INVESTIGATION ON OVINE HAEMOPHILOSIS

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ABSTRACT

This study was performed during winter 2002 in a sheep flock manifesting respiratory embarrassments in 44 out of 321 animals of different ages and sex. Bacteriological examination of nasopharyngeal swabs taken from diseased sheep revealed the detection of Haemophilus somnus (H.somnus) either alone (55.6%) or in combination with Streptococcus pneumoniae (22.2%), Klebsiella pneumoniae (11.1%), and Staphylococcus aureus (11.1%). Also H.somnus was detected from the nasopharyngeal swabs collected from in contact apparently healthy cattle.

Electrophoretic pattern of Haemophilus somnus isolated from diseased sheep and incontact clinically normal cattle proved the similarity of ovine and bovine strains, such criteria have potential value for epidemiological studies of hemophilosis among farm animals. Clinical signs exhibited on the diseased animals was described and some epidemiological data and the treatment of diseased animals was studied and discussed

INTRODUCTION

Sheep are considered to be one of the most valuable sources for wool industry, milk and meat production in Egypt. Pneumonia causes serious financial wastes to the entire sheep industry resulting from deaths, reduced gross weights, delayed marketing and from costs of expensive treatment. Sheep and goats of all ages are infected but lambs and kids are susceptible to bacterial pneumonia with high morbidity and mortality rates **(Smith and Olubunmi., 1983).**

Respiratory infection in sheep is caused by several agents namely bacterial, viral, fungal or parasitic agents synchronized with stress and/or environmental factors. Many of bacterial agents as *Pasteurella* sp, *E-Coli*, *Staphylococcus epidermidis*, *Corynebacterium ovis* or *Corynebacterium pyogens*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*

and *Haemophilus* sp **(Misra et al., 1970, Vyas et al., 1984; Elyas., 1993 and Radostits et al., 2000)**.

Haemophilus species are commensals or parasites of the mucous membranes of humans and animals, most commonly in the upper respiratory and lower genital tract **(Quinn et al., 1994)**. H.Somnus is an important veterinary pathogen that causes respiratory disease, arthritis, septicemia and abortion in cattle and sheep **(Yang et al., 1998)**. Moreover *H.somnus* is considered as a major significant cause of pneumonia **(Corboz and wild., 1981)**.

H.ovis has been serological cross-reaction with H.somnus and both recognized as commensal and pathogenic organisms in cattle and sheep **(Killan and Biberstein., 1984)**. *H. Somnus*, *H.agni* and *Histophilus ovis* should be considered a single species and have been recognized as important pathogens in domestic ruminants **(Walker et al., 1985 and Ames., 1987)**. Although they have been described under various names, these organisms are very similar and may be identical **(Humphery and Stephens., 1983 and Ward et al., 1995)**. The nasal and genital discharges, urine and expired air could all potentially act as source of infection and transmission of the haemophilus within a flock of sheep **(Philbey et al., 1991)**.

Regarding to clinical manifestation of hemophilosis in sheep. Histophilus ovis was incriminated as the cause of natural cases of epididymitis in rams, polyarthritis in lambs, mastitis and abortion in ewes **(Webb., 1983)**. The respiratory form of *H.somnus* infection has been gaining in importance **(Harris and Janzen., 1989)**. Fatal pneumonia of sheep with fever, prostration, dyspnea, cyanosis and bloody diarrhea **(Hungerford., 1990)**. The clinical and pathological findings of hemophilosis were consistent with previous reports and included polyarthritis, epididymoorchitis, meningoencephalitis, pneumonia, septicaemia, mastitis and metritis **(Philbeyet al., 1991)** An outbreak of Haemophilus aegyptius infection in a sheep farm characterized by meningoencephalomyelitis **(Akpavie., et al. 1994)**. *H.agni* and *H.ovis* have been associated with ovine septicemia, pneumonia, mastitis and epididymitis **(Radostits et al., 2000)**.

Because of the acute nature of the disease, vaccination is likely to be the only satisfactory method of control. Haemophilus species was sensitive to various antibiotics namely penicillin, ampicillin, oxytetracycline, chlortetracycline, chloramphenicol, nitrofurantoin, erythromycin and polymyxin B but were resistant to bacitracin and vancomycin **(Hajtos., 1987 and Euzeby., 2001)**.

So the fundamental goal of this study was aimed to investigate problem of respiratory distress among sheep through:

A- Identification and characterization of Haemophilus sp and other incriminated bacteria.

B- Studying the pathogenicity of Haemophilus sp in mice and chicken embryos.

C- Trials for the isolation of Haemophilus sp from incontact clinically healthy cattle.

D- Eleetrophoretic protein profile of Haemophilus somnus isolated from investigated sheep (ovine strain) and incontaet clinieally normal cattle (bovine strain).

E- Description of clinical picture and assessments of some epidemiological data.

F- Serological investigation of the animals under study.

G- Trials of treatment after the application of antiblogram on the isolated *Haemophilus somnus.*

MATERIAL AND METHODS

1- Animals : The study was conducted on a total of 321 sheep of different ages and breeds. this flock belonged to private sheep flock at Kaluobia Governorates, 44 out of them showing respiratory distress. Besides 50 clinieally healthy contact cattle All animals were subjected to careful and fully clinical investigation **(Pugh., 2002)**.

2- History of the investigated animals : This study was applied during end of winter 2002. The animals under investigation had previous history of recurrent respiratory distress circulating among these animals during winter seasons. The investigated animals were housed overcrowded under inadequate ventilation, received poor feeding quality and exposed to inclement weather during this period and the floor of the stable have wet straw bedding. This flock was in contact with cattle population (50) at the same vacinity and the attendants share the work in the two farms.

3-Sampling :

A- Nasopharyngeal swabs: Two nasopharyngeal swabs were taken from 44 diseased sheep, the first one was used for the possible existence of Haemophilus while the second one exposed to bacteriological examination against other incriminated bacteria. Also nasopharyngeal swabs collected from contact clinically normal cattle for detection of haemophilus species.

B- Serum samples: It were taken from diseased sheep and exposed to agglutination test.

4- Bacteriological examination:

A- For haemophilus: The swabs were directly streaked on selective media **(Brewer et al., 1986)**, the colonies were identified from microscopic examination of stained smears, colony

morphology and biochemical reactions **(Mackie and Macarteny., 1996)**.

B- For other incriminated bacteria: The swabs were taken into nutrient broth, then subcultures on specific media and the isolated bacteria were identified and characterized from colony morphology and biochemical reaction **(Quinn et al., 1994)**.

5- Identification of the haemophilus isolates : The isolated agents were confirmed and identified by:

A- Sero-identification: The colonies identified serologically using standard hyperimmune sera (Veterinary Laboratory Agency., UK) according to the methods of **Hoerlein et al., (1973)**.

B- Pathogenicity test: It was performed by mice inoculation (Intraperitoneally) with 0.2 ml of twenty-four hours broth culture of the isolate and observed for a week **(Kennedy et al., 1960)**, and by inoculation of 0.2 ml of 24 hours broth culture of *H.somnus* into yolk sac of 6 days embryonated chicken eggs and candling of eggs and the mortalities were detected **(Livard et al., 1982)** .

6- Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE): It was applied using 12.5% separating gel with discontinuous buffer system, gel stained with 0.25% (w/v) coomasie brilliant blue in 50% methanol 10% (w/v) acetic acid, destaining by 10% methanol 7.5% acetic acid and the gel photographed and read by computer program through scanner **(Laemmli., 1970 and Tagawa et al., 1993)**. The test was done to compare between ovine and bovine strain of Haemophilus somnus.

7- Tube agglutination test : It was performed on serum from diseased sheep for detection of agglutinin antibodies against Hemophilus somnus.

8- Antibiogram procedures : The haemophilus isolates were tested for sensitivity to antibiotics according to the method of **Sogka et al., (1972) and Quinn et al., (1994)**.

9- Therapeutic trials of diseased animals : The diseased sheep were treated after sensitivity test. The diseased animals were allocated into two groups, the animals of first one were treated specifically by gentamicin sulfate as 5mg/kg. b/w. I/M for 5 days (gentamicin 5% Inj., Bremer Pharma Gmbh., Germany), beside symptomatic and supportive treatment and non-specific immunostimulant as levamisole in dose of 0.03 ml/kg. b/w. S/C for three days, repeat after 3 days (Vermisole Inj., Bimeda Chemicals Limited., Dublin). While the second group received the same regimens without immunostimulant therapy **(Humphrey and Stephens., 1983; Radostits et al., 2000 and Zaitoun., 2001)**.

RESULTS AND DISCUSSION

Haemophilus species have been implicated elsewhere in ovine respiratory disease, which is a major problem in sheep flocks. A shift in the normal upper airway bacterial flora or stress activation of latent H.somnus in the upper airway may contribute to a lower airway infection. Regarding to the results of bacteriological examination, it showed identical Hemophilus somnus colonies, which identified from morphology and biochemical reaction as shown in table (1). Such results were similar to that obtained by **Corboz and Wild., (1981); Quinn et al., (1994); Ibrahim., (2001) and Euzeby., (2001)**.

Further identification of haemophilus achieved by application of the pathogenicity test in mice which declared deaths of the mice within one week and inoculation of chicken embryos where deaths of embryos occured within 48.h. The above mentioned results were justified by the prior work of **Kennedy et al., (1960); Livard et al., (1982); Kwiecien and Little., (1992) and Mohamed., (2000)**. Serological identification of the isolated pathogen using standard hyperimmune serum proved infection by *Hemophilus somnus*. The current results were in agreement to that obtained by **Hoerlein et al., (1973) and Mohamed., (1996)**.

Table (2) declared isolation of 9 strains of Haemophilus somnus from 44 diseased animals (20.45%) taken from clinically ill sheep either alone (55.6%) or in combination with *Streptococcus pneumoniae* (22.2%), *Klebsiella pneumoniae* (11.1%) and *Staphalyoccus auerus* (11.1%). The lower number of Haemophilus isolates in this study contributed to the mixed infection with other pathogens, which overgrown the growth of haemophilus. It should be borne in mind that *haemophilus somnus* when cultured is a relatively slow grower and may be outgrown by other bacteria when mixed infections are present **(Coetzer et al., 1994)**. The detection of other pathogens in combination with haemophilus was similar to that reported by many workers **(Misra et al., 1970, Vyas et al., 1984; Elyas., 1993 and Radostits et al., 2000)**.

The current results revealed detection of *haemophilus somnus* (2 isolates) from contact clinically healthy cattle (50). The obtained results was highly augmented with the prior work of **Lees et al., (1990)** who reported that, purchasing replacement animals and having cattle on the farm were risk factors for Haemophilus infection in sheep flock and it is possible interspecies transmission may play a role in the epidemiology of the disease.

SDS-PAGE electrophoresis was performed (Fig 1&2) to compare between the soluble protein of *H.somnus* ovine and bovine strains, the results showed similarity between the two strains. Such finding nearly identical to that gained by **Lees et al., (1994)** who proved that, protein profiles of bovine and ovine *H. somnus* done by sodium dodecyl sulphate-polyacrylamide gel electrophoresis showed similar patterns for virulent bovine and ovine isolates and indicated the similarity in

pathogenicity and in surface antigens.

Clinical signs exhibited on the investigated animals revealed fever, respiratory distress as cough, dyspnea, nasal discharges (Fig 3), abnormal lung sounds on auscultation and alteration in appetite and body condition. Such observations were previously described by **Hungerford., (1990); Quinn et al., (1994) and Ibrahim., (2001).** However few animals exhibited chronic cough, this might be contributed to occurrence of laryngitis, tracheitis in addition to the pneumonia due to Haemophilus **(Humphery and Stephens., 1983 and Harris and Janzen., 1989).**

The absence of CNS involvement was supported previously by the work of **Little., (1986)** who declared that, first outbreak of *haemophilus somnus* predominately characterized by thrombotic meningoencephalitis but as the infection becomes endemic, there is a gradual change in its manifestation and the respiratory form attains a high profile.

The epidemiological data cleared that, the disease was occurred during end winter 2002 and the flock was exposed to stress factors namely bad inclement weather during this period, confinement of sheep for long time overcrowded in inadequate ventilated stables had wet straw bedding and received poor quality food. Such data was explained by **Humphery and Stephens., (1983) and Quinn et al., (1994)** who documented that, stresses such as inclement weather, housing and overcrowding are thought to be of triggering the haemophilus infection.

The incidence of the haemophilosis in relation to the age (table 3). The results of this study concluded that, incidence of hemophilosis was higher in young s than adults. This might attributed to the levels of natural serum bactericidal activity against *Haemophilus somnus* infection during this age **(Simnson and Maheswaran., 1981).** These results came in accordance to that of **Quinn et al., (1994)** who published that young or previously unexposed animals are most susceptible to Haemophilus infections..

The incidence of the disease in relation to breed and sex (Table 4) cleared that, the incidence was higher in Rahmany than in Baladi sheep while the incidence was nearly equal in both male and female.

The use of serological tests for the diagnosis of clinical cases of hemophilosis is equivocal **(Coetzer et al., 1994).** The current results of tube agglutination test (Table 5) revealed positive results in 24 samples (54.5 %) out of 44 samples collected from diseased animals and the titer of agglutination antibodies reached to 1/800. However serological data is lacking in specificity as *H.somnus* cross reacts with other bacteria as other *Haemophilus* spp. *Actinobacillus lignieresii, Listeria monocytogenes, Bordetella bronchispetica* and others **(Humphery and Stephens., 1983).** In this context **Ruegg et al., (1988)** concluded that titers of 1:256 to 1: 512 of *H.somnus* in non-vaccinated herds indicate a chronic infection. Therefore the above results indicative of chronic

haemophilosis. Also **Safacon and Higgins., (1982)** cited that high agglutinating titers against *H.somnus* are transient as the titers in affected animals don't persist.

The results in table (6) illustrated the correlation between bacteriological and serological results, it is cleared that, the serological incidence of haemophilosis was higher (54.5%) than bacteriological incidence (20.5%), this data support the view that clinical haemophilosis should be confirmed with both bacteriological and serological investigation.

The results in table (7) showed that *Haemophilus somnus* sensitive in variable degrees to many antibiotic and the sensitivity to gentamicin reached to 92.86% while it was completely resist to bacitracin and vancomycin, such criteria justified by the prior work of **Hajtos., (1987) and Euzeby., (2001)**.

With respect to treatment, the diseased animals showed clinical improvement after specific treatment with gentamicin sulfate beside symptomatic therapy within 1-2 weeks in the first group while in the second one extended to 3 weeks. These results were in harmony to that reported by **Humphery and Stephens., (1983)**. This might be contributed the non-specific immune stimulant action of levamisole which enhance and accerlate the clinical improvement. Such result was in harmony to that proved by **Zaitoun., (2001)**.

Finally, the main points recommended in control of ovine haemophilosis were detection and treatment of diseased animals after application of sensitivity test, careful management to reduce the influence of the predisposing factors, interspecies transmission of haemophilus somnus or contact of sheep with cattle or other reservoirs should be avoided and vaccination of sheep against Haemophilus somnus (local strain) either alone or in combination with other bacterial or viral vaccines used in Egypt.

Table (1): Results of biochemical reactions

Sugar fermentation						Oxidase	Nitrate	Catalase	Urease	Indole
G	F	M	A	L	T					
+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve

G: Glucose F: Fructose M: Mannitol A: Arabinose L: Lactose T: Trehalose

Table (2): Results of bacteriological examination

Number of sheep	Number of clinically ill	Isolates								Total isolates	
		H.somnus alone		H+Strept		H+Klebsiella		H+Staph			
		No	%	No	%	No	%	No	%	No	%
321	44	5	55.6	2	22.2	1	11.1	1	11.1	9	20.45

H:Haemophilus

Table (3): Incidence of hemophilosis in relation to age.

Animals examined			Animals age								
			>6.m			6->1.y			1-3.y		
Total	CI	%	No	CI	%	No	CI	%	No	CI	%
321	44	13.71	32	17	53.1	68	18	26.5	102	9	8.8

CI: clinically ill

Table (4): Incidence of hemophilosis in relation to breed and sex

Animals			Breed						Sex					
			Baladi			Rahmany			Males			Females		
Total	CI	%	No	CI	%	No	CI	%	No	CI	%	No	CI	%
321	44	13.71	184	14	7.6	137	30	21.9	131	20	15.3	190	24	12.6

Table (5): Results of tube agglutination test on sera taken from diseased sheep

Total number of sheep sera samples	Negative samples		Positive samples		End titer					
	No	%	No	%	1/25	1/50	1/100	1/200	1/400	1/800
44	20	45.5	24	54.5	8	5	2	5	3	1

Table (6) Correlation between bacteriological and serological results

Number of clinically ill sheep	Bacteriology		Serology	
	No +Ve	%	No +Ve	%
44	9	20.5	24	54.5

Table (7) Results of sensitivity test on the isolated haemophilus somnus.

Antibiotic	Resistant %	Sensitivity %
Gentamycin	7.14	92.86
Enrofloxacin	14.29	85.71
Kanamycin	21.43	78.57
Tetracycline	38.6	61.4
Ampicillin	42.86	57.14
Erythromycin	48.26	51.74
Bacitracin	100	0.0
Vancomycin	100	0.0

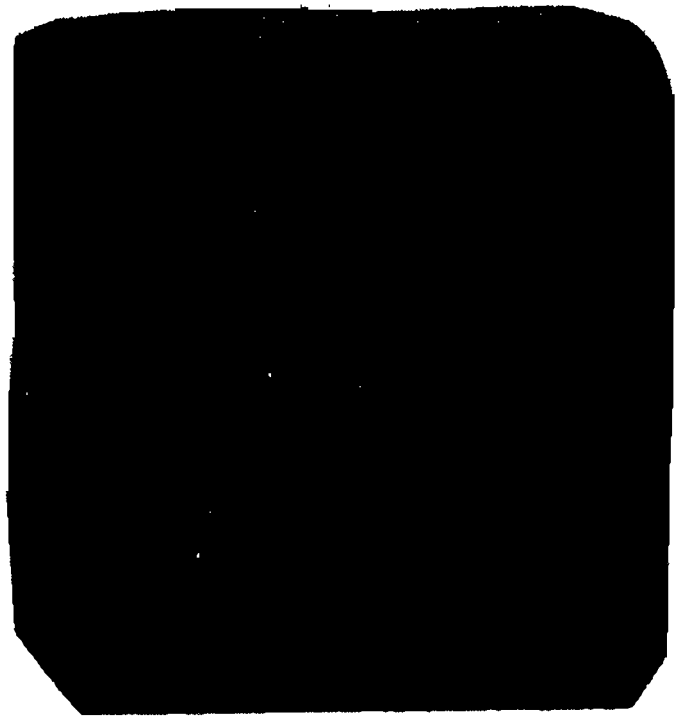

Fig. (1) : SDS-PAGE of whole cell protein of H.somnus (ovine strain).

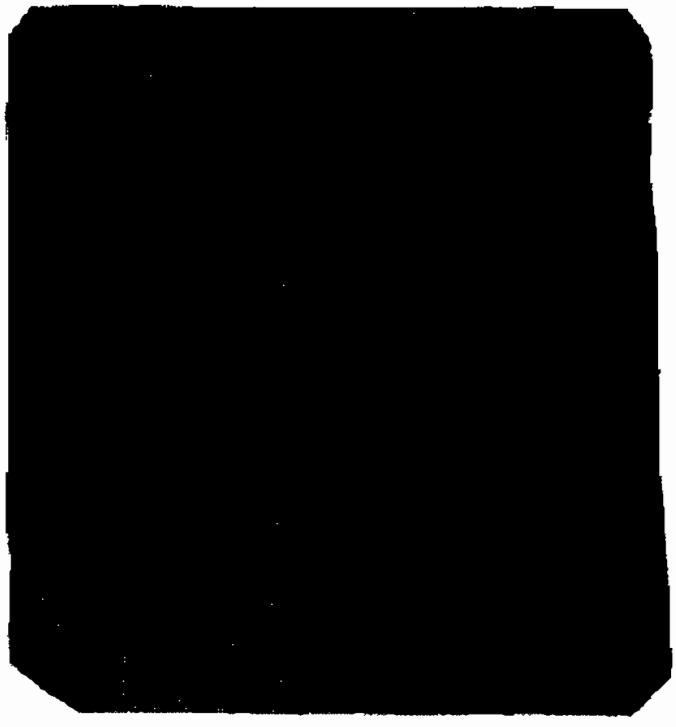

Fig. (2) : SDS-PAGE of whole cell protein of *H.somnus* (Bovine strain)

Fig. (3) : Diseased lamb showing nasal discharges

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الملخص العربى

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قسم الأمراض الباطنة والأمراض المعدية والأسماك - كلية الطب البيطرى - جامعة المنصورة**

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إصابة الجهاز التنفسي بالأمراض المعدية المختلفة تؤثر علي الحالة الصحية والانتاجيه للاغنام وقد تؤدي إلي وفيات بنسب مختلفة مؤديه إلي خسائر اقتصادية كبيرة. لذا استهدف هذا البحث لمحاولة التعرف علي دور ميكروب الهيموفيلس سومنس كمسبب رئيسي في إحداث أعراض تنفسية في قطيع من الأغنام وكذلك التعرف علي الصورة الإكلينيكية والوبائية للمرض واستبيان دور العوامل الضاغطة كعامل مساعد في إحداث المرض هذا بالاضافه إلي محاولة عزل ميكروب الهيموفيلس سومنس من الأبقار المخالطة لهذا القطيع وعمل تحليل للبروتين الكلي للعتره المعزولة من الأغنام ومقارنتها بتلك التي عزلت من الأبقار وكذلك عمل اختبار حساسية لميكروب الهيموفيلس سومنس علي أطباق الزرع واختيار المضاد الحيوي المناسب في العلاج مع وضع التوصيات اللازمة في التحكم والسيطرة علي المرض. أجريت الدراسة علي عدد ٣٢١ من الأغنام متفاوتة في الأعمار والأجناس والسلالات بالاضافه إلى ٥٠ بقره مخالطه لهذا القطيع وذلك بمحافظه القليوبية أثناء شتاء عام ٢٠٠٢ وقد تم اخذ مسحات من التجويف الأنفي البلعومي للاغنام المصابة والأبقار المخالطة هذا بالاضافه إلي انه تم تجميع عينات سيرم من الأغنام المصابة وذلك للفحص المعملي. الفحص الإكلينيكي للاغنام اظهر أن عدد ٤٤ من الأغنام كانت تعاني من الأعراض التنفسية المختلفة. الفحص البكتريولوجي للمسحات اظهر وجود ميكروب الهيموفيلس سومنس منفردا بنسبه عالية إلي حدا ما وصلت ٥٥ر٦٪ بينما قد تم عزله مع بعض البكتريا الأخرى في صوره ثنائية مثل الاستربتوكوكس والكلبسيلا والاستافيلوكوكس بنسب ٢٢ر٢ و ١١ر١ و ١١ر١٪ على التوالي. أيضا تم عزل ميكروب الهيموفيلس سومنس من الأبقار المخالطة. هذا وقد تم توصيف وتصنيف عترات الهيموفلس المعزولة علي أساس التفاعلات الكيمائية للميكروب و التشخيص السيرولوجي للميكروب باستخدام أجسام مضادة قياسيه وكذا حقن الميكروب في اجنه البيض المخصب وفي فئران التجارب. فحص ومقارنة البروتين الكلي الخلوي لميكروب الهيموفيلس سومنس عن طريق اختبار الهجرة الكهربائي (الالكتروفوريسس) لعتره الهيموفيلس سومنس المعزولة من الأغنام والتي تم عزلها من الأبقار وجد أنها متشابه إلي حد كبير وهذا يؤكد امكانيه انتقال الميكروب من الأغنام إلى الأبقار والعكس صحيح. الدراسات الوبائية للمرض أظهرت أن النسبة الكلية للإصابة كانت

١٣٫٧١٪ وسجلت الحيوانات ذات العمر الأقل من ست شهور نسبة ٥٣٫١٪ بينما كانت بين الحيوانات ذات العمر من ست شهور إلى أقل من سنة وكذا الحيوانات التى عمرها من سنة إلى ثلاث سنوات ٢٦٫٥٪ و٨٫٨٪ على التوالى. كما أن معدل الإصابة فى الأغنام الرحمانى كانت أعلى ٢١٫٩٪ عنها فى الأغنام البلدى ٧٫٦٪ إختبار تاذن السيرم على عينات السيرم المجمعة من الأغنام المصابة أثبت أن ٥٤٫٥٪ من الحيوانات به أجسام مناعية بعيارات مختلفة (١/٢٥-١/٨٠٠) بينما الفحص البكتريولوچى أعطى نتائجه إيجابية فى ٢٠٫٥٤٪ فقط من الحيوانات المصابة. هذه النتائج تشير إلى ضرورة إجراء الفحوصات البكتريولوچية والسيرولوچية معاً لتحقيق أفضل النتائج. تم علاج الحيوانات بعد إجراء إختبار الحساسية لميكروب الهيموفيلس ووجد أن الميكروب حساس بنسبة ٩٢٫٨٦٪ لعقار الجنتاميسين وقد تم علاج الحيوانات المصابة بعد تقسيمها إلى مجموعتين متساويتين المجموعة الأولى تم حقنها بعقار الجنتاميسين مع استخدام عقار اليفاميزول لرفع المناعة الغير نوعيه للحيوانات مع إعطائها العلاج العرضي والمساعد المناسب أما المجموعة الثانية فقد عولجت بنفس العلاج السابق فيما عدا استخدام عقار اليفاميزول. وجد أن حيوانات المجموعة الأولى قد تحسنت حالتها الصحية وتماثلت للشفاء في خلال١-٢ أسبوع بينما وصلت إلي ثلاث أسابيع في المجموعة الثانية.

هذا البحث خلص إلى الآتى : ١- مرض الهيموفيلوزس من الأمراض التنفسية الخطيرة التي تصيب الأغنام في مصر وتشارك العوامل الضاغطة التي يتعرض لها الحيوانات كعامل مساعد في إحداث المرض. ٢- ميكروب الهيموفيلس سومنس له القدرة علي اصابة الأغنام والماشية لذا يجب وضع هذه النتيجة في الحسبان عند دراسة وبائية المرض. ٣-ميكروب الهيموفيلس سومنس حساس لعقار الجنتاميسين ويفضل استخدام العقارات التي ترفع المناعة الغير نوعيه للحيوانات المصابة للاسراع في شفائها. ٣- ضرورة تحضير لقاح من ميكروب الهيموفيلس سومنس منفردا أو مركبا مع ميكروبات أخرى ودراسة امكانيه استخدامه في التحكم في المرض في مصر.