, Stein B, Fuchs K, Guetler S, Baumgartner W. Serological, bacteriological and molecular biological survey of paratuberculosis (Johne's disease) in Austrian cattle. Vet Med 2006;53:477–81.
[20] Park KT, Ahn J, Davis WC, Koo HC, Kwon NH, Jung WK. Analysis of the seroprevalence of bovine paratuberculosis and

the application of modified absorbed ELISA to field sample testing in Korea. Vet Sci 2006;7:349–54.
[21] Anna Rita A, victor NN, Silvia P, Luciana P, Anastasia D, Vincenzo C. Ovine paratuberculosis: a seroprevalence study in dairy flocks reared in the Marche region, Italy. Vet Med Int 2011;7:82–7.
[22] Lee KW, Jung BY, Moon OK, Yang DK, Lee SH, Kim JY, et al. Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Korean black goats (*Capra hircus aegagrus*). J Vet Med Sci 2006;68:1379–81.
[23] Mercier P, Baudry C, Beaudeau F, Seegers H, Malher X. Estimated prevalence of *Mycobacterium avium* subspecies *paratuberculosis* infection in herds of dairy goats in France. J Vet Rec 2010;167:412–5.
[24] Khan FA, Chaudhry ZI, Ali MI, Khan S, Mumtaz N, Ahmad I. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in tissue samples of cattle and buffaloes. Trop Anim Health Prod 2010;42:633–8.
[25] Sivakumar P, Tripathi BN, Singh N. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in intestinal and lymph node tissues of water buffaloes (*Bubalus bubalis*) by PCR and bacterial culture. Vet Microbiol 2005;108:263–70.
[26] Salem MA. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds and comparative molecular characterization (Dissertation). Giessen, Hessen: Germany Justus-Liebig-University; 2009.
[27] Mendoza JL, Abreu MT. Biological markers in inflammatory bowel disease: practical consideration for clinicians. Gastroenterol Clin Biol 2009;33:158–73.
[28] Reumaux D, Sendid B, Poulain D, Duthilleul P, Dewit O, Colombel JF. Serological markers in inflammatory bowel diseases. Best Pract Res Clin Gastroenterol 2003;17:19–35.
[29] Bernstein CN, Blanchard JF, Rawsthorne P, Collins MT. Population-based case control study of seroprevalence of *Mycobacterium paratuberculosis* in patients with Crohn's disease and ulcerative colitis. Clin Microbiol 2004;42:1129–35.
[30] Norton S, Johnson WO, Jones G, Heuer C. Evaluation of diagnostic tests for Johne's disease (*Mycobacterium avium* subsp. paratuberculosis) in New Zealand dairy cows. Vet Diagn Invest 2010;22:341–51.
[31] Naser S, Ghobrial G, Romero C, Valentine JF. Culture of *Mycobacterium avium* subsp. *paratuberculosis* from the blood of patients with Crohn's disease. Lancet 2004;364:1039–44.