

**SEROPREVALENCE OF *MYCOPLASMA MYCOIDES* CLUSTER IN SMALL RUMINANT USING MONOCLONAL ANTIBODY- BASED C ELISA IN DAKAHILIA PROVINCE**

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**ABSTRACT**

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In this study, 1103 sheep and goat at five different localities in Dakahilia Governorate, Egypt, during a period from November 2013 until March 2014 were examined clinically for respiratory manifestation. a total of 212 sheep and goat with different ages showing respiratory manifestations. Serum samples from diseased animals were examined using monoclonal antibody-based C ELISA Kit for Diagnosis of CBPP to study the prevalence of *Mycoplasma mycoides* cluster. Our result revealed that, prevalence of *Mycoplasma spp* was (52.35%) in both sheep and goat while there are higher significance between species  $P < 0.05$  and odds ratio = 0.3381 it was higher in goat (67.81%) than in sheep (41.6%) moreover, they are higher significance between two age groups  $P < 0.05$  and odds ratio equal 2.2722 it was higher in young animals  $< 2$  year than in adult  $> 2$  year old age (60.29%) and (38.15%) respectively.

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**Keywords:** *Mycoplasma*, C ELISA, small ruminant.

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**INTRODUCTION**

Members of the genus *Mycoplasma* belong to the most important bacterial livestock pathogens worldwide. Of particular importance are *Mycoplasma mycoides* subsp. *mycoides* (Mmm) and *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), two members of the 'Mycoplasma mycoides cluster' (Cottew *et al.*, 1987), which are responsible for contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP), respectively. Both diseases cause significant losses in livestock, in particular in Africa and Asia, and are a threat to disease-free countries. The *Mycoplasma mycoides* cluster is made up of six species, subspecies or group of strains that are pathogenic for ruminants. It includes two biotypes of *M. mycoides* subsp. *mycoides*, the small-colony (MmmSC) and large-colony (MmmLC) biotypes, *M. mycoides* subsp. *capri* (Mmc), *M. capricolum* subsp. *capricolum* (Mcc), *M. capricolum* subsp. *capripneumoniae*, formerly *M. sp* type F38 (Mccp) (Bonnet *et al.*, 1993; Leach *et al.*, 1993) and the bovine group 7 of Leach (BG7). They all share numerous genotypic or phenotypic traits. The 16S rRNA genes of *Mmc* and *MmmLC* are 99.9% similar, suggesting they should be considered as two phenotypes of the same species, distinct from *MmmSC* (Monnerat *et al.*, 1999 and Pettersson *et al.*, 1996); (iv) Animal host specificity, previously thought as very specific and used as a clue to identification, has

proven to be unreliable (Nicolet, 1996). Contagious caprine pleuropneumonia (CCPP) is a highly contagious, infectious fibrinous pleuropneumonia of goats caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), characterized by fever, respiratory distress with coughing, nasal discharge, high morbidity and mortality rates (Radostitis *et al.*, 2006). Its economic importance is due to direct loss which result from its high mortality, reduced milk yield, cost of treatment and vaccination of the disease and indirect loss due to the imposition of trade restrictions. It has been reported from more than 35 countries most of which are in Africa. However, the exact distribution of the disease is not yet known mainly due to the lack of sensitivity and specificity of the diagnostic tests and difficulty of identification of the organism causing the disease (Nicholas, 2002).

Competition enzyme-linked immunosorbent assay (c-ELISA) is a newly developed test, which permits the specific detection of antibodies in animals, which have been affected by CCPP (Thiaucourt *et al.*, 1994). This test is based on the use of a monoclonal antibody (MAb), which is competing with goat antibodies to bind to the antigen that is coated on the plates. The specificity of the test depends on the epitope that recognized by the MAb. The introduction of the c-ELISA for CCPP will permit the implementation of serological enquiries on a large scale for the first time. This test combines the well-known advantages of the

ELISA format with the specificity provided by the use of a MAbs. Thiaucourt *et al.* (1996)

However, information on seroprevalence and associated risk factors were scanty. Therefore, the objectives of this study were to determine the seroprevalence of CCPP in small ruminant in five localities in Dakahlia Governorates, Egypt, and to identify the risk factors responsible for the occurrence of the disease.

## MATERIALS and METHODS

### Animals (sheep and goat):

**Table 1:** Sheep and goat examined according to localities.

Flock No	Total (Sheep + Goat)	Sheep	Goat
El-Mansoura	165	140	25
Talkha	224	154	70
Sherbin	192	110	82
Belkas	282	180	102
Dikrins	240	180	60
<b>Total</b>	<b>1103</b>	<b>764</b>	<b>339</b>

### 1-Samples

Two hundred and twelve blood samples collected from each sheep and goat showing respiratory manifestation through the jugular vein of each animal using plain vacutainer tubes. The blood allowed clotting for 1-2 hrs. at room temperature, stored horizontally overnight at 4° C. Serum separated from the clot by centrifugation at 2000-3000 rpm for 10-15 minutes, the serum labeled and stored at -20° C, then transported on ice in icebox to Mycoplasma Department, Animal Health Research Institute.

### 2- Serological detection of mycoplasma antibodies (OIE 2014) and SANCO/AH/R25/2001:

ELISA kits {monoclonal antibody-based C ELISA, named Mab 117/5 (CIRAD / Institute POURQUIER CBPP serum competition ELISA - Version P05410/02 – Page 1/5). were used for detection of mycoplasma *mycoides subspecies mycoides* (large Colony *Mmm* LC) antibodies. This kit has been evaluated by the Joint Division FAO/AIEA within the framework of a Coordinated Research Project (CRP).

### The principle of the test is:

1) Serum samples to be tested are diluted and mixed with the specific monoclonal antibody (Mab 117/5) in a dilution plate or “pre-plate”. This mixture transferred into the *Mmm*SC coated micro plate. Any specific antibodies present in the test sera will bind to the *Mmm* SC antigen, competing with the Mab for the specific epitope.

During a period from November 2013 until March 2014 a total of 1103 sheep and goat (764 sheep and 339 goats), located in five different localities in Dakahlia province, were examined clinically for respiratory manifestation, each animal show one or more from the following signs (dyspnea, cough, nasal discharge ranged from serous to purulent, rapid breathing) were blood sampled and their data were recorded for seroprevalence of *mycoplasma spp.* in sheep and goat in Dakahlia province. The sheep and goat under study were raised mixed in the same area in morning and at night were kept in closed pen, the sheep and goat were illustrated in table (1).

2) After washing, an anti-mouse IgG serum conjugated to horseradish peroxidase (HRP), which will bind to any Mab fixed to the wells, is added. If specific *Mmm*SC antibodies are present in the bovine sera, they will displace the Mab and the conjugate will not be able to bind.

4) Following another series of washes, the HRP substrate (TMB) is added, forming a blue compound that will turn to yellow when the reaction is stopped. The intensity of the color is an inverse measure of the proportion of *Mmm*SC antibodies present in the test sera. The cut-off point is calculated using the results obtained from a monoclonal control (Cm, 0% inhibition) and a conjugate control (Cc, 100% inhibition). Positive and negative control sera delivered within the kit.

### Reading and Interpretation.

a) Read the OD at 450 nm (OD450). The photometer could first be blanked on air.

b) Calculate the mean value of the Cm (0% inhibition) and Cc (100% inhibition) controls.

c) Calculate the percentage of inhibition (PI) for each serum as follows:

$$PI = 100 \times [(OD\ Cm - OD\ Test) / (OD\ Cm - OD\ Cc)]$$

- Sera with a PI equal to or lower than 40% considered negative.
- Sera with PI between 40 and 50% considered

doubtful.

- Sera with PI equal to or greater than 50% considered positive.

**Statistical Analysis**

The odds ratio and P value were calculated according to the method described by Altman (1991).

**RESULTS**

**Clinical examination:**

Clinical examination of 1103 sheep and goat revealed that, total of 212 (19.2%), sheep and goat show

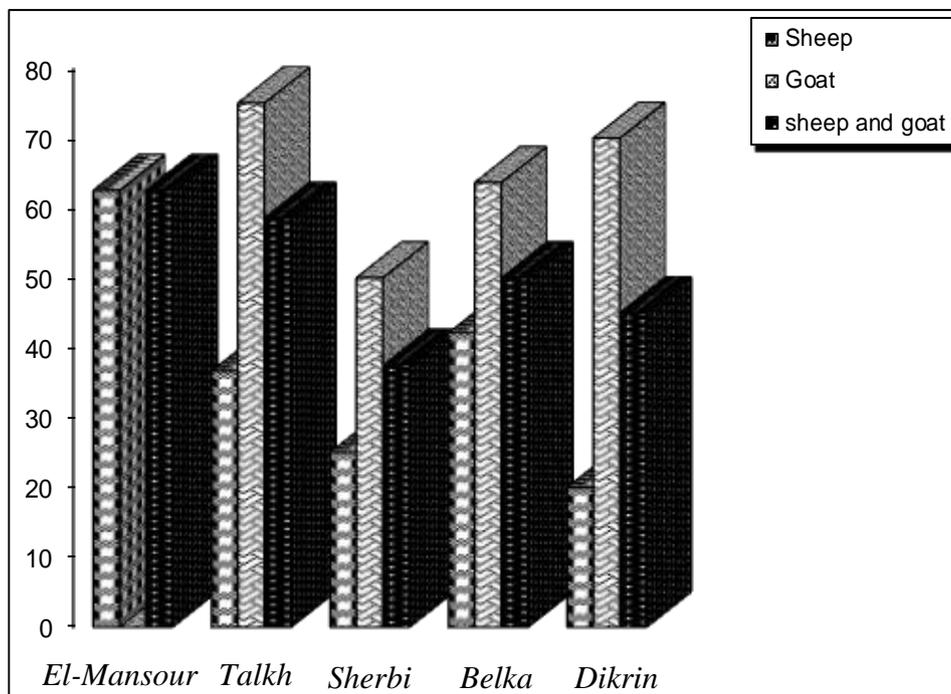
respiratory manifestation as dyspnea, cough, nasal discharge, for sheep 125 out of 764 ( 16.36%) sheep examined show respiratory manifestation while in goat the respiratory manifestation reach 25.66% (87 from 339 examined).the respiratory manifestation. Testing of blood samples from diseased animals (212) using ELISA technique, revealed that, Table (2) & Fig (1) *mycoplasma mycoides* seroprevalence in sheep and goat has respiratory manifestation reach 52.35% (111 out of 212 examined), while in goat only reach 67.81% (59 out of 87 examined), in sheep only reach 41.6% (52 out of 125 examined).

**Table 2:** Seroprevalence of *Mycoplasma mycoides cluster* in sheep and goat using ELISA kit.

Species Locality	Total sheep and goat			Goat			Sheep		
	Total	Positive	%	Total	Positive	%	Total	Positive	%
El-Mansoura	40	25	62.5	0	0	0	40	25	62.5
Talkha	70	41	58.57	40	30	75	30	11	36.66
Sherbin	32	12	37.5	16	8	50	16	4	25
Belkas	30	15	50	11	7	63.63	19	8	42.1
Dikrins	40	18	45	20	14	70	20	4	20
<b>Total</b>	<b>212</b>	<b>111</b>	<b>52.35</b>	<b>87</b>	<b>59</b>	<b>67.81</b>	<b>125</b>	<b>52</b>	<b>41.6</b>

Between species Odds ratio= 0.3381 95 % CI= 0.1905 to 0.5998 P = 0.0002

**Fig. 1:** Seroprevalence of *Mycoplasma Mycoides* in sheep and goat using ELISA kit

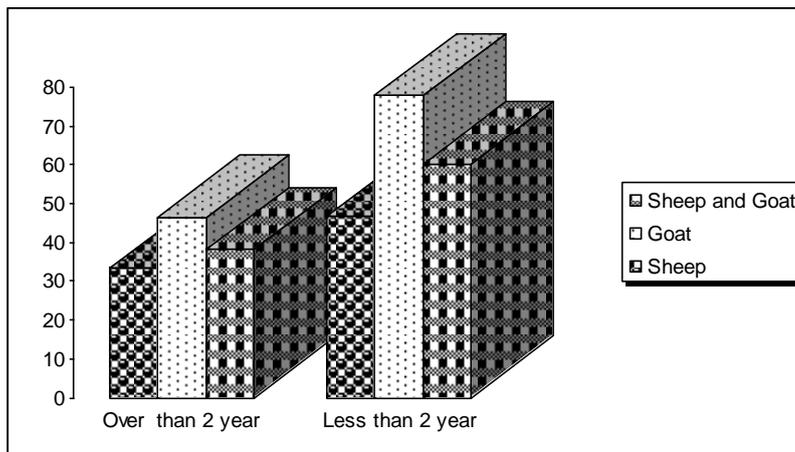


Age seroprevalence of mycoplasma mycoides revealed that, the higher seroprevalence were recorded in age less than 2 year old, (60.29%), (77.96%) and (46.75%) in (sheep and goat) , goat only and sheep only respectively , while adult sheep recorded low seroprevalence as it reach 38.15%, 46.42% and 33.33% in (sheep and goat), goat only and sheep only respectively. (Table 3).

**Table 3:** Age seroprevalence of *Mycoplasma mycoides* in sheep and goat using ELISA kit.

Age	Total sheep and goat			Goat			Sheep		
	Total	positive	%	Total	Positive	%	Total	Positive	%
Less than 2 year	136	82	60.29	59	46	77.96	77	36	46.75
Over than 2 year	76	29	38.15	28	13	46.42	48	16	33.33
<b>Total</b>	<b>212</b>	<b>111</b>	<b>52.35</b>	<b>87</b>	<b>59</b>	<b>67.81</b>	<b>125</b>	<b>52</b>	<b>41.6</b>

Between Age P = 0.0053 Odds ratio = 2.2722 95 % CI= 1.2756 to 4.0473

**Fig. 2:** Age seroprevalence of *Mycoplasma mycoides* in sheep and goat using ELISA kit.

## DISCUSSION

On examining seroprevalence of *Mycoplasma spp* in sheep and goat in Dakahlia governorate and studying their role in respiratory manifestation and risk factors associated with its seroprevalence a total of 1103 sheep and goat were examined clinically for respiratory signs, the result revealed that, total of 212 (19.2%), sheep and goat show respiratory manifestation as dyspnea, cough, nasal discharge, for sheep 125 out of 764 (16.36%) sheep examined show respiratory manifestation while in goat the respiratory manifestation reach 25.66% (87 from 339 examined).

The OIE reference method for CBPP serology is the complement fixation test (CFT). This technique has been used for CBPP eradication campaigns in many countries. However, it presents some disadvantages, particularly due to the existence of non-specific positive results and to difficulties encountered in the standardization of antigen production. For these reasons CIRAD-UMR15 (FAO and OIE world reference center for (CBPP) has developed another test, a competition ELISA (C-ELISA) based on a monoclonal anti-Mmm SC antibody, named Mab 117/5. This test is an alternative to the CFT for the OIE and can be used for official CBPP testing. Regalla

*et al.* (2000). According to phenotypic and genotypic characteristics that cause cross-reactions in conventional diagnostic techniques between the five *Mycoplasmas*, spp. that called “*Mycooides cluster*” (Manso-Silvan *et al.*, 2007) Its closest relatives are *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma leachii*, which may cross-react with *Mccp*, but the other members of the mycooides cluster, such as *Mycoplasma mycoides* subsp *capri* or *Mycoplasma mycoides* subsp. *mycoides*, may also share similarities. *MmmSC* is a mycoplasma, i.e. a wall-less bacteria (mollicute), belonging to the so-called “mycooides cluster” The closest relative to *MmmSC* is *M. mycoides* subsp *capri* (*Mmc*), which is usually found in goats. (OIE 2014) and SANCO/AH/R25/2001.

In our study the overall seroprevalence of *mycoplasma spp* in sheep and goat were 52.35% (111 out of 212 examined), while there are higher significance between species  $P < 0.05$  and odds ratio = 0.3381. In goat only the seroprevalence reach 67.81% (59 out of 87 examined), while in sheep only the seroprevalence reach 41.6% (52 out of 125 examined). Moreover, their variations in seroprevalence between localities as showed in Table (2) & Fig. (1), the obtained result is agreed with the result obtained by Sharew *et al.* (2005). They made a comparison of serological tests

for CCPP; sera from 767 goats examined. They were subjected to three tests: complement fixation test (CFT) with *Mycoplasma capricolum* subspecies *capripneumoniae* antigen; blocking ELISA (B-ELISA) with *Mycoplasma capricolum* subspecies *capripneumoniae* antigen; and CFT with *Mycoplasma mycoides* subspecies *mycoides* small colony type antigen. Antibodies were detected by these three tests in 23%, 2% and 12%, respectively, of sera from districts in which CCPP had not been reported, and in 60%, 83% and 87%, respectively, in sera from areas in which CCPP had been reported. Moreover, added that the use of B-ELISA test for the diagnosis and for epidemiological studies of CCPP strongly recommended. And the obtained result are nearly higher than the result obtained by Al-Momani *et al.* (2011) they examined 18 sheep flocks, 27 goat flocks, and 59 mixed flocks containing both sheep and goats in northern Jordan against *ycoplasma mycoides* subspecies *capri* using the latex agglutination test. To increase the chances of detecting positive flocks, sick or older ewes were sampled. Specific information was obtained using a questionnaire to identify potential risk factors for *M. mycoides* subsp. *capri* seropositivity in small ruminants. The true flock-level seroprevalences of *M. mycoides* subsp. *capri* were 34%, 32%, and 38% in small ruminants (sheep and goats), sheep, and goats respectively, the difference in result may be due to difference in test using and locality.

In regarding to age as second risk factors they are higher significance between two age groups  $P < 0.05$  and odds ratio equal 2.2722, (Table 3) in our study the higher seroprevalence higher seroprevalence were recorded in age less than 2 year old, (60.29%), (77.96%) and (46.75%) in (sheep and goat), goat only and sheep only respectively, while adult sheep recorded low seroprevalence as it reach 38.15%, 46.42% and 33.33% in (sheep and goat), goat only and sheep only respectively. The obtained result is differ from the result obtained by Eskindir *et al.* (2012), they studied seroprevalnce of *mycoplasma Capri* in two age group of sheep, age group  $\leq 2$  years(young), age group  $>2$  years (adult). The seroprevalence of CCPP was significantly ( $P < 0.05$ ) higher in adult (7.38%) than in young (1.05%) goats added that, there was non-significant variation concerning the risk involvement of she-goat (4.67%) and buck (5.32%) in the flock. In addition, APHRD (2010) stated that age is an important factor and all ages can be affected. Seropositivity may be high in adult but mortality is higher in young animals than in adults Radostits *et al.* (2007). The differences may be attributed to flock size increase, the chance of contact between animals increase, which enhance chance of acquiring the infection. Being a contagious infection, the chances of spread of CCPP was maximum in large flock where the husbandry practices were not efficiently available and the individual animal care was not appropriately

possible.

The goat has a higher seroprevalence than sheep (Table 2), these results are discussed by Elsawalhy (2012), who mentioned that, Goats are highly susceptible to natural infection and the pathogen has been isolated from sheep with typical lesions, Added that Housing goats together facilitate spread of the disease.

From our result we can concluded that, CCPP can be diagnosed by ELISA kit used to diagnose CBPP and CCPP need further studies in studying areas and isolation and identification of *Mycoplasma Mycoides subspp Capri* and studying all risk factors associated with disease transmission to initiate preventive control measures.

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## معدل انتشار الميكوبلازما في مصل المجترات الصغيرة باستخدام اختبار الاليزا في محافظة الدقهلية

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في هذه الدراسة تم فحص عدد 1103 من الأغنام والماعز في خمس مناطق مختلفة في محافظة الدقهلية وذلك خلال الفترة من نوفمبر 2013 حتى مارس 2014 تم فحص هذه الحيوانات للأعراض التنفسية وتبين أن من بينهما 212 من الأغنام والماعز من مختلف الأعمار تظهر عليها الأعراض التنفسية مثل الكحة والإفرازات الأنفية وضيق التنفس تم سحب عينات دم من هذه الحيوانات وفصل السيرم لاختبار استخدام الاليزا لميكروب الميكوبلازما وتبين من النتائج أن معدل انتشار الميكوبلازما في سيرم الأغنام والماعز بلغ (52.35%) في كل من الأغنام والماعز في حين ان هناك ارتباط معنوي  $p < 0.05$  بين الأنواع المختلفة حيث أن النسبة كانت اعلى في الماعز 67.81% بينما كانت في مصل الأغنام 41.6% وعلاوة على ذلك كان هناك تباين في الإصابة بالنسبة للأعمار  $p < 0.05$  حيث أنها كانت 60.29% & 38.15% في الأعمار أقل من سنتين وأكبر من سنتين على التوالي. مما سبق يتبين أن ميكروب الميكوبلازما ينتشر في الأغنام والماعز بمحافظة الدقهلية مما يحتاج الى مزيد من الدراسة لعزل الميكروب وتصنيفه علاوة على دراسة دورة في الأمراض التنفسية الأخرى التي تصيب الاغنام والماعز.