

EXPERIMENTAL STUDIES ON THE PATHOGENESIS OF *HAEMOPHILUS SOMNUS* INFECTION IN LABORATORY ANIMALS

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Abstract

A total of 466 cows and 286 buffaloes, suffering from endometritis, metritis, retained placenta, and pyometra were investigated to isolate and identify the causative agent. Moreover, the pathogenicity of the isolate was studied in guinea pigs and rats which were intraperitoneally (IP) or intracerebrally (IC) injected with 0.5 ml of 24 hours bactothiol broth culture. Specimens from the uterus, brain, kidneys, lungs, and spleen of the inoculated laboratory animals were collected and fixed in 10% neutral buffered formol saline for histopathological examination. *Haemophilus somnus* was isolated from cows and buffaloes showing retained placenta and pyometra and from cows suffering endometritis and abortion with overall percentages of 4.93 for cows and 1.75 for buffaloes.

The encountered lesions in guinea pigs were hemorrhages and congestion, besides degeneration of the internal organs. In rats, the lesions were nearly similar to those in guinea pigs besides oedematous and congested uterine lamina propria, demyelination of the brain, and hemorrhagic and emphysematous lungs.

INTRODUCTION

The genital diseases may be either nonspecific or specific. The nonspecific diseases are sporadic, but the specific genital diseases are of major importance and are caused by certain organisms (*Brucella abortus*, *Campylobacter fetus var venerealis*, *Listeria monocytogenes*, *Tritrichomonas fetus*, infectious bovine rhinotracheitis virus, and bovine viral diarrhoea virus) which are characterized by their definite predilection seats and definite pattern of involvement for the genital tract. A bovine disease, which was characterized by a brief stage of fever and stiffness followed by rapid development of neurologic signs and death was described by Kennedy *et al.* (1960). Macroscopically, single or multiple hemorrhagic areas were present in the brain. Microscopically, these lesions showed vasculitis which led to thrombosis and infarction. The nomenclature

"*Haemophilus somnus*" was proposed for the haemophilus-like organism causing infectious thrombotic meningioencephalitis of cattle (Dierks *et al.*, 1973). *Haemophilus somnus* was isolated from the brain, lungs, heart, and uterus of sterile cows, besides the lungs of young calves with pneumonia, and the semen of bulls with purulent infection of the genital tract (Corboz and Nicolet, 1975). The placental lesions due to infection with *Haemophilus somnus* were thrombosis, oedema, and placentitis (Van Dreumel and Kierstead, 1975). The lesions, in the fetus were vascular necrosis, thrombosis, and leukocytosis. The inoculation of *Haemophilus somnus* into the middle uterine artery of pregnant cows resulted in abortion associated with thrombosis and necrotizing vasculitis in the fetal membranes. Also, intraamniotic inoculation of *Haemophilus somnus* resulted in fetal death, abortion, birth of weak calves, and retained placenta (Miller, 1980).

Haemophilus somnus was considered as a major cause of endometritis, metritis, abortion between 7th-9th month of gestation and retained placenta in cows (Humphrey and Stephens, 1983). Placentitis, retained placenta, and pyometra were described in cows infected with *Haemophilus somnus*. Infected laboratory animals showed thrombotic meningioencephalitis besides acute orchitis, and epididymitis, characterized by necrosis and arteritis (Dewey and Little, 1984). This work was initiated so as to throw more light on the pathogenicity of *Haemophilus somnus* infection of the reproductive tracts of buffaloes and its pathological effect on the organs of laboratory animals.

MATERIALS AND METHODS

A total of 446 cows and 286 buffaloes, suffering from endometritis, metritis, retained placenta and pyometra were investigated during the present study. These animals were located in Sabeh farm (Giza governorate), El-Waha farm (Nobaria, El-Behera governorate), El-Khanka farm (Kaliobia governorate), Adah farm (Daminhour, El-Behera governorate) and Samir Wasfy farm (Aja, Dakahlia governorate). A complete history of each case was taken and proven to be free from any congenital or anatomical abnormalities, as well as being free from signs of endocrine dysfunction and nutritional disorders. Vaginal and uterine samples were obtained aseptically (Jerry *et al.*, 1984). The vulva was cleaned with dry paper towels and disinfected by equal mixture of lugol's iodine and absolute ethyl alcohol. The pipette was passed into the vagina until near the external os of the cervix, and mucus was drawn into the pipette by tracing the syringe plunger. Then, the pipettes collecting mucus samples were transported to the laboratory in ice box. The specimens were cultivated on the nutrient agar and blood agar media. Smears from the colonies after incubation were stained with Gram's method and exam-

ined microscopically. *Haemophilus* organisms were identified according to Sneath *et al.* (1984). The culture for inoculation was prepared in the research section of venereal diseases, Animal Reproduction Research Institute (Giza, Egypt).

Tables 1 and 2 show the guinea pigs and rats which were injected intraperitoneally (IP) and intracerebrally (IC) with 0.5 ml of 24 hours old bactothiol broth culture. The mortality rate was recorded, and direct smears were obtained from the peritoneal fluid of the dead laboratory animals (guinea pigs and rats). Trials for reisolation of the organism from infected organs were made. Postmortem examination was done on all inoculated animals directly after death or after sacrifice. Specimens from uterus, brain, kidneys, liver, lungs, and spleen of the inoculated laboratory animals were collected and fixed in 10% neutral buffered formol saline. Five micron thick paraffin sections were prepared and stained with Harris hematoxylin and eosin (Drury and Wallington, 1967). The stained sections were examined microscopically.

Table 1. Experimental infection of guinea pigs with *Haemophilus somnus* isolated from cow and buffalo.

Group	Source of isolate	Route of injection	No. of animals
1	Cow	Intraperitoneal (IP)	4
2		Intracerebral (IC)	4
3	Buffalo	Intraperitoneal (IP)	4
4		Intracerebral (IC)	4

Table 2. Experimental infection of rats with *Haemophilus somnus* isolated from cow and buffalo.

Group	Source of isolate	Route of injection	No. of animals
5	Cow	Intraperitoneal (IP)	6
6		Intracerebral (IC)	6
7	Buffalo	Intraperitoneal (IP)	6
8		Intracerebral (IC)	6

RESULTS

Haemophilus somnus was isolated from cases of pyometra (1.12% cow and 1.05% buffalo), endometritis (1.35% cow), retained placenta (0.90% cow and 0.70% buffalo), and aborted cow feti (1.57%). Generally, the prevalence of *Haemophilus somnus* infection was 4.93% in cow and 1.75% in buffalo as shown in Table 3.

Table 3. Incidence of *Haemophilus somnus* as regards to various reproductive disorders.

Reproductive disorders	Cows		Buffaloes	
	No. of examined cases	No. & % of positive cases	No. of examined cases	No. & % of positive cases
Pyometra	98	5 (1.12%)	61	3 (1.5%)
Endometritis	236	6 (1.35%)	140	0 (0%)
Retained placenta	64	4 (0.90%)	42	2 (0.70)
Aborted feti	48	7 (1.57%)	43	0 (0%)
Total	446	22 (4.93%)	286	5 (1.75%)

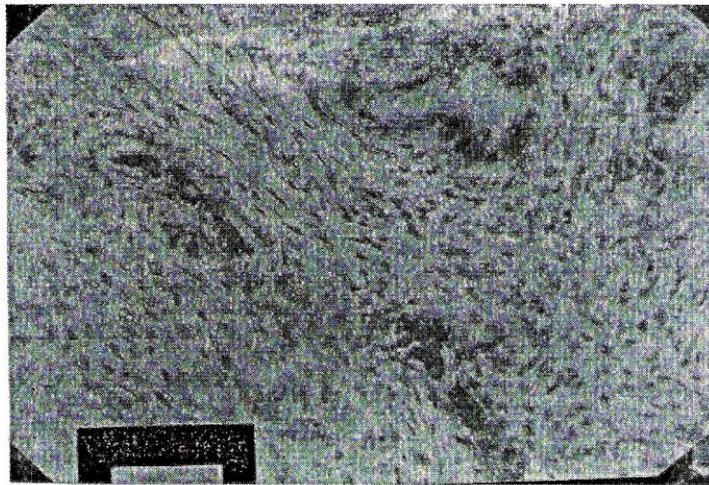
After 24 hours of IP and IC inoculation of rats and guinea pigs with *Haemophilus somnus*, direct films from the peritoneal fluid showed numerous organisms inside the neutrophils. The organisms were reisolated from the peritoneal cavity, brain, and the other organs. The guinea pigs resisted the IP and IC inoculation with *Haemophilus somnus* (cow isolates), while, the rats resisted the IP injection, and were susceptible to the IC inoculation as they showed tremors and rough hair coat 24 hours post-injection. On the other hand, the intracerebral inoculation of buffalo isolates resulted in death of guinea pigs within 24 hours post-injection and tremors and rough hair coat in rats 24 hours post-injection. However, rats and guinea pigs inoculated IP with buffalo isolates showed no clinical symptoms.

The dead and sacrificed laboratory animals (24 hours post-injection) were necropsied. Macroscopically, the rats (groups 5, 6, 7, and 8) showed enlarged and congested lungs, friable liver with necrotic foci, and congested brain. Microscopically, the rats in groups 5 and 6 showed oedematous and congested uterine lamina propria (Fig. 1) with the presence of some siderocytes. The epithelial lining of the endometrium was partially desquamated. The liver showed hydropic degeneration and activated Kupffer cells. The brain was congested. Microscopically, the rats of groups 7 and 8 showed edematous endometrium with hemosiderosis and lymphocytic infiltration. The liver showed congested sinusoids, lymphocytic infiltration, and degenerative changes in the

hepatocytes. The brain showed focal hemorrhages and area of demyelination (Fig. 2). The kidneys showed cloudy swelling, and or hydropic degeneration in the epithelial lining of the renal tubules besides hemorrhages infiltration with lymphocytes and macrophages. The lungs showed emphysematous and hemorrhagic areas with heavy infiltration of mononuclear cells, mainly lymphocytes and macrophages around the blood vessels and bronchioles. The spleen contained numerous siderocytes.

The guinea pigs of groups 1 and 2 resisted the IP and IC inoculation with *Haemophilus somnus* (cow isolates). They were macroscopically and apparently normal, and microscopically all the internal organs were within normal limit. The guinea pigs in groups 3 and 4 which were inoculated with the buffalo isolates showed congested brain and enlarged liver. Microscopically, the brain was congested and hemorrhagic, besides focal perivascular lymphocytic infiltration and neuronal degeneration. The liver showed cloudy swelling and fatty change (Fig. 3) surrounded with numerous lymphocytes and macrophages. Activated Kupffer cells were noticed. The kidneys showed cloudy swelling, lymphocytic infiltration, congestion, and hemorrhage.

Fig. 1. Uterus rat, intraperitoneally inoculated with *Haemophilus somnus* (cow isolate) showing edematous and congested lamina propria (H&E X 400).



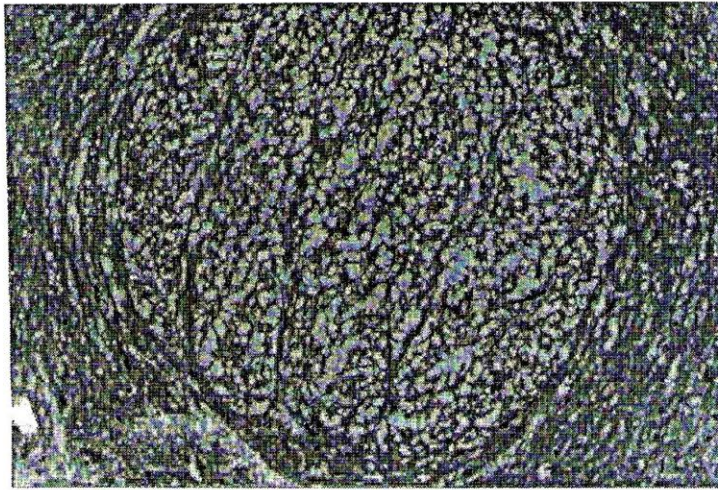


Fig. 2. Brain rat, intracerebrally inoculated with *Haemophilus somnus* (buffalo isolate) showing area of demyelination (H&E X 400).

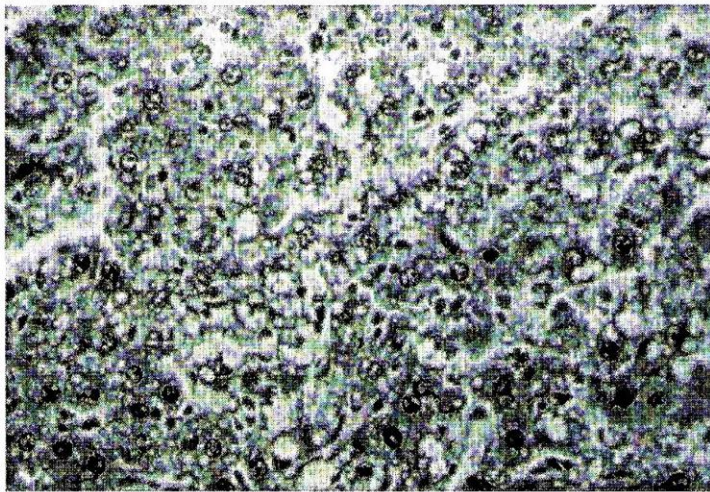


Fig. 3. Liver guinea pig, intraperitoneally inoculated with *Haemophilus somnus* (buffalo isolate) showing cloudy swelling and fatty change (H&E X 400).

DISCUSSION

Haemophilus somnus (22 isolates) was demonstrated in 4.93% of total cows showing pyometra (1.12%), endometritis (1.35%), retained placenta (0.90%), and abortion (1.57%) a result which is in agreement with Humphrey and Stephens (1983), Miller *et al.* (1983), Stephens *et al.* (1983), and Weisser and Albert (1987). The latter observed that *Haemophilus somnus* was isolated from 184 cows with an incidence of 6.8% out of 2711 vaginal and uterine swabs of examined cows. *Haemophilus somnus* (5 isolates) was isolated from 1.75% of total buffaloes suffering pyometra (1.05%) and retained placenta (0.70%), but the organism could not be recovered from buffaloes suffering endometritis or abortion. Such findings are almost in agreement with Miller (1980), Humphrey and Stephens (1983), and Miller *et al.* (1983). Although increasing the incidence of isolation of *Haemophilus somnus* from cows and buffaloes suffering reproductive disturbances, the role of the organism remains undefined. Isolation of *Haemophilus somnus* from the cervico-vaginal region of the cows was frequently reported, but the description of the clinical signs and microscopic observations are generally lacking. Vulvitis was experimentally induced with *Haemophilus* species (Miller *et al.*, 1983), but the role of *Haemophilus somnus* in such condition is unknown. However, the present work shows that *Haemophilus somnus* is involved in the severe pyometra, but the disease has not been induced by the infusion of *Haemophilus somnus* or by breeding of infected bulls experimentally (Rauth *et al.*, 1986).

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دراسات تجريبية باثولوجية على ميكروب الهيموفيلس سومنس في حيوانات التجارب

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أجريت هذه الدراسة على عدد ٤٤٦ بقرة و٢٨٦ جاموسة تعاني من التهابات الرحم وبطانة واحتباس المشيمة وكذا التهابات رحمية صديدية وتم عزل ميكروب الهيموفيلس سومنس من بعض الحالات السابقة.

وتم حقن حيوانات التجارب (خنزير غينيا والفئران) داخل الغشاء البريتوني والغشاء السحائي بمعدل ٠.٥ مليلتر من شورية الباكثوثيول للميكروب المعزول وأخذت عينات من أنسجة الرحم والمخ والكلية والكبد والرئة والطحال لحيوانات التجارب ثم وضعت في محلول الفورمالين المتعادل ١٠٪ وذلك للدراسة الباثولوجية. تم عزل ميكروب الهيموفيلس سومنس من حالات أبقار وجاموس تعاني من احتباس المشيمة والتهابات رحمية صديدية وكذا من أبقار تعاني من التهابات بطانة الرحم وحالات إجهاض وبلغت نسبة عزل الميكروب ٩٢,٩٢٪ في الأبقار و ١,٧٥٪ في الجاموس. كانت التغيرات الباثولوجية في خنزير غينيا هي أنزفة مخية وفراغات في بعض الخلايا العصبية وكذلك استحالة بخلايا الكبد واحتقانات وأنزفة بالكلية. أما التغيرات الباثولوجية التي حدثت في فئران التجارب فكانت ارتشاحات سائلية واحتقان للطبقة الحشوية للرحم ومراحل مختلفة من الاستحالة بخلايا الكبد والكلية والمخ هذا بالإضافة إلى بعض الأنزفة وتمدد الأكياس الهوائية للرئة.