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SEROLOGICAL INVESTIGATIONS FOR DETECTION OF CHLAMYDIA INFECTION IN ABORTED CATTLE IN ASSIUT GOVERNORATE

(With 3 Tables)

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فحوصات سيرولوجية للتعرف على مدى انتشار الإصابة بميكروب الكلاميديا في الأبقار في محافظة أسيوط

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قامت الدراسة بعمل مسح سيرولوجى للكشف عن الإصابة بميكروب الكلاميديا في الأبقار في محافظة أسيوط وذلك لدراسة نسبة الإصابة بهذا الميكروب بين الأبقار. أجريت الدراسة على خمس وثمانون بقرة من مزارع مختلفة (ستون بقرة كانت تظهر عليها علامات الإصابة من الإجهاض في مراحل متأخرة، في أربعة مزارع كمجموعة مختبرة وخمس وعشرون بقرة سليمة ظاهريا، في ثلاثة مزارع كمجموعة ضابطة). قسمت المجموعة المختبرة إلى ثلاثة مجموعات، المجموعة الفرعية الأولى من عمر ثلاثة إلى خمس سنوات (20 بقرة)، المجموعة الفرعية الثانية من عمر خمس حتى ثمانية سنوات (25 بقرة) والمجموعة الفرعية الثالثة عمرها أكثر من ثمانية سنوات. وباستخدام اختبار الأليزا تم فحص عينات السيرم للكشف عن الأجسام المضادة لميكروب الكلاميديا. بينت الدراسة أن عدد 21 بقرة من الحيوانات الكلية (85 بقرة) كانت تحتوي علي الأجسام المضادة لميكروب الكلاميديا بنسبة 24.7% (33.3% من مجموعة الأبقار المختبر ة و4% من مجموعة الأبقار الضابطة). وأظهرت الفحوصات السيرولوجية أن نسبة الإصابة كانت مرتفعة في المجموعة الفرعية الأولي المريضة (3-5 سنوات)، حيث كانت 35% من الأبقار في تلك المجموعة تحتوي علي الأجسام المضادة لميكروب الكلاميديا. أيضا كانت 32% و33.3% من الأبقار المريضة المختبرة في المجموعتين الفرعيتين الثانية والثالثة إيجابية للفحص السيرولوج ي، علي التوالي. ويمكن الخلاصة أن اختبار الأليزا المستخدم يتميز بسهولة إجراؤه ومتخصص للكشف عن الأجسام المضادة لميكروب الكلاميديا في عينات السيرم للأبقار، ترى الدراسة أن هذا الاختبار ملائم للمسح الوبائي الشامل.

SUMMERY

Serological study was carried out on the chlamydial related infections in cattle at Assiut Governorate. Eighty five cows in different localities were examined (sixty cows with reproductive disorders in four farms as tested group and twenty five clinically healthy cows in three different farms as a control group). The diseased tested cows were divided into three subgroups; the first tested subgroup (20 cows) with age 3-5 years; the second tested subgroup (25 cows) with age 5-8 years and the third tested subgroup (15 cows) with age more than eight years. Blood samples were taken from each animal with different ages (three years to more than eight years). Samples were examined for both IgG and IgM *Chlamydia* antibodies with an indirect ELISA. Twenty-one cows of the total examined cows (85) were serologically positive for both IgG and IgM *Chlamydia* antibodies with a percentage 24.7% (33.3% of the diseased tested cows and 4% of the clinically healthy cows). The serological investigation referred that the infection was higher in diseased tested cows at age 3-5 years; where 35% of these tested cows were serologically positive for both IgG and IgM *Chlamydia* antibodies. Also seropositivity was 32% and 33.3% of the diseased tested cows in the second (5-8 years) and the third (>8 years) tested subgroups, respectively. It can be concluded that the enzyme immunoassay (ELISA) was easily to perform and can used as rapid screening test for detection of recent, latent chlamydial infections and apparently healthy cows.

Key words: *Chlamydia psittaci, ELISA, cattle, abortion.*

INTRODUCTION

The Chlamydiaceae are ubiquitous through out the world and infect both humans and animals. The obligate intracellular bacteria exhibit a unique life cycle with two morphologically different infectious and reproductive forms: The elementary body (EB) and reticular body (RB) forms (Rekiki, *et al*., 2002).

Phylogenetic analysis based on 16 S-23 S ribosomal RNA sequences has led to the classification of the Chlamydiaceae family into two genera: *Chlamydia* and *Chlamydophila* (Everett, *et al*., 1999).

Chlamydiphila abortus and other *Chlamydia* are the most important causative agent of the enzootic abortion infections in cattle,

sheep and goat. It may also impair the over all reproductive performance. The organism has a zoonotic potential, and therefore must be dealt with great care with adequate microbiological precautions and laboratory containment (Travnicek, *et al.*, 2003). In cattle infections with *Chlamydophila abortus* can cause illnesses like pneumonia, genital infections, abortion, enteritis, keratoconjunctivitis, polyarthritis, encephalomyelitis and mastitis (Abd El-Rahim, 2002 and Twomey, *et al.*, 2006).

Examination of field samples indicated regular clinical inapparent chlamydial infections in cattle breeds. Clinically abortions occur in the last months of pregnancy or the production of weak and generally premature calves. Abortions due to chlamydial infections have major economic implications in ruminant breeding. Infected cattle often excreted Chlamydia with their feces which play an important role for further spreading of the infection for other animals (Wittenbrink, *et al*., 1993).

Diagnosis of *Chlamydia* infections depends on laboratory examinations for chlamydial antigen and / or antibodies detection. Serological methods could be used for diagnosis of latent and chronic chlamydial infections through detection of the specific persistent antibodies. All chlamydiae carry a common diagnostically relevant cell-wall lipopolysaccharide (LPS)–antigen, which can be detected by both monoclonal and polyclonal antibodies (Ward and Ridgway, 1998).

In case of direct EIA (Enzyme Immuno Assay) test, enzyme labeled antibodies that recognize all species of *Chlamydia* bind to LPS extracted from elementary bodies in the specimen. In indirect EIA test it detects reactivity to genus specific antigen, or LPS, of chlamydial elementary or reticulate bodies (Moss, *et al*., 1993). The indirect ELISA was efficient useful screening test for Chlamydial abortion on the flock level, performed well, being more easier, sensitive and specific. It is of great help for the epidemiological control of the disease (Longbottom, *et al.*, 2001; Buendia, *et al.*, 2001 and McCauley, *et al.*, 2007).

The present study aimed to investigate the incidence of *Chlamydia* related abortions in cattle during breeding seasons also to compare seroprevalence to *Chlamydia psittaci* in aborted cow populations and in a randomly selected control group.

MATERIALS and METHODS

Materials:

Samples:

Blood samples were collected from eighty-five cows from different farms in Assiut Governorate (sixty cows with a history of abortion within the previous seasons in four farms as a tested group and twenty-five apparently healthy cows without a history of abortion as a control group).

The tested group cows(60) was subdivided into three subgroups according to their age; the first tested subgroup (20 cows) were from 3-5 years; the second tested subgroup (25 cows) were from 5-8 years and the third tested subgroup (15 cows) were over eight years.

Sample collection: (according to Black, *et al.*, 1997).

1. The animal was examined clinically; symptoms and data including the number of animal, animal's owner, or name of the farm, age of the cattle, and its general conditions were recorded.
2. 5 ml blood sample was collected from coccygyal vein from each examined animal.
3. Blood samples were centrifuged for ten minutes at 4000 rpm and separated sera were stored at -20 ºC in epindorf tube.
4. Indirect enzyme linked immunosorbent assay (ELISA) ChlamyEIA Vierotech ELISA kit for detection of antichlamydia serum IgG and IgM antibodies against *Chlamydia psittaci* commercially supplied from Human Company –Germany.

Preparation of reagents used in ELISA:-

1. Dilution of RF sorbent, 5ul from tested serum was added to 50 ul RF reagent and 445 ul diluting buffer to give a final dilution of 1:100 in a sterile epindorf tube and mixed well by vortex.
2. Dilution of the antibovine conjugate in a ratio 1:100 was done using a diluting buffer which in the ELISA kit. 4950 ul diluting buffer was added to 50 ul antibovine conjugate.

Methods:

Detection of IgM, IgG antichlamydia antibodies by ELISA:

- Principles of EIA virotech ELISA:-

The ELISA was intended for semiquantitave detection of IgG, IgM in serum. The antibodies form an immune complex with the antigen coated on the test strips. Unbounded immunoglobulins were removed by

washing processes. After adding the substrate solution (TMB), a blue dye was produced by the bound enzyme (peroxidase), the colour changed to yellow when the stopping solution was added.

Preparation of reagents:-

1- All reagents were brought to room temperature before opening package of microtiter strips.
2- All liquid components were shaked well before use.
3- Washing solution concentrate had to be filled up to one liter with double distilled water.

High IgG titer disturbs the specific detection of IgM antibodies and may cause false negative or false positive results. For proper IgM determination it was therefore necessary to treat sera with RF – sorbent.

Procedure:

1- For each test run, 100 ul each of ready to use dilution buffer were pipetted (blank), IgG and IgM positive, negative and cut off controls.
2- The working dilution of the serum samples was 1:100.
3- The test plate after pipetting were incubated for 30 min at 37ºC (with cover).
4- The microtiter strips were washed four times with 300 ul washing solution per well. All residues were removed on a cellulose pad.
5- 100 ul of diluted antibovine IgG and IgM conjugate were dispensed to each well and were incubated for 30 min at 37 ºC with cover .
6- Conjugate incubation was stopped by washing several times.
7- 100 ul of ready to use TMB were pipetted into each well.
8- In dark place the substrate solution was added then incubated for 30 min at 37 ºC with cover.
9- The substrate reaction was stopped with 50 ul of citrate stopping solution in each well, the plate was shacked carefully and thoroughly until mixed completely and a homogeneous yellow color was visible.
10- Optical density (OD) was measured at 450/620 nm. The photometer was set such a way that the blank value was deducted from all other extinctions.

Statistical data analysis was done using Chi-square by SPSS, 2005 program (Statistical Package for Social Sciences for Windows Release 14.0.0.).

RESULTS

Table 1: Serological results of ELISA for antichlamydia IgG and IgM of different age groups.

Group	No. of examined cows	Serological results			
		positive	%	negative	%
Tested group aborted cows**	Subgroup (1) 20 cows from 3-5 years*	7	35	13	65
	Subgroup (2) 25 cows from 5-8 years*	8	32	17	68
	Subgroup (3) 15 cows more than 8 years *	5	33.3	10	66.6
	60 cows	20	33.3	40	66.7
Clinically healthy cows**	8 cows (3-5 years)	0	0	8	100
	10 cows (5- 8 years)	1	10	9	90
	7 cows (>8 years)	0	0	7	100
	25 cows	1	4	24	96
Total	85 cows	21	24.7	64	75.3

* No statistical difference $\chi^2 = 0.045$ p= 0.978
** Highly significant statistical variations $\chi^2=8.163$ p> 0.004

Table 2: Results of ELISA for detection of antichlamydial IgG in sera of tested cows of different age group:

Age group	Number of cows	ELISA Results			
		positive	%	negative	%
3-5 years	20	3	15	17	85
5-8 yeas	25	5	20	20	80
>8 years	15	4	26.7	11	73.3
Total	60	12	20	48	80

Table 3: Results of ELISA for detection of antichlamydial IgM antibodies in sera of tested cows of different age group:

Age group	Number of cows	ELISA Results			
		positive	%	negative	%
3-5 years	20	4	20	16	80
5-8 yeas	25	3	12	22	88
>8 years	15	1	6.7	14	93.3
Total	60	8	13.3	52	86.7

DISCUSSION

Chlamydophila abortus and other chlamydial infections is the most important causative agent of abortions of cattle. It responsible for a multiplicity of syndromes such as polyarthritis, keratoconjunctivitis, mastitis. The frequency of the latent infection and the difficulties of diagnosis, prophylaxis as well as treatment of *Chlamydia* infections cause a number of field problems to veterinarians, Abd El–Rahim (2002). Serological methods for examination of cattle herds are necessary, so ELISA was often preferred because it was easy to perform, its higher sensitivity and specificity for detection of Chlamydia antibodies in bovine sera, (Sting and Hafez, 1992).

Table (1) showing that 21 cows (24.7%) out of totally 85 cows were serologically positive for antichlamydial antibodies IgG or IgM. This result is nearly similar to that obtained by Schmatz, *et al.* (1978) in Egypt (22%) and Abd El-Rahim (2002) in North East Germany (19.6%). The high rate of *Chlamydia* infection (68%) was recorded in Germany by Sting and Hafez (1992). The present study showing that the tested cows at age 3-5 years had high titer of both antichlamydial IgG and IgM, as 35% of these tested cows were serologically positive. This result was nearly similar to findings of Abd El–Rahim, (2002) who carried out a serological survey on the *Chlamydia* infection in cattle dairy herds in Germany and he concluded that the infection was more frequent among young cows (3-6 years). Also Sting and Hafez (1992) concluded that chlamydial infections were more frequent among young cows.

The serological investigation in the present work, Table (1), referred that 32% and 33.3% of the tested cows subgroup two (5-8 years) and subgroup (3) (>8 years) were positive, respectively, this may

due to LPS ELISA are superior for serodiagnosis of ruminant infections with abortigenic *Chlamydia* and this result was in agreement with those of Kaltenboeck, *et al.* (1997), Baud, *et al.* (2008) and Pantchev, *et al.* (2009), they concluded that mixed chlamydial infections were not rare and suggested an extended host range of individual species and probably emerging obstetrical pathogens.

The results of ELISA in the serum samples of apparently healthy control cows, as showing in Table (1), showing that one cow out of (25) cases with percentage of (4%) was serologically positive. Cavirani, *et al.* (2001) compared between seroprevalnce to *Chlamydia psittaci* in aborted cow populations (45%) and in the control group (24%).

Table (2) shows the results of ELISA for antichlamydia IgG in the sera of tested cattle of different age groups, the infection was higher among cattle at age more than eight years (4)cows (26.7%) out of (15) cows were serologically positive, this due to old age cows at which latent and chronic chlamydial infection were high, this result emphasized the results of Wang, *et al.* (2001) who found that 71% of aborted cows had IgG against *Chlamydia abortus* in sera of tested cattle. Travnicek, *et al.* (2003) concluded that there is a significant level of IgG antibodies against *Chlamydia abortus* was detected up to eight weeks after abortion or parturition and a rise in antibodies titer provides a basis for retrospective diagnosis. Detection of specific IgG antibodies in aborted bovine sera against *Chlamydia psittaci* using indirect ELISA revealed 39.1% positive seroreactors, Ghazy, *et al.* (2005).

Table (3) shows that antichlamydia IgM was higher in tested cows at age three to five years with percentage of 20% this due to the recent chlamydial infections, this result was in agreement with findings of Wehrend, *et al.* (2000) and Wang, *et al.* (2001), they investigated the prevalence of *Chlamydia psittaci* infections in cattle farms with fertility disorders and determined *Chlamydia* specific IgM antibodies in sera of tested cattle. Also they found that with increased incidence of abortions showed detectable antichlamydia antibodies and in follow up examinations seroconversion show increase in IgG titer in these farms the results support the hypothesis that there was a high prevalence of latent *Chlamydia* infection in cattle.

It can be concluded that the enzyme immunoassay (ELISA) was easily to perform and can used as rapid screening test for detection of recent, latent chlamydial infections and apparently healthy cows.

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