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SEROLOGICAL INVESTIGATION ON SPREADING OF BRUCELLOSIS, CHLAMYDIOSIS AND LEPTOSPIROSIS IN FARM ANIMALS

BY

A.S. AMIN ; E.R.H. ATTIA; T.M. AHMED and M.N. SAKRAN

Animal Reproduction research Institute, Giza, Egypt.

SUMMARY

Three thousands and two hundred seventy one blood sera (2663 from cattle, 558 from buffaloes, and 50 from horses) were tested for antibodies against *Brucella abortus, Chlamydia psittaci* and Leptospira serovars. Antibodies against *Brucella abortus* were detected by Rose Bengal Plate and standard serum agglutination tests in sera of cattle, buffaloes and horses in 120 (4.51%), 30 (5.38%) and 4 (8%), respectively. Antibodies against *Chlamydia psittaci* were detected by micro-immunofluorescence test in 414 (15.55%), 185 (33.15%) and 8 (16%) serum samples of cattle, buffaloes and horses, respectively. While, antibodies against *Leptospira hardjo, Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae* were detected by microscopic agglutination test in 187 (7.1%), 67 (12%) and 6 (12%) serum samples of cattle, buffaloes and horses, respectively.

INTRODUCTION

Brucellosis, chamydiosis and leptospirosis are common zoonotic infections capable of affecting most mammals and man. Brucellosis is considered to be the most important reproductive disease of livestock in Egypt, causing abortion and infertility in animals and malta fever in human beings (Christie, 1980; El-Gibaly et al., 1993 and Abdel-Hafez et al., 1995). Standard tube serum agglutination test (SAT) is commonly used for serological investigation of brucellosis because it allows titration of the antibody levels but the Rose Bengal Pengal Plate test (RBPT) is a rapid screening test and can be used in the field diagnosis (Refai, 1989).

Chlamydia psittaci is widely distributed obligate intracellular pathogens which exhibit a broad pathogenic potenitial (Fukushi and Hirai, 1992). Depending on factors, such as virulence of the strain, physiological state of the host and environmental factors, chlamydial infection may

lead to abortion, still birth, pneumonia, polyarthritis, gastroenteritis, conjunctivitis, encephalomyelitis, infetility or seminal vesiculitis (Storz, 1971). The organism can cause placentitis and abortion in women (Buxton, 1986) and can also cause pneumonia i.e. ornithosis or psittacosis in human (Macfarlane and Macrae, 1983). Laboratory diagnosis of chlamydial infections among farm animals is based on the serological procedures because the isolation techniques remains more difficult and the staining methods are not totally reliable because intracellular chlamydia are almost indistinguishable from mycoplasmas and rickettsias (Page, 1978).

Leptospirosis can cause economic losses due to abortion, still birth, infertility, reduced milk production, decrease growth rate and death in affected livestock. In the horse, there is an association between recurrent uveitis and Leptospirosis (Hanson et al., 1969; Williams et al., 1971; Verma et al., 1977; Matthews et al., 1987; Stillerud et al., 1987; Verma et al., 1977; Matthews et al., 1987; Stillerud et al., 1987 and Donahue et al., 1992).

The present study was designed to spot light on the seropositivity of brucellosis, chalamydiosis and leptospirosis in cattle buffaloes and horses.

MATERIAL AND METHODS

Samples:

Blood was collected from 2663 cattle, 558 buffaloes and 50 horses from different governorates in Egypt. Collected blood was allowed to clot and the serum were decanted and stored at -20°C till tested.

Brucella antigen:

Standard *Brucella abortus* antigen was obtained from Serum and Vaccine research Institute (Abbasia), Cairo, Egypt.

Chlamydia psittaci antigeen:

Chlamydial antigen was kindly supplied by Prof. Dr. Andersen, National Animal Diseases Center, Ames, Iowa, USA.

Leptospiral serovars:

Leptospiral reference serovars namely: hardjo, grippotyphosa, pomona, canicola and icterohaemorrhagiae were kindly obtained from C. Sulzer, CDC, Atlanta, USA.

Serological diagnosis of Brucellosis:

Serological evidence of brucellosis was determined by using RBPT and standard SAT (Alton et al., 1988). A titer of 1/40 or more was considered to be positive for SAT.

Serological diagnosis of Chlamydiosis:

A micro-immunflourescence (MIF) test of *Chlamydia psittaci* and *Chlamydia pecorum* was carried out according to method of Andersen (1991). A titer of 1/16 or more considered to be positive.

Serological diagnosis of Leptospirosis:

Microscopic agglutination test (MAT) was used to screen sera for the presence of antibodies against leptospira (Alexander et al., 1970). A serial double fold serum dilutions was done using

phosphate buffer saline (PBS) begining with dilution 1/100. Titers of 1/200 or more were considered positive.

RESULTS

Three thousands and two hundred seventy one serum sampels were collected from 2663 cattle, 558 buffaloes and 50 horses from different governorates in Egypt. The presence of antibodies against *Brucella abortus, Chlamydia psittaci* and leptospira species were detected by different serological tests.

The results of serological prevalence of brucellosis, chlamydiosis and leptospirosis in different farm animals are shown in tables 1,2 &3 respectively.

Correlation between symptoms exhibited and its seropositively to brucellosis, chlamydiosis and leptospirosis are shown in table 4.

Table (1): Prevalence rate of Brucella abortus antibodies in sera of different examined animals

Animal Species	Number of serum samples	Number of Positive sera	Percentage
Cattle	2663	120	4.51
Buffaloes	558	30	5.38
Horses	50	4	8.00

Positive = At titer 1/40 or more

Table (2): Prevalence rate of Chlamydial antibodies in sera of different examined animals

Animal Species	Number of serum samples	Number of Positive sera	Percentage
Cattle	2663	414	15.55
Buffaloes	558	185	33.15
Horses	50	8	16.00

Positive = At titer 1/16 or more

Table (3): Prevalence rate of Leptospiral antibodies in sera of examined animals

Animal Species	Number of serum samples	L. hardjo		L. grippotyphosa		L. icterohaemorrhagiae		Total Positive No.	%
		Positive No.	%	Positive No.	%	Positive No.	%		
Cattle	2663	100	3.8	87	3.3	0	--	187	7.1
Buffales	558	25	4.5	30	5.4	12	2.1	67	12
Horses	50	2	4	3	6	1	2	6	12

Positive = At titer 1/200 and more

Table (4): Correlation between exhibited clinical signs and seropositivity to brucellosis, chlamydiosis and leptospirosis

Symptoms	Brucellosis						Chlamydiosis						Leptospirosis					
	Cattle		Buffaloes		Horses		Cattle		Buffaloes		Horses		Cattle		Buffaloes		Horses	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Abortion	19	0.71	7	1.30	0	--	2	0.08	0	--	0	--	0	--	0	--	0	--
Still Birth	3	0.11	1	0.18	0	--	18	0.68	2	0.36	0	--	15	0.56	20	3.58	0	0.56
Retained placenta	24	0.90	11	1.97	0	--	0	--	0	--	0	--	0	-	0	-	0	-
Repeat breeder	8	0.3	3	0.50	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Pneumonia	0	--	0	--	0	--	41	1.54	14	2.51	1	2	0	--	0	--	0	--
Enteritis	0	--	0	--	0	--	32	1.20	13	2.33	1	2	0	--	0	--	0	--
Arthritis	1	0.04	0	--	0	--	37	1.39	21	3.76	1	2	0	--	0	--	0	--
Orchitis	3	0.11	0	--	0	--	2	0.08	0	--	0	--	0	--	0	--	0	--
Fistulous withers	0	--	0	--	4	8-	0	--	0	--	0	--	0	--	0	--	0	--
Laminitis	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	5	10.0
No clinical signs	62	2.33	8	1.43	0	--	282	10.6	135	24.2	5	10	172	6.46	47	8.42	1	2.00
Total	120	4.50	30	5.38	4	8	414	15.6	185	33.2	8	16	187	7.1	67	12.0	6	12.0

Total serum samples = 2663 from cattle, 558 from buffaloes and 50 from horses.

DISCUSSION

One of the major constraints to livestock production is infertility and abortions. It has decreased production in dairy industry and is of major economic and public health importance. The etiology of these conditions are microbial infections, nutritional disorders and bad management practices. Microbial infections play a vital role in precipitating the disease conditions of genitalia and abortions. The present study was designed to spot light on the sero-prevalence of brucellosis, chlamydiosis and leptospirosis in cattle, buffaloes and horses.

The control program for brucellosis in Egypt depends mainly on serological testing. The use of serological tests for the diagnosis of brucellosis represent a practical tool to identify infected herds (Refai, 1989). The results in this investigation showed that the prevalence rate of Brucella agglutinins among cattle and buffaloes was nearly similar (4.51% and 5.38%, respectively), while, it was 8% in horses (Table 1). The obtaining results are in harmony with that obtained by Barsoum (1980) and significantly less than the incidence obtained by Ghoneim et al. (1985); Shalaby (1986) and Selim (187). The strict husbandry mesures, improvement in management and use of S 19 vaccine, seem to play an important role in the reducion of brucellosis in many herds.

By analysis of the results, according to the symptoms exhibited among examined animals (Table 4), it was found that about 0.71%, 0.11%, 0.9%, 0.3, 0.04 and 0.11% of cattle (serologically positive for Brucellosis), showed abortion, still birth, retained placenta, repeated breeder, arthritis and orchitis, respectively. In serologically positive buffaloes, 1.30%, 0.18%, 3.67% and 0.50% showed abortion, still births, retained placenta and repeat breeder, respectively. While in horses had antibrucella antibodies 8% showed fistulous withers. These results may support what was previously reported by Shalaby (1986); Bassiony et al. (1995) and Osman et al. (1997).

Serological studies have added little information if compared with the isolation results of *Chlamydia psittaci.* The group specific complement fixation test has been of little value because of inadequate sensitivity and realibility (Schachter et al., 1974). However the MIF test (Andersen, 1991) offers advantages more than the other serological tests because it is highly sensitive, rapid and simple to perform in addition it requires neither the highly trained personnel nor the specialized equipment demanded by other techniques (Amin, 1993).

Antichlamydial antibody was detected by MIF test in 414 out of 2663 (15.55%) serum samples collected from cattle at a titer of 1/16 or above against the chlamydial antigens (Table 2). Several authors demonstrated an incidence range from 11.5 up to 30% (Kaadan and Lieberman, 1966; Neuvonen and estola, 1974 and Schmatz et al., 1978). While, ata (1982) estimated incidence rate up to 39% in sera collected from herds with clinical signs of abortion.

In buffalo-sera, the antichlamydial antibodies was detected by MIF test in 185 of 558 (33.15%) at a titer of 1/16 or above (Table 2). Schmatz et al.

(1978) have shown an incidence of 42.5% on random samples collected from buffaloes in Egypt while, Ata (1982) reported an incidence reached 36%.

Antichlamydial antibodies was detected by MIF test in 8 out of 50 (16%) serum samples collected from horses (Table 2). Previous serological studies revealed an incidence from 4% up to 23% (Friis, 1967; Schmatz et al., 1978 and Ata, 1982).

By analysis of the results, according to the symptoms exhibited by animals (Table 4), it was found that about 0.08%, 0.68%, 1.54%, 1.20% 1.39% and 0.08% of cattle had chlamydial antibodies showed abortion, still birth, pneumonia, enteritis, arthritis and orchitis, respectively. In buffaloes had chalmydial antibodies 0.08%, 2.5%, 2.3%, 3.8% showed still birth, pneumonia, enteritis and arthritis, respectively. While, in horses had chlamydial antibodies 2% and 2% showed pneumonia, enteritis and arthritis, respectively. Nealry similar were previously recorded by Eugster et al. (1970); Storz (1971); Hafez and Krauss (1979); Jones (1981); Ata (1982); Sharma and Baxi (1983); Charan et al. (1986); Purohit et al. (1986); Reggiardo et al. (989); Nabeya et al. (1991); Fukushi and Hirai (1992) and Amin (1993).

The answer to the question of leptospiral serological reactions in confirmed non-infected cattle populations seems to be even more difficult, and relvenant information has not been available in literature. From the absence of clinical signs such as abortions, still births or sudden drop of milk yield together with negative or only low level antibody reactions it can only be concluded that Leptospirosis does not exist in a given herd.

Therefore, it may be justified to apply two different systems to the classification of serological antileptospirosis reactions. First, if a herd is suspected of being infected with leptospirosis, repeated serum samples should be taken and even low antibody reactions are to be recorded in addition to attempts to isolate the causative organism (Ellinghausen et al., 1977). Second, for cross-sectional studies more importance should be attributed to specificity rather than sensitivity (Pritchard, 1984) by accepting only higher titer levels as a positive result. According to Ellis et al. (1982) a positive titer of 1/10 in confirmed *L. hardjo* infections of cattle produced a sensitivity of 0.67 and specificity of 0.86. When the classification of serological reactions was based on titer levels >1/100 the sensitivity dropped to 0.41 but the specificity was 1. From this, it is concluded that serological results referring to leptospirosis should be interpreted on a herd basis and not for individual animals.

Concerning the results of serological examination of cattle revealed the prevalence of significant seropositivity with (7.1%) to both *L. hardjo* and *L.grippotyphosa* (Table 3). The obtained results are in harmony with that obtained by Attia (1993) who detected agglutinins to leptospira in cows with an incidence of 6.5% and higher than obtained by Hatem (1979) who detected 5.3% leptospiral agglutininis in cows and Tawfik (1977) who detected leptospiral agglutinins in 3.5% of cattle sera.

Concerning the serological examination of buffaloes revealed leptospiral agglutinins against *L. hardjo, L. hardjo, L. grippotyphosa* and *L. icterohaemorrhagia* (12%). The obtaining results agree with that obtained by Attia (1993) who detected 13.33% leptospiral agglutinins in buffaloes and with that obtained by Tawfik (1977) who detected leptospiral agglutinins in 33.4% at titer of 1/100 in buffalo-sera. Also, Hatem (1979) detected 10.86% leptospiral agglutinins in buffalo-sera.

Concerning the results of serological examination of horses showed the prevalence of significant seropositivity to *L. hardjo, L. grippotyphosa* and *L. icterohaemorrhagiae* (12%) total percentage. The obtained results agree with that obtained by Deborah et al. (1994), Swart et al. (1982) and Verma et al. (1977).

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