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Pathology and colonization of internal organs after experimental infection of broiler chickens with *Salmonella* Gallinarum through oral or intraperitoneal routes

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Keywords

Broiler chicken – Chick – *Salmonella enterica* serovar Gallinarum – Typhoid – Experimental infection – India.

Summary

This paper describes pathological changes and the frequency of isolation of *Salmonella enterica* subsp. *enterica* serovar Gallinarum (O: 9, 12) from internal organs in broiler chicks experimentally infected through oral or intraperitoneal routes. The experiment was conducted on 110 one-week-old chicks divided into three groups: the CR group (30 chicks) was kept uninfected and served as control, the OR group (40 chicks) was inoculated orally with *Salmonella* Gallinarum (10^9 organisms/ml), and the IP group (40 chicks) was infected intraperitoneally with *Salmonella* Gallinarum (10^9 organisms/ml). Three birds from each group (dead or sacrificed) were observed at 3, 5, 7, 14, 21, 28, 35, and 42 days postinfection for evaluation of gross and histopathological changes in visceral organs, and for frequency of isolation of *Salmonella* Gallinarum from internal organs. Gross and histopathological changes were compared between infected groups by measuring mean lesion scores. The gross and histopathological changes in visceral organs, although similar in both infected groups, were more severe and observed at earlier stages of infection and in more birds in the IP group. There was however no significant difference between the two infected groups in the frequency of isolation of *Salmonella* Gallinarum from internal organs, even in fecal sheddings. It was therefore concluded that the intraperitoneal route should be primarily considered for inducing *Salmonella* Gallinarum infection in experimental trials.

■ INTRODUCTION

Fowl typhoid (FT) is a septicemic disease of poultry that causes considerable economic losses through mortality and increased morbidity. Infection of birds of all ages, in the field or experimentally, can result in very high mortality (5, 10). The disease is caused by the gram-negative bacterium *Salmonella enterica* serovar Gallinarum (31), a member of the Enterobacteriaceae family which is widely distributed throughout the world (34). *Salmonella* Gallinarum is highly adapted and seldom causes significant problems in hosts other than chickens, turkeys and pheasants (30, 34). No difference in susceptibility to *Salmonella* Gallinarum has been observed between local and commercial chickens (26). It was formerly known as *Shigella gallinarum*, when first isolated by Klein in England in 1889 (30). The disease was called fowl typhoid in 1902 (31).

FT has been eradicated in the commercial poultry production of developed countries, but is still a major problem in developing countries (22). In India, FT has long been plaguing the poultry industry, causing heavy economic losses due to mortality in young and adult chickens. Since it was first reported by Cooper and Naik (9) in India, the incidence of FT is on the increase and illustrated by the fact that *Salmonella* Gallinarum alone accounted for 32% of *Salmonella* of avian origin typed at the National Salmonella Centre (Veterinary), Izatnagar, India, from 1987 to 1995 (14). *Salmonella* Gallinarum has been found to be the predominant serotype and the major cause of mortality in poultry in India (28, 32).

Various strategies, i.e. novel antibiotics, vaccines, immunotherapeutics and antimicrobial feed additives, are currently explored to control *Salmonella* infection in poultry (3, 4, 23). The birds are

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routinely experimentally infected by *Salmonella* Gallinarum to evaluate efficacy of different drugs and vaccines. The alimentary tract is the natural route of *Salmonella* infection in poultry. Following oral ingestion, *Salmonella* penetrates the mucosal epithelium of the small intestine, interacting with columnar epithelium cells and microfold cells. *Salmonella* has been shown to survive and replicate within macrophages residing in the lymphoid follicles in the intestines. Macrophages have been found to play an important role in the dissemination of *Salmonella* to organs of the reticulo-endothelial system such as the liver, spleen and bursa (17). The experimental reproduction of FT in adult chickens *via* oral *Salmonella* Gallinarum challenge requires a very high titer as well as treatment with some reagents to reduce the effects of gastric juice (6, 35). This inherent difficulty in the reproduction of FT has been an obstacle to the experimental evaluation of vaccines as well as to understanding FT outbreaks in the field (6). Alternate routes, i.e. intraperitoneal and respiratory routes, have also been reported to induce experimental FT (5, 6). However, the comparative study of *Salmonella* Gallinarum infection through intraperitoneal and oral routes is scanty. Thus we explored the feasibility of using the intraperitoneal route in experimental birds as a cost effective alternative model to study FT further. The clinico-hematobiochemical changes have already been described in a previous paper (29); this article focuses on the gross and histopathological lesions and the isolation of the bacteria from visceral organs.

■ MATERIALS AND METHODS

Experimental birds and their management

The study was carried out in the experimental house of the Department of Veterinary Pathology, Faculty of Veterinary Science and Animal Husbandry, Shere-Kashmir University of Agricultural Sciences and Technology, Kashmir, India, with 110 unsexed one-day-old broiler chicks procured from a local hatchery. The birds were treated humanely during the whole period of the experiment and the work was agreed upon by the Institutional Animal Ethics Committee on ethical standards in animal experimentation (No AU/FVS/Estt/C-12/16638-40). The chicks used were from the same breeding flock. They were reared for a period of 49 days under strict hygienic conditions and maintained on broiler mash from day 1 till the end of experiment. Feed and water were given *ad libitum*. Bacteriological and serological examination showed that the birds were negative for *Salmonella* at the beginning of the study.

Salmonella *strain*

The *Salmonella* Gallinarum strain (from here on called "SG") used for inducing infection was isolated according to the standard method from a disease outbreak in a private broiler farm in Ganderbal area in October 2009 (21). The isolate was serotyped as *Salmonella enterica* subsp. *enterica* serovar Gallinarum with the antigenic structure O: 9, 12 by the National Salmonella and Escherichia Research Institute, Kasauli, Himachal Pradesh, India. The SG strain was selected after it was shown to be virulent following a preliminary infection experiment using seven-week-old commercial broiler chickens.

Experimental design

At day 7, before infecting the chicks with the SG strain, they were divided in three groups: uninfected (n = 30, control birds, group CR), orally infected (n = 40, group OR) and intraperitoneally infected (n = 40, group IP). The chicks of groups OR and IP were challenged with 10^9 organisms of SG strain in one millimeter of normal saline. The three groups of birds were kept separately in different rooms of the experimental house.

Pathological findings

Birds from each group were observed thrice daily (morning, noon and evening) for clinical signs and mortality. Following SG infection on day 7 (day 0 of infection), three birds from each group were euthanatized at 3, 5, 7, 14, 21, 28, 35 and 42 days postinfection (DPI) by cervical dislocation for gross and histopathological studies. However if any bird died due to infection during these specific days, the number of euthanatized birds was reduced to keep the total number (sacrificed + dead birds) equal to 3. Representative tissue samples from the liver, spleen, heart, lungs, bursa of Fabricius, kidneys and intestines were taken from both dead and euthanatized birds and fixed in 10% neutral buffered formalin. These were processed for paraffin embedding using alcohol as dehydrating agent and benzene as clearing agent. The sections were cut at 4-5 µm thickness and stained by the routine hematoxylin and eosin method (24).

Lesion scoring

Gross and histopathological lesions in birds of all groups were scored. Dead birds in OR and IP were also taken into consideration for scoring. Each of the gross and histopathological lesions in different organs was graded as mild, moderate or severe with corresponding intensity scores of 1, 2, and 3. The lesion score was determined for each of the organs in sacrificed and dead birds of a group by multiplying the gross lesion intensity by the number of birds showing that particular intensity of lesion and then by dividing the total number of birds (sacrificed + dead) examined for lesions.

Bacterial isolation and identification

The samples from the liver, spleen, heart and ceca as well as fecal samples were collected from each sacrificed or dead chick at 7, 21 and 35 DPI for bacterial isolation and identification. The samples were individually collected in Rappapart Vassiadis (Oxid, UK) and incubated at 37°C for 18-24 hours. They were then streaked onto brilliant green agar (BGA) and xylose-lysine-desoxycholate agar (XLD) and incubated at 37°C for 24 hours. The identity of suspected blank colonies from XLD and pink colonies from BGA were biochemically confirmed (40).

Statistical analysis

An analysis of variance (ANOVA) was used to test for level of significance of gross lesion scores (36). When differences were significant, Tukey test was used for comparison of mean gross lesion scores between different groups at 95% confidence level using SPSS 17 software (19). Similarly, total histopathological lesion scores were determined for each group and analyzed by ANOVA, and mean values in different groups were compared by Tukey test.

■ RESULTS

Table I shows the number of birds that died in the different experimental groups at various DPI. No mortality was recorded in the uninfected birds of the control group. Maximum mortality was observed in IP (47.50%) with most of the birds dying from 1 to 7 DPI, and peak mortality (four chicks) observed at 4 DPI. Clinical signs, previously described (29), were observed in IP as early as 12 hours after infection and in OR at 3 DPI. Recovery was noticed in OR from 21 DPI, and in IP from 15 DPI.

Table II shows the intensity of gross lesions in different organs of the experimentally-infected birds. The birds in CR did not exhibit any gross lesion in any of the organs examined throughout the

experiment. Gross lesions were first detected in the visceral organs of IP birds at 1 DPI and were not detected in OR birds until 3 DPI.

Initial changes in OR birds included congestion of visceral organs, enlargement of the liver and spleen, and thickening of intestinal mucosae. Necrotic foci on the surface of the spleen and liver were observed at 9 and 14 DPI. Other changes included a bronze discoloration of the liver and mild grayish nodular areas on the ventricular region of the heart.

In IP birds, the initial changes, in addition to congestion of visceral organs, included severe enlargement of the liver and spleen, and distention of the gall bladder. Prominent necrotic foci on the spleen were observed at 5 DPI. Small necrotic foci were visible on the liver as early as 5 DPI, however larger necrotic areas on the liver were observed in a bird which died at 10 DPI (Figure 1). Severe congestion and swelling of the kidneys were observed in IP. In this group, clearly visible grayish white nodules of various sizes were observed at 21 DPI projecting above the surface of the heart (Figure 2). In general, the gross changes in IP infected birds were similar to those observed in OR infected birds but the lesions were more severe and observed at earlier stages of infection and in a higher number of birds.

Table II also shows the intensity of histopathological lesions recorded in different organs of experimentally-infected birds. The histopathological changes in the liver of OR infected birds at 3 DPI were characterized by congestion of blood vessels, hemorrhages, and mononuclear cell infiltration around blood vessels, besides isolated foci of necrosis along with infiltration of heterophils observed at 14 DPI. IP birds showed similar lesions in the liver at 1 DPI, whereas at 3 DPI, aggregates of heterophils were observed in the parenchyma (Figure 3). There were numerous large-sized necrotic foci along with infiltration of heterophils at 7 DPI and 10 DPI (Figure 4).

Large necrosis areas causing severe depletion of the lymphoid tissue along with reticular endothelial cell hyperplasia were noticed in the spleen as early as at 5 DPI in IP (Figure 5). A similar type of lesions but with less intensity was observed in the spleen of OR chicks at 7 DPI onward (Figure 6). The heart showed degeneration of myocardial muscles at 14 DPI and 21 DPI due to infiltrating mononuclear cells, which was mild to moderate in OR (Figure 7) and extensive in IP resulting in atrophy, necrosis, and replacement of the heart muscles (Figure 8). The lungs showed congestion in the interlobular septa and hemorrhages in the parabronchi of both infected groups. The interlobular septa were infiltrated with mononuclear cells mixed with heterophils. The kidneys showed congestion, interstitial hemorrhages, mononuclear cell infiltration in the interstitial tissue along with mild degenerative changes in the tubular epithelium from 3 to 21 DPI in OR. These changes were accompanied by moderate to heavy degenerative changes in the tubular epithelium at 5 and 7 DPI in IP. In the bursa of Fabricius, a mild depletion of lymphoid tissues in the follicles along with infiltration of lymphocytes in interfollicular spaces were noticed from

Table I

Mortality pattern and number of birds sacrificed in the different groups of broiler chickens infected by oral or intraperitoneal routes with *Salmonella* Gallinarum

Days postinfection	CR		OR		IP	
	Died	Sacrificed	Died	Sacrificed	Died	Sacrificed
0	0	_	0	_	0	_
1	0	_	0	_	2	_
2	0	_	0	_	3	_
3	0	3	1	2	2	1
4	0	_	1	_	4	_
5	0	3	1	2	1	2
6	0	_	1	_	3	_
7	0	3	2	1	2	1
8	0	_	0	_	1	_
9	0	_	1	_	0	_
10	0	_	0	_	1	_
11	0	_	1	_	0	_
12	0	_	0	_	0	_
13	0	_	0	_	0	_
14	0	3	1	2	0	3
21	0	3	0	3	0	3
28	0	3	0	3	0	3
35	0	3	0	3	0	3
42	0	3	0	3	0	3
Total	0	24	9	19	19	19
Mortality (%)	0		22.50		47.50	

CR: control group; OR: chicks inoculated orally with *Salmonella* Gallinarum; IP: chicks infected intraperitoneally with *S*. Gallinarum

Table II

Number of birds showing gross and histopathological lesions of different intensities in various organs in broiler chickens infected by oral or intraperitoneal routes with *Salmonella* Gallinarum during the entire experiment[1]

	Num. of birds showing lesions of various intensities						Mean lesion score	
	OR[2] (n = 19 + 9 = 28)[4]			IP[3] (n = 19 + 19 = 38)			OR (n = 19 + 9 = 28)	IP (n = 19 + 19 = 38)
Intensity score[5]	**1**	**2**	**3**	**1**	**2**	**3**		
Gross lesion score								
Liver	10	4	1	17	10	3	0.75 ± 0.160[a]	1.21 ± 0.142[b]
Spleen	7	4	-	14	7	1	0.54 ± 0.140[a]	0.82 ± 0.135[a]
Heart	7	1	-	8	4	2	0.32 ± 0.103[a]	0.57 ± 0.144[a]
Kidneys	7	1	-	11	3	-	0.32 ± 0.103[a]	0.45 ± 0.116[a]
Lungs	6	-	-	9	-	-	0.21 ± 0.078[a]	0.23 ± 0.069[a]
Bursa	5	-	-	8	-	-	0.17 ± 0.073[a]	0.21 ± 0.067[a]
Intestines	11	2	-	10	-	-	0.54 ± 0.120[a]	0.26 ± 0.072[b]
Histopathological score								
Liver	17	9	2	14	18	6	1.48 ± 0.120[a]	2.12 ± 0.114[b]
Spleen	12	9	-	18	12	3	1.07 ± 0.145[a]	1.34 ± 0.134[b]
Heart	4	5	1	9	8	6	0.60 ± 0.173[a]	1.13 ± 0.181[b]
Kidneys	5	6	-	9	8	1	0.60 ± 0.157[a]	0.73 ± 0.153[a]
Lungs	11	-	-	17	-	-	0.39 ± 0.093[a]	0.44 ± 0.081[a]
Bursa	9	-	-	12	1	-	0.32 ± 0.089[a]	0.37 ± 0.087[a]
Intestines	11	3	2	12	2	-	0.89 ± 0.171[a]	0.42 ± 0.097[a]

[1] From day 0 of infection (i.e. at 7 days of age) until 42 days postinfection
[2] OR: chicks orally inoculated with *Salmonella* Gallinarum
[3] IP: chicks intraperitoneally infected with *Salmonella* Gallinarum
[4] Data in parentheses indicate the number of birds in the group (sacrificed + dead, respectively).
[5] 1 = mild; 2 = moderate; 3 = severe
[a,b] Means on the same row with different superscripts differ significantly ($p < 0.05$).
Note: no lesions were observed in any of the organs of the control birds throughout the experiment.

14 DPI onward in OR. In IP, in addition to depletion of lymphocytes, atrophy of bursal follicles, degenerative changes and slight metaplastic changes (in the epithelium separating the cortex from the medulla) were also evident at the early stage of infection. In OR birds severe catarrhal enteritis was observed, characterized by congestion, marked goblet cell hyperplasia, infiltration of heterophils and mononuclear cells in the lamina propria, mucosa and submucosa, and degeneration and desquamation of the epithelium. These changes were less prominent in IP birds.

Lesion scores varied between the different organs of a group as well as between the two infected groups. Mean gross lesion scores for the liver and mean histopathological scores for the liver, spleen and heart were significantly higher ($p < 0.05$) in IP than in OR birds (Table II). However, mean gross lesion scores and mean histopathological scores for the intestines were significantly higher ($p < 0.05$) in OR than in IP birds (Table II).

SG was isolated from all the cultured samples of dead chickens. No significant differences between the two infected groups in their frequency of isolation from internal organs were observed at any time postinfection. Mean SG isolation in OR was 67% from the liver, 33% from the spleen and the heart blood, 55% from ceca and 44% from fecal samples, whereas in IP it was 78% from the liver, 44% from the spleen, heart and ceca, and 33% from fecal samples (Table III).

Table III

Isolation of *Salmonella* Gallinarum from visceral organs of broiler chickens infected by oral or intraperitoneal routes at various days postinfection

	OR			IP		
	Positive samples			Positive samples		
	7 DPI	21 DPI	35 DPI	7 DPI	21 DPI	35 DPI
Liver	3/3	2/3	1/3	3/3	2/3	2/3
Spleen	2/3	1/3	0/3	3/3	1/3	0/3
Heart blood	2/3	1/3	0/3	2/3	2/3	0/3
Ceca	2/3	1/3	1/3	2/3	1/3	1/3
Feces	2/3	0/3	2/3	2/3	0/3	1/3

OR: chicks orally inoculated with *Salmonella* Gallinarum
IP: chicks intraperitoneally infected with *Salmonella* Gallinarum
DPI: days postinfection
Note: *Salmonella* Gallinarum was not isolated from any organs at any stage from the control group.

Figure 1: *Liver of a bird intraperitoneally infected with* Salmonella *Gallinarum showing areas of necrosis at 10 days postinfection.*

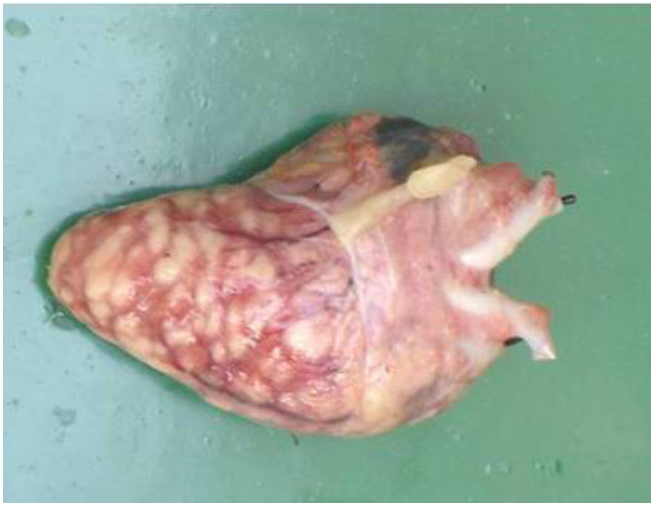

Figure 2: *Heart of a bird intraperitoneally infected with* Salmonella *Gallinarum showing white nodules at 21 days postinfection.*

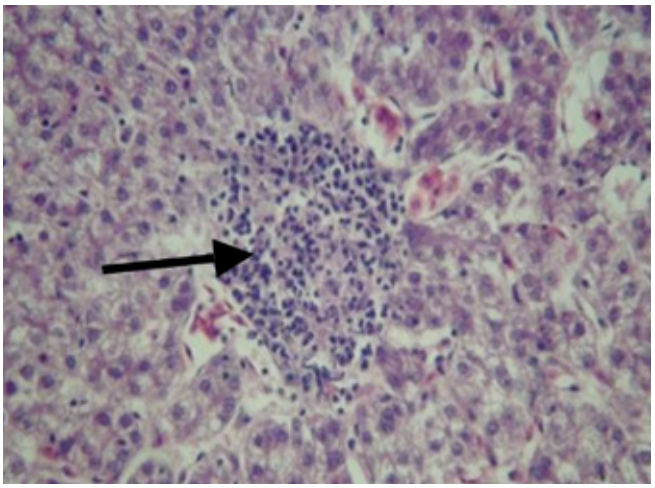

Figure 3: *Liver of a bird intraperitoneally infected with* Salmonella *Gallinarum showing aggregates of heterophils in parenchyma (arrow) at 3 days postinfection. Hematoxylin and eosin (x 1280).*

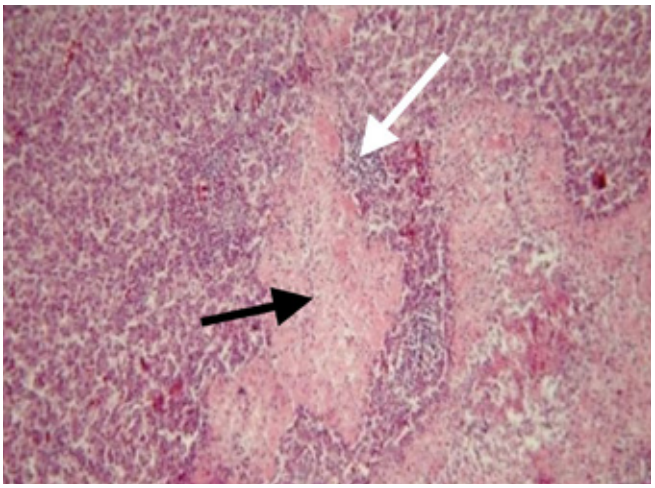

Figure 4: *Liver of a bird intraperitoneally infected with* Salmonella *Gallinarum showing large areas of necrosis (black arrow) surrounded by heterophilic infiltration (white arrow) at 7 days postinfection. Hematoxylin and eosin (x 960).*

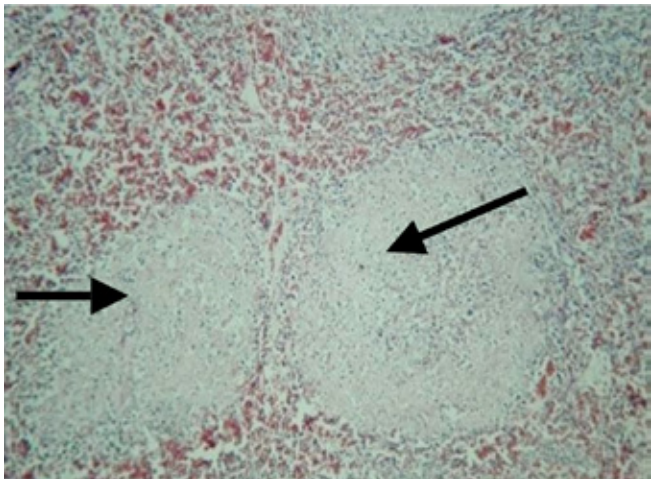

Figure 5: *Spleen of a bird intraperitoneally infected with* Salmonella *Gallinarum showing severe necrosis (arrow) and congestion along with marked depletion of lymphocytes at 5 days postinfection. Hematoxylin and eosin (x 960).*

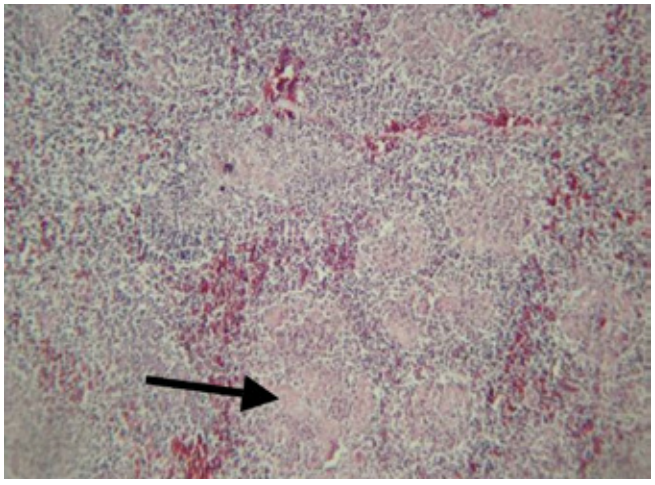

Figure 6: *Spleen of a bird orally infected with* Salmonella *Gallinarum showing depletion of lymphocytes along with areas of necrosis (arrow) at 7 days postinfection. Hematoxylin and eosin (x 240).*

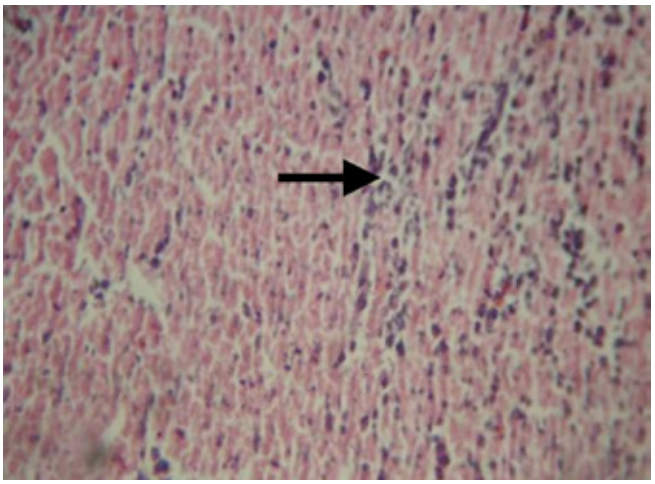

Figure 7: *Heart of a bird orally infected with* Salmonella *Gallinarum showing disruption of myocardial muscles due to infiltrating mononuclear cells (arrow) at 21 days postinfection. Hematoxylin and eosin (x 12,500).*

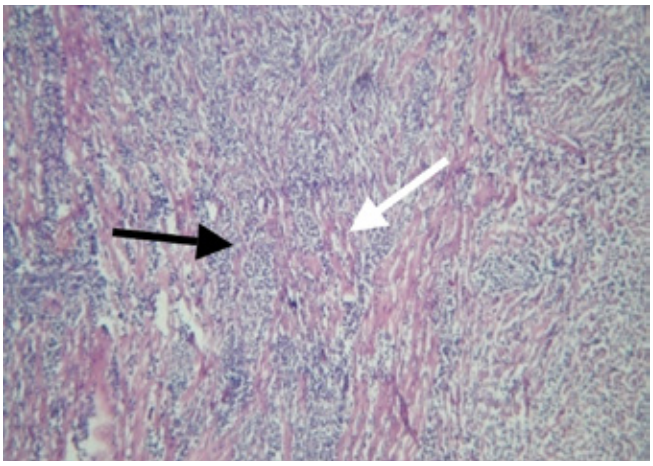

Figure 8: *Heart of a bird intraperitoneally infected with* Salmonella *Gallinarum showing fragmentation (white arrow) and replacement of myocardial fibers with heavy infiltrating mononuclear cells (black arrow) at 21 days postinfection. Hematoxylin and eosin (x 960).*

■ DISCUSSION

In the present study, FT was reproduced experimentally through both oral and intraperitoneal routes by locally isolated *Salmonella* Gallinarum strain to study various pathological alterations and the frequency of bacterial isolation from internal organs. We previously described the clinical signs, mortality and hematobiochemical changes (29). They correlate with the bacterial isolation, and gross and microscopic lesions of the disease.

The most common lesions observed were necrosis, degeneration, hemorrhages and infiltration of leukocytes, in conformity with earlier reports (12, 15, 26). However, the distribution and intensity of these lesions in various organs following the two routes of inoculation differed. The earlier appearance of clinical signs (29) and lesions in IP birds could be used to reduce expense and timing by diminishing the number of studied birds and hours of experimentation.

Gross and microscopic lesion scores suggested that the liver and spleen were the primary target organs involved in SG infection, irrespective of the route of inoculation. These observations are similar to those from Al-Shabibi (2) on *Salmonella* Typhimurium infection. Severe catarrhal enteritis, which was more prominent in orally infected birds, has also been reported by Prasanna et al. (33). The sloughing of superficial layers of villi revealed the damage to the integrity of the intestinal epithelium, resulting in the translocation of bacteria to other tissues in OR birds. Colonization of visceral organs including the liver and spleen occurs when *Salmonella* is not cleared by the host immune system, resulting in systemic infection (1). The lesions in the liver, spleen, heart, kidneys, bursa, intestines and lungs revealed the invasive potential of the *Salmonella* Gallinarum strain used and its pathogenicity.

In general, pathological changes were of less intensity in OR birds than in IP birds, as suggested by the fact that mean gross lesion scores for the liver, and mean histopathological scores for the liver, spleen and heart were significantly higher in IP birds than in OR birds. This could be because only a small proportion of the bacteria in OR (compared to IP) were able to reach visceral organs due to the antagonistic effects of low gastric pH (37) and inhibitory effects of the normal intestinal flora (8). As reported by Christensen et al. (7), viable counts of approximately 10^4 colony-forming units of *Salmonella* Gallinarum in the spleen and liver are necessary for the development of significant pathological and hematological changes. Environmental conditions (including pH, temperature and growth in chicken tissues) can also affect the expression of *Salmonella* Gallinarum virulence factors such as flagella and fimbriae, outer membrane proteins and iron uptake systems (39). The presence of B and T lymphocytes in the upper gastrointestinal tract (25, 38) and anti-*Salmonella* IgA in the crops of birds have also been reported to counter oral infection (18). These conditions make difficult the experimental reproduction of FT through the oral route. The intraperitoneal route of infection could be an alternative to overcome these difficulties in experimental trials where the oral route of infection is not essential.

Isolation of SG in the liver and spleen of dead chicks suggested that death originated from FT. No bacteria were isolated from the birds of the control group. A large degree of similarity between OR and IP birds was also observed in the frequency of isolation of samples from liver, spleen, heart blood, cecum and feces in the present study. Most of these samples were positive at one week postinfection but only a small percentage were still positive at three weeks postinfection. This observation concurs with that of Wigley et al. (41). The ability of *Salmonella* Gallinarum strain to invade the liver and spleen, although indicative of a systemic infection, has not always correlated with the frequency of fecal shedding of the pathogen. The decrease in the rate of fecal shedding of the bacterium after one week postinfection in both groups agrees with earlier findings (13, 19, 20). Ishola (20) reports that the rate of fecal shedding decreases from one to four weeks postinfection with *S. enteritidis*. The decline in the rate of fecal shedding or re-isolation from visceral organs indicates a reduction in the level of systemic infection in birds, probably through a humoral and cell mediated immune response (16, 27). Both responses peak at three to four weeks postinfection, a point that coincides with bacterial clearance (41). The percentage increase of birds shedding the organism at week 5 postinfection in both infected groups could be due to a gradual reduction in the immune response. Oral challenge at relatively low doses, as it is likely to occur in broiler chickens under natural outbreaks, may not cause systemic infection but rather intestinal carriage which is more persistent (11). The presence of more than 50% birds as silent carriers in orally infected birds in the present study indicates that the majority of birds may act as carriers for other birds.

■ CONCLUSION

From the present study, it can be deduced that the intraperitoneal route can be considered as one of the alternative cost-effective methods for inducing *Salmonella* Gallinarum infection in experimental trials of novel drugs, feed additives, etc., as the induction of FT in birds using that route revealed similar clinical signs and pathological lesions (although more severe) as those observed with the oral route.

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Résumé

Nazir S., Kamil S.A., Riyaz A., Mir M.S., Darzi M.M., Yasine A., Goudar K.S. Pathologie et colonisation des organes internes après infection expérimentale des poulets de chair par *Salmonella* Gallinarum par voies orale ou intrapéritonéale

Cet article décrit les changements pathologiques et la fréquence d'isolement de *Salmonella enterica* subsp. *enterica* serovar Gallinarum (O : 9, 12) des organes internes des poussins de chair infectés expérimentalement par voie orale ou intrapéritonéale. L'expérience a été menée sur 110 poussins, âgés d'une semaine et divisés en trois groupes : le groupe CR (30 poussins), non infecté, a servi de groupe témoin, le groupe OR (40 poussins) a été inoculé par voie orale (10^9 organismes/ml), et le groupe IP (40 poussins) a été infecté par voie intrapéritonéale (10^9 organisms/ml). Trois poussins de chaque groupe (morts ou sacrifiés) ont été examinés 3, 5, 7, 14, 21, 28, 35 et 42 jours après l'infection afin d'évaluer l'importance des lésions macroscopiques et histopathologiques, et de déterminer la fréquence d'isolement de *Salmonella* Gallinarum dans les organes internes. Les différences entre les groupes infectés ont été évaluées par la comparaison des scores moyens des lésions macroscopiques et histopathologiques. Bien que similaires dans les groupes OR et IP, les lésions des organes viscéraux ont été plus sévères, plus précoces et présentes chez un plus grand nombre de volailles dans le groupe IP. Cependant, il n'y a pas eu de différence significative entre les deux groupes infectés en termes de fréquence d'isolement de *Salmonella* Gallinarum dans les organes internes ni dans les fientes. Il a été conclu que la voie intrapéritonéale devrait être privilégiée pour induire une infection à *Salmonella* Gallinarum lors d'essais expérimentaux.

Mots-clés : Poulet de chair – Poussin – *Salmonella enterica* serovar Gallinarum – Typhoïde – Infection expérimentale – Inde.

Resumen

Nazir S., Kamil S.A., Riyaz A., Mir M.S., Darzi M.M., Yasine A., Goudar K.S. Patología y colonización de órganos internos después de una infección experimental en pollos de engorde con *Salmonella* Gallinarum por vías oral e intraperitoneal

El presente artículo describe los cambios patológicos y la frecuencia de aislamiento de *Salmonella enterica* subsp. *enterica* serovar Gallinarum (O: 9, 12) de órganos internos en pollos de engorde, infectados en forma experimental por vías oral e intraperitoneal. El experimento fue llevado a cabo en 110 pollos de engorde de 1 semana de edad, divididos en tres grupos: grupo CR (30 pollos) mantenido sin infección y sirvió de control, grupo OR (40 pollos) inoculados oralmente con *Salmonella* Gallinarum (10^9 organismos/ml) y grupo IP (40 pollos) infectados intraperitonealmente con *Salmonella* Gallinarum (10^9 organismos/ml). Tres aves de cada grupo (muertas y/o sacrificadas) fueron observadas al día 3, 5, 7, 14, 21, 28, 35 y 42 post infección, para evaluar cambios macro e histopatológicos en los órganos viscerales y asesorar la frecuencia del aislamiento de *Salmonella* Gallinarum de órganos internos. Los cambios macro e histopatológicos fueron comparados entre los grupos infectados mediante medidas de graduación promedio de las lesiones. Los cambios macro e histopatológicos en los órganos viscerales, a pesar de ser similares en ambos grupos infectados, fueron más severos y observados a un estadio más temprano de infección y en más aves en el grupo IP. Sin embargo no hubo diferencia significativa entre los dos grupos infectados en cuanto a la frecuencia del aislamiento de *Salmonella* Gallinarum de los órganos internos, incluyendo en efusiones fecales. Por lo tanto se concluye que la ruta intraperitoneal debe ser considerada ante todo para inducir la infección por *Salmonella* Gallinarum en estudios experimentales.

Palabras clave: Pollo de engorde – Pollito – *Salmonella enterica* serovar Gallinarum – Tifoidea – Infección experimental – India.

Occurrence of bovine tuberculosis at Nyala abattoirs in South Darfur State, Sudan

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Keywords

Cattle – *Mycobacterium tuberculosis* infection – Slaughterhouse – Sudan.

Summary

The aim of this study was to determine the occurrence of tuberculosis in cattle slaughtered at Nyala abattoirs, South Darfur State, Sudan during the period April 2006 – May 2008. In total 2794 cattle were examined for tuberculous lesions. Tuberculous lesions (n = 163) were found in 40 (1.4%) animals, among which seven had generalized tuberculosis and 33 localized tuberculosis, mainly in the lungs, thoracic lymph nodes, and/or in the liver, spleen, kidneys and mesenteric lymph nodes. Tissue samples were collected either in 10% formal saline for routine histopathology or in ice packs for direct microscopy and culturing. Direct microscopy showed that 124 (76.1%) tuberculous lesions harbored acid fast bacilli, whereas 17 (10.4%) isolates of *Mycobacterium* spp. were recovered in pure Lowenstein-Jensen medium cultures and identified as *M. bovis* (n = 11) and *M. farcinogenes* (n = 6). Granulomatous inflammation was evident in all sections of tuberculous lesions. Further studies are needed to identify mycobacteria species causing tuberculosis in other animal species.

■ INTRODUCTION

Bovine tuberculosis (BTB) is a chronic zoonotic bacterial disease characterized by progressive development of specific granulomatous tubercles in affected tissues and organs, more significantly in bronchial, mediastinal, retropharyngeal and portal lymph nodes. In addition, the lungs, liver, spleen and surface of body cavities are commonly affected (13, 15). Bovine tuberculosis is caused by slow growing non-photochromogenic members of the *Mycobacterium tuberculosis* complex: *M. bovis*, *M. caprae*, *M. microti*, *M. africanum*, *M. canettii* and *M. pinnipedii*. However, *M. bovis* is the most universal pathogen among mycobacteria and affects many domestic and wild animals. Cattle, goats and pigs are most susceptible, whereas sheep and horses have a high natural resistance (19, 24). Bovine tuberculosis is widely distributed throughout the world and causes serious economic losses in animal production (6). In cattle, inhalation of *M. bovis* is considered to be the most frequent route of infection but ingestion of contaminated material can also cause infection (3). Tuberculous lesions usually have a yellowish appearance and are caseous, caseo-calcareous, or calcified in consistency. A tubercle is described as a granulomatous lesion, characteristically composed of a caseous or necrotic center bordered by a zone of epithelioid cells, some of which may form multinucleated giant cells, accumulation of lymphocytes, a few granulocytes, and encapsulation of fibrous connective tissue of varying thickness (20, 25, 16). Tuberculosis is detected in live cattle by tuberculin test, in addition to other tests such as the cellular test based on the quantification of gamma interferon. At postmortem examination it

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is diagnosed and confirmed by bacteriological, histopathological and molecular methods (7, 9, 10, 14, 17).

In Sudan, studies conducted in the 1960's and 1970's indicated that the incidence was high in the humid southern part of the country where the animals are in close contact, and low in the dry zones where the nomadic cattle of Western provinces, the seminomadic cattle of Eastern and Central Sudan are reared (11, 23). Sulieman and Hamid (22) found that 64 (53.3%) of 120 caseous lesions from cattle in Eastern and Central Sudan were due to acid-fast bacilli (AFB), whereas 56 (46.7%) were due to other causes. Growth on Lowenstein-Jensen slants was obtained in 52 of the 120 samples and identified as follows: 25 (48.1%) *Mycobacterium bovis*, 21 (40.4%) *M. farcinogenes*, 4 (7.7%) *M. tuberculosis*, 1 (1. 9%) *M. avium*, and 1 (1.9%) *Nocardia* sp.

In South Darfur State, El Tigani-Asil et al. (8) report a bovine tuberculosis prevalence of 0.18% in slaughtered cattle. The true epidemiological picture of the disease in different parts of Sudan is still unclear. Determination of the prevalence of the disease in various regions of the country will help establish control and prevention policies. This study aimed to detect bovine tuberculosis and identify the causative agents among cattle slaughtered at Nyala abattoirs.

■ MATERIALS AND METHODS

The study was conducted in Nyala abattoirs, South Darfur State, Sudan, from April 2006 to May 2008. Cattle (n = 2794) slaughtered at Nyala abattoirs were examined by visual and physical inspection of external and internal organs, with special consideration to systemic portal lymph nodes for presence of caseous nodules. Tuberculous tissue samples (n = 163) were collected from infected animals (n = 40) either in 10% neutral buffered formalin for routine histopathology or in sterile plastic containers which were kept in ice packs and carried to Nyala Veterinary Research Laboratory within two hours for direct microscopy and culturing. Ten percent neutral formal saline fixed tissues were processed and embedded in paraffin wax; 5 μm-thick sections were stained with hematoxylin and eosin for histopathological examination (2). Direct smears were prepared from caseous tissue material and Ziehl-Neelsen stained for acid fast bacilli (18).

A portion of each sample was homogenized with 5 ml of sterile normal saline using a sterile mortar and pestle. Then 2 ml of the homogenate was transferred into sterile centrifuge tubes, decontaminated by adding an equal volume of sterile 4% NaOH (2 ml) for 10 min, and centrifuged at 3000 rpm for 15 min. After centrifugation, to recover sediment, the supernatant was rejected and the sediment neutralized with 1% HCl, with phenol red as indicator. Neutralization was achieved when the color of the solution changed from purple to yellow (5). The sediment from some samples was inoculated onto a set of Lowenstein-Jensen (LJ) medium slants supplemented with 4% sodium pyruvate (LJ pyruvate) and the rest was enriched with glycerol (standard LJ). Cultures were incubated at 37ºC for up to 12 weeks. Mycobacteria growth was evidenced by phenotypic characteristics of pure visible colonies and confirmed by Ziehl-Neelsen stained films from the cultures (18).

Identification of *M. tuberculosis* complex was carried out according to growth rate, colony morphology, niacin production, pyrazinamide deamination, nitrate reduction, urease production, 5% NaCl tolerance, inhibition by thiophen-2-carbonic acid hydrazide (TCH) 10 mg/ml, and catalase activity and its thermolability to 68ºC (18, 12).

■ RESULTS

Yellowish caseous necrotic lesions of various sizes enclosed in hard white to light grey fibrous tissue (Figure 1) were observed in infected carcasses (n = 40). Seven (17.5%) had generalized tuberculosis, whereas 33 (82.5%) had localized tuberculosis mainly in the lungs and thoracic lymph nodes, and/or liver, spleen and mesenteric lymph nodes.

Gross examination revealed typical tuberculosis lesions with caseous or mineral material in various organs (Figure 1). Histopathology revealed necrotic centers bordered by zones of epithelioid cells, accumulation of lymphocytes, granulocytes, some of which forming multinucleated giant cells. Lesions were encapsulated by fibrous tissue of varying thickness (Figure 2). In some sections there were central necrotic areas with some mineralization surrounded by epithelioid cells and lymphocytes encapsulated by a thick zone of fibrous tissue (Figure 2). Microscopy evidenced that 124 (76.1%) smears harbored acid fast bacteria, whereas 39 (23.9%) were negative (Figure 2).

Seventeen (10.4%) samples grew on pyruvate LJ medium during three-month incubation, whereas 146 (89.6%) failed to grow or promoted contaminant growth (Table I). Eleven isolates (64.7%) were identified as *M. bovis* and six (35.3) as *M. farcinogenes*.

Table I

Direct microscopy and isolation of *Mycobacterium* sp.

Organ/tissue	Number (%)	Direct microscopy		Isolation	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
Lungs	30 (18.4)	24 (80.0)	6 (20.0)	7 (23.3)	23 (76.7)
Liver	17 (10.4)	12 (70.6)	5 (29.4)	2 (11.8)	15 (88.2)
Spleen	9 (5.5)	8 (88.9)	1 (11.1)	2 (22.2)	7 (77.8)
Kidneys	5 (3.0)	3 (60.0)	2 (40.0)	1 (20.0)	4 (80.0)
Pleura	6 (3.7)	4 (66.7)	2 (33.3)	0 (0.0)	6 (100.0)
Peritoneum	4 (2.5)	3 (75.0)	1 (25.0)	0 (0.0)	4 (100.0)
Lymph nodes	92 (56.4)	70 (76.1)	22 (23.9)	5 (5.4)	87 (94.6)
Total	163	124 (76.1)	39 (23.9)	17 (10.4)	146 (89.6)

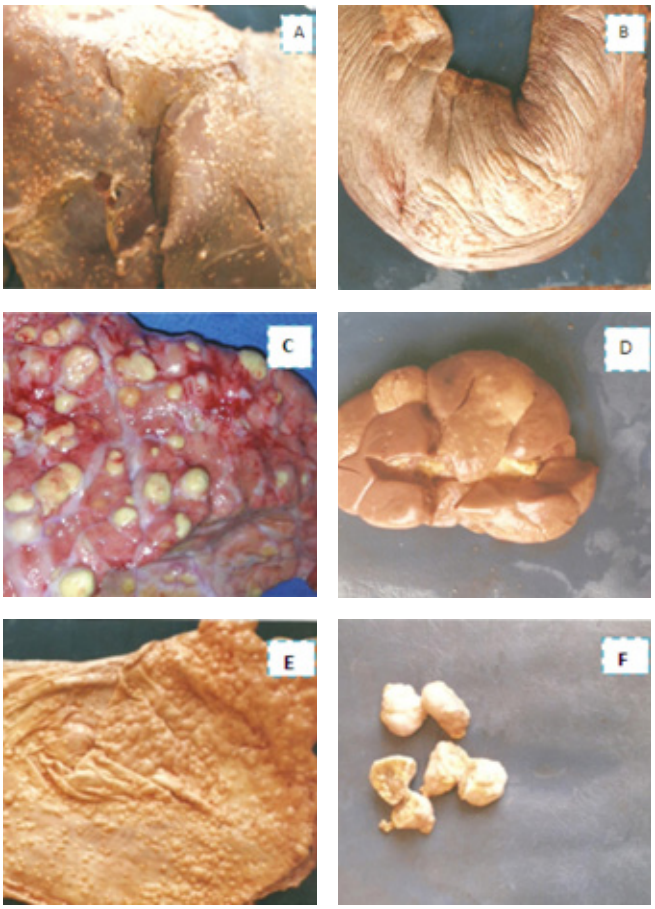

Figure 1: *Liver (A), spleen (B), lung(C), kidney (D), peritoneum (E) and bronchial lymph node (F) showing tubercles with caseous necrotic areas and calcifications.*

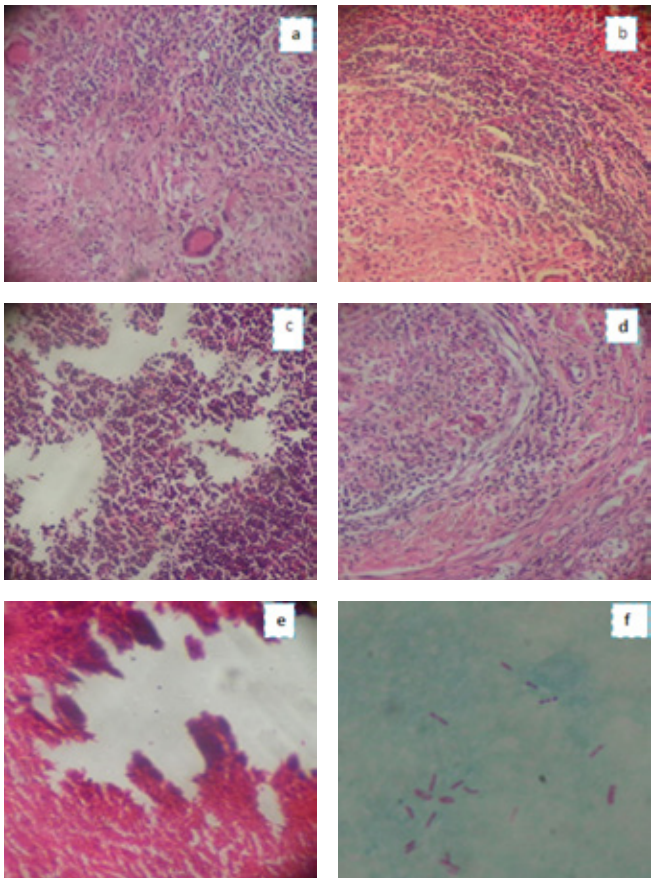

Figure 2: *Lung (a), spleen (b), bronchial lymph node (c), and kidney (d) showing a necrotic center surrounded by epithelioid cells, lymphocytes, multinucleated giant cells (a and b); liver (e) showing a central necrotic areas with mineralization surrounded by fibrous connective tissues. Hematoxylin and eosin stains (x 40 magnification). Ziehl-Neelsen's stained film (f) showing acid fast bacilli (100 x oil immersion objective).*

■ DISCUSSION

Eradication programs and control of BTB based on test and slaughter of tuberculin positive reactors have been adopted in some countries. Application of these programs in developed countries has eradicated or drastically reduced the infection rate in farm animals (1). In Sudan, especially in South Darfur, control of the disease through the test-and-slaughter policy has not been adopted yet because of the lack of knowledge on the actual prevalence of the disease, the absence of cattle identification and control of animal movements, and prevailing technical and financial limitations. The control of bovine tuberculosis is only based on the detection of gross lesions in abattoirs and subsequently partial or total condemnation of carcasses. The incidence of tuberculosis in this study was low compared to previous reports (22), and high to some extent compared to results obtained by El Tigani-Asil et al. (8). BTB prevalence might be underestimated in tuberculous cattle because of undetected lesions in early infection or because small lesions might be missed as a result of difficulties in carrying out inspection without pressure.

In the 40 carcasses with tuberculous lesions, localized tuberculosis was higher than generalized tuberculosis in the infected cattle which may indicate that cattle in the region were infected by aerosol and/or ingestion of contaminated material (3). Histopathological examinations of lesions showed typical granulomas characterized by central necrosis surrounded by multinucleated, Langhans, epithelioid and lymphocyte cells. This is consistent with findings by Whipple et al. (25) who observed typical granulomatous lesions in tissues with evident gross lesions. These granulomas were characterized by a central necrotic area and focal mineralization (20). Microscopic examination evidenced a high number of AFB (76.1%) and confirmed that microscopy is essential to establish BTB diagnosis especially in developing countries, echoing OIE which states that microscopic examination provides presumptive confirmation (15). Our finding is not consistent with that of Sahraoui et al. (21) who only found 28.85% positive smears.

In the present study (n = 163) a low culture positive rate (10.4%) was recorded. Sahraoui et al. (21) reported 51.6% positive cultures. It might be caused by the absence of viable mycobacteria in calcified lesions or the toxic adverse effect of decontaminants which kill the organism during long incubation periods. It has been established that in completely calcified lesions, tuberculous bacilli are usually dead and do not grow on LJ media (18). Furthermore, mycobacteria cannot be isolated from healed lesions (4). The isolation of mycobacteria on selective culture media and their subsequent identification by cultural and biochemical tests or DNA techniques such as PCR are needed to confirm the infection in different animal species.

■ CONCLUSION

To the best of our knowledge, tuberculosis research in Sudan, especially in conflict areas, is scarce. It is however essential to

improve animal health, increase productivity and control this zoonotic disease. Furthermore, comparative tuberculosis studies are also crucial to map the disease and define national or international control policies.

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Résumé

Aljameel M.A., Abdel Wahab M.B., Fayza A.O., El Tigani A.E., Abdellatif M.M. Incidence de la tuberculose bovine dans les abattoirs de Nyala dans l'Etat du Darfour du Sud au Soudan

L'objectif de l'étude a été de déterminer l'incidence de la tuberculose chez les bovins abattus dans les abattoirs de Nyala, Etat du Darfour du Sud au Soudan, pendant la période d'avril 2006 à mai 2008. Au total, 2 794 bovins ont été examinés pour la présence de lésions tuberculeuses. Ces dernières (n = 163) ont été retrouvées dans 40 (1,4 p. 100) animaux, sept ayant présenté une tuberculose généralisée et 33 une tuberculose localisée, principalement dans les poumons, les ganglions lymphatiques thoraciques, et/ou dans le foie, la rate, les reins et les ganglions lymphatiques mésentériques. Des échantillons tissulaires ont été placés soit dans une solution de formol à 10 p. 100 pour un examen histopathologique de routine, soit dans de la glace pour un examen en microscopie directe et une mise en culture. La microscopie directe a montré que 124 (76,1 p. 100) lésions tuberculeuses contenaient des bacilles acido-alcoolo-résistants, tandis que 17 (10,4 p. 100) isolats de *Mycobacterium* spp. ont pu être cultivés en milieu de culture de Löwenstein-Jensen pur et identifiés comme étant *M. bovis* (n = 11) et *M. farcinogenes* (n = 6). Une inflammation granulomateuse a été observée dans toutes les coupes de lésions tuberculeuses. D'autres études seront nécessaires pour identifier les espèces de mycobactéries responsables de la tuberculose chez d'autres espèces animales.

Mots-clés : Bovin – Infection *Mycobacterium tuberculosis* – Abattoirs – Soudan.

Resumen

Aljameel M.A., Abdel Wahab M.B., Fayza A.O., El Tigani A.E., Abdellatif M.M. Ocurrencia de tuberculosis bovina en ganado de sacrificado en mataderos Nyala en el Estado de Darfur del Sur, Sudan

El estudio se llevó a cabo con el fin de detectar tuberculosis en ganado sacrificado en los mataderos de Nyala, estado de Darfur del Sur, Sudan, durante el periodo entre abril 2006 y mayo 2008. Un total de 2794 cabezas fueron inspeccionadas para la observación de lesiones tuberculosas. Las lesiones tuberculosas (n = 163) se encontraron en 40 (1.4%) animales, siete con tuberculosis generalizada y 33 con tuberculosis localizada, principalmente en el pulmón y los linfonodos torácicos y/o hígado, baso, riñones y linfonoos mesentéricos. Se colectaron muestras de tejido, sea en 10% formol salina para histopatología de rutina o en paquetes de hielo para microscopia directa y cultivo. La microscopia directa mostró que 124 (76,1%) de las lesiones tuberculosas albergaron bacilos ácidos-alcohol resistentes, mientras que 17 (10,4%) *Mycobacterium* spp. fueron aislados en cultivos en medio de Lowenstein-Jensen puro e identificados como *M. bovis* y *M. farcinogenes* (n = 6). Inflamación granulomatosa fue evidente en todas las secciones de las lesiones tuberculosas. Más estudios son necesarios para identificar las especies de micobacterias causantes de tuberculosis en otras especies.

Palabras clave: Ganado bovino – Infección *Mycobacterium tuberculosis* – Matadero – Sudán.

Prévalence de la brucellose bovine et comportements à risque associés à cette zoonose dans la zone périurbaine de Dakar au Sénégal

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Mots-clés

Bovin laitier – Brucellose – Morbidité – Zoonose – Agriculture périurbaine – Sénégal.

Résumé

L'objectif de cette étude a été d'évaluer la prévalence et la fréquence des comportements à risque de transmission zoonotique de la brucellose bovine dans la zone périurbaine de Dakar. Le statut sérologique individuel de 300 bovins répartis dans 30 élevages de cette zone a été déterminé par les tests au rose Bengale et de fixation du complément. La fréquence des comportements à risque envers cette zoonose a été déterminée à travers deux questionnaires épidémiologiques qui ont permis de faire le recensement de facteurs connus de risque de transmission de la brucellose entre animaux et humains. En considérant la sensibilité et la spécificité des tests au rose Bengale et de fixation du complément utilisés en série, respectivement de 85 et 98,75 p. 100, la prévalence réelle a été évaluée à 36,36 p. 100. Au moins un animal a été infecté dans 96,6 p. 100 des troupeaux. La positivité au test de fixation du complément a été significativement associée à l'âge, la race, l'avortement et la présence d'hygromas chez les bovins. Les comportements à risque les plus fréquemment observés chez les humains dans cette zone ont été l'assistance aux mises bas et aux avortements, la manipulation de l'avorton sans gant, la consommation de lait cru ou de lait caillé non pasteurisés, et de fromage frais. Ces résultats montrent que la brucellose existe dans les élevages bovins laitiers en périphérie de Dakar. Etant donné que le lait produit par ces élevages sert à ravitailler la ville de Dakar, des mesures adéquates doivent être prises afin d'orienter les moyens de prévention contre la brucellose chez les habitants.

■ INTRODUCTION

Les zones périurbaines des villes africaines ont subi des transformations structurelles de leur agriculture du fait d'une urbanisation rapide provoquant une forte demande en produits d'origine animale (16, 18). Pour répondre à cette demande sans cesse croissante, les acteurs de la filière laitière, en l'absence de politique clairement définie, ont adopté de nouvelles stratégies et des innovations techniques (3). Dans cette dynamique, on observe une intensification des systèmes d'élevage, un développement du secteur laitier informel et des changements des modes de production (4) et de consommation (6, 8, 17). Certaines de ces évolutions aboutissent à mettre sur le marché des produits de qualités très diverses, échappant pour la plupart au contrôle des services publics (8). Ainsi, le lait produit dans les élevages bovins laitiers en périphérie de Dakar sert à ravitailler la ville en lait cru et produits laitiers non pasteurisés.

Cependant, la brucellose bovine est une zoonose majeure qui peut avoir un impact important sur la santé publique, la transmission se faisant généralement par la consommation de lait cru contaminé (10, 11, 13). La brucellose est l'infection zoonotique

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la plus fréquente au monde, avec chaque année plus de 500 000 nouveaux cas déclarés (10). Elle est présente à travers le monde avec une prédominance dans le Bassin méditerranéen, l'ouest de l'Asie, le Moyen-Orient, l'Amérique du Sud, l'Amérique centrale et l'Afrique subsaharienne (10). Au Kirghizistan, la brucellose est une priorité en santé publique car l'incidence annuelle est supérieure à 50 cas pour 100 000 habitants avec une séroprévalence de 8,8 p. 100 chez les humains et de 2,8 p. 100 chez les bovins (9). Elle a affecté 1 014 personnes en Bosnie-Herzégovine en 2008 et 458 (cas officiellement déclarés) en 2009 (10).

L'élevage occupe une place importante pour les populations africaines (19). Le développement de cet élevage est cependant sous la contrainte de nombreux facteurs dont les contraintes pathologiques (27). Outre leur impact sur la santé des animaux, certaines de ces pathologies peuvent aussi causer des problèmes de santé publique : c'est le cas de la brucellose (27). Ainsi, en Afrique, la brucellose bovine a été rencontrée partout où elle a été recherchée. Dans la zone périurbaine d'Abéché au Tchad, la prévalence de cette pathologie a été évaluée à 2,6 p. 100 et la prévalence cheptel a été estimée à 20 p. 100 (15). Chez les humains au Tchad elle est de 2 p. 100 (28). La prévalence de la brucellose humaine est de 2,6 p. 100 en Ethiopie (2), 3 p. 100 en Egypte (1) et 6,2 p. 100 en Tanzanie (23). La prévalence de la brucellose bovine dans le centre de la Côte d'Ivoire est de 8,8 p. 100 (27). Des études sur le lait de bovin au Mali par Bonfoh et coll. (7) indiquent son importance avec 53 p. 100 des fermes infectées et une prévalence de 15 p. 100 chez les bovins. Chez les humains à Mopti au Mali, la séroprévalence de la brucellose est de 58 p. 100 (13). Dans environ 30 p. 100 des échantillons de lait de vache en zone rurale et périurbaine au Mali, il y a présence d'anticorps anti-*Brucella* (5).

Au Sénégal, très peu de données existent (10). Néanmoins, la brucellose est incriminée dans de nombreux cas d'avortement chez des vaches après l'insémination artificielle (20). Dans ce contexte, une étude de la brucellose dans les élevages bovins laitiers en périphérie de Dakar paraît nécessaire. L'hypothèse énoncée ici est que la brucellose est présente dans les élevages bovins laitiers en périphérie de Dakar au Sénégal. La consommation de lait cru et de produits laitiers non pasteurisés provenant de ces élevages n'est donc pas sans conséquence sur la santé de la population. L'objectif de cette étude a été d'évaluer la prévalence et la fréquence des comportements à risque de la brucellose bovine dans la zone périurbaine de Dakar. De façon spécifique, il s'agissait de déterminer la séroprévalence individuelle des bovins de cette zone, d'estimer la corrélation intratroupeaux, de calculer la prévalence réelle à partir des sensibilités et spécificités des tests utilisés, d'identifier quelques facteurs de risque chez les bovins et de fournir la fréquence des comportements à risque observés chez les humains dans cette région.

■ MATERIEL ET METHODES

Zone de l'enquête

L'étude a été réalisée entre le 2 janvier et le 2 juin 2012 dans la région de Dakar, dans un rayon de trente kilomètres correspondant approximativement au bassin laitier de la région de Dakar. Les élevages laitiers périurbains de Dakar utilisent parfois des races exotiques pures et pratiquent aussi l'insémination artificielle afin d'améliorer génétiquement le cheptel autochtone et d'intensifier la production laitière locale. La population bovine sédentaire dans cette région a été évaluée en 2010 à 21 270 têtes (29). La distribution spatiale des élevages enquêtés a été faite à partir des coordonnées géographiques de chaque élevage enquêté, obtenues avec le logiciel GPS Garmin®.

Population étudiée et méthode d'échantillonnage

La population étudiée était constituée, d'une part, des troupeaux de bovins de plus de dix têtes (prélèvements sanguins) et, d'autre part, des personnes en contact direct avec ces troupeaux situés en périphérie de Dakar. Les critères retenus pour participer à l'étude ont été limités aux bovins de plus d'un an. L'accent a été mis sur les femelles en raison du faible impact des mâles dans l'épidémiologie de la brucellose. Toutefois, quelques mâles, surtout les reproducteurs, ont été retenus. La méthode d'échantillonnage aléatoire à deux degrés a été utilisée. Le premier degré a porté sur le tirage aléatoire d'élevages dans la zone périurbaine de Dakar. Ne disposant pas de listes exhaustives des unités successives d'échantillonnage, une enquête préliminaire a été menée. Cette enquête a permis de recenser 58 élevages dont 36 répondaient aux critères d'inclusion. Parmi les 36 élevages, 30 ont été tirés au hasard. Le deuxième degré a porté sur le tirage aléatoire de 10 bovins au sein de chaque élevage sélectionné, soit 300 bovins au total. Dans chaque élevage, deux visites ont été effectuées : la première pour la sensibilisation et le consentement écrit de chaque éleveur pour les deux études (animaux et humains), et la seconde pour les prélèvements sanguins sur les animaux.

Recueil des données

Deux questionnaires épidémiologiques, l'un pour les humains et l'autre pour les animaux, comportant chacun principalement des questions de type fermé, ont été élaborés afin d'établir les comportements à risque pour cette zoonose. Les entretiens ont duré en moyenne 20 minutes par personne et se sont déroulés en wolof, en pulaar ou, dans certains cas, en français. Chez les animaux, la situation sanitaire des élevages, l'âge, le sexe, la race, la vaccination contre la brucellose et quelques symptômes connus de la brucellose bovine comme les antécédents d'avortement et la présence d'hygroma (figure 1) ont été relevés. Les questions sur les éleveurs ont porté sur l'ethnie, l'habitat, les pratiques courantes et à risque des éleveurs enquêtés, comme les déplacements saisonniers, le mode d'élevage, la manipulation d'un avorton sans port de gant, l'assistance des vaches gravide lors des mises bas ou des avortements, le mode alimentaire (consommation de lait cru et de produits laitiers non pasteurisés), et la vente et le circuit de cette vente.

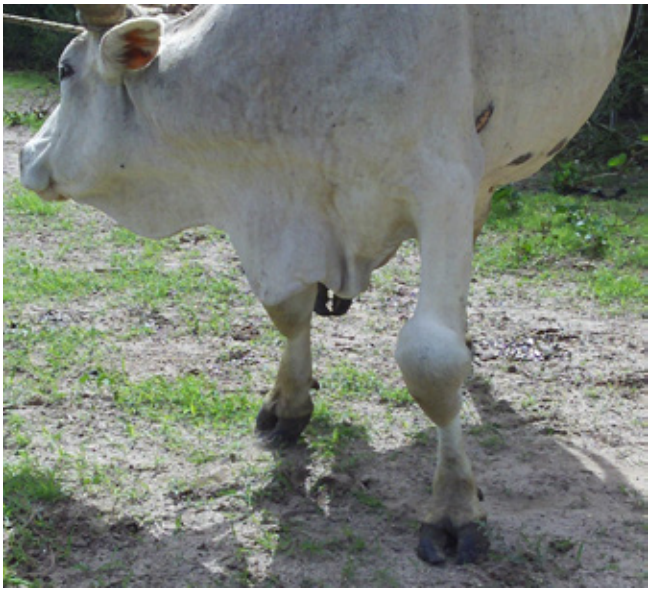

Figure 1 : *bovin présentant un hygroma.*

Méthodes de diagnostic

Chez les animaux, les prélèvements sanguins ont été effectués à la veine jugulaire sur tube sec identifié par le code de l'élevage et le numéro de l'animal. Après rétraction du caillot, les sérums ont été prélevés après centrifugation et mis dans des microtubes à congélation à l'aide de pipettes jetables stériles. Deux tests sérologiques ont été utilisés : le test au rose Bengale et le test de fixation du complément (TFC) selon la technique de Kolmer à froid. Le test au rose Bengale est un test rapide, simple, économique, réputé sensible (90 p. 100) et relativement peu spécifique (75 p. 100) (24). TFC est considéré comme très sensible (≥ 95 p. 100) et très spécifique (≥ 95 p. 100) (15). Il a permis de rechercher les anticorps anti-*Brucella* par microméthode en plaque selon les recommandations de l'Organisation mondiale de la santé animale (OIE). Afin de déceler les faux positifs, une réaction au TFC a été effectuée sur les prélèvements positifs ou douteux au rose Bengale. Elle a été considérée comme positive lorsque le titre du sérum a été supérieur ou égal à un quart (12). La prévalence réelle a été estimée d'après la méthode décrite par Toma et coll. (30) selon la formule :

$P_a = P_{réelle} \times (Se + Sp - 1) + (1 - Sp)$ et ainsi

$P_{réelle} = P_a - (1 - Sp) / (Se + Sp - 1)$

où P_a est la prévalence apparente mesurée dans l'échantillon initial, $P_{réelle}$ la prévalence réelle dans l'échantillon initial, Se la sensibilité et Sp la spécificité.

Les tests au rose Bengale et de FC ont été utilisés en série, ce qui diminue la sensibilité et augmente la spécificité (30). Ainsi, la sensibilité finale a été : Se = Se1 x Se2, soit Se = 85 p. 100 ; et la spécificité finale a été : Sp = 1 – (1 – Sp1) x (1 – Sp2), soit Sp = 98,75 p. 100.

Analyse statistique

Les données ont été saisies avec Epidata® et traitées avec les logiciels Epidata Analysis® et Epi Info®. L'analyse statistique a été réalisée pour prendre en compte simultanément divers facteurs pouvant influer sur la prévalence de la brucellose. Les variables d'intérêts, codées en présence/absence, ont été la positivité au test de diagnostic (fixation du complément). Les variables explicatives ont été des caractéristiques individuelles et collectives. Les facteurs de risque chez les bovins et les comportements à risque chez les humains ont été identifiés grâce un modèle multivarié. Un modèle de régression logistique (proc logistic, SAS 9.3) a été utilisé afin d'analyser la positivité au test de diagnostic en fonction des variables explicatives considérées comme facteur de risque ou comportement à risque. Le seuil de significativité a été fixé à 5 p. 100.

■ RESULTATS

La figure 2 montre la situation géographique des élevages enquêtés.

Le tableau I indique le pourcentage de femelles, l'âge des animaux, les antécédents d'avortement, la présence d'hygroma, et si les bovins étaient de races locales (N'Dama, Gobra, zébu Maure, zébu Peul) ou exotiques. Aucun bovin n'avait été vacciné contre la brucellose.

Le tableau II présente les résultats des diagnostics sérologiques de la brucellose pour les bovins de l'étude. Sur 300 sérums, 75 (25 p. 100) et 35 (11,7 p. 100) ont été respectivement positifs et douteux au rose Bengale. Après l'analyse de ces 110 échantillons au TFC, les 75 échantillons positifs au rose Bengale l'ont

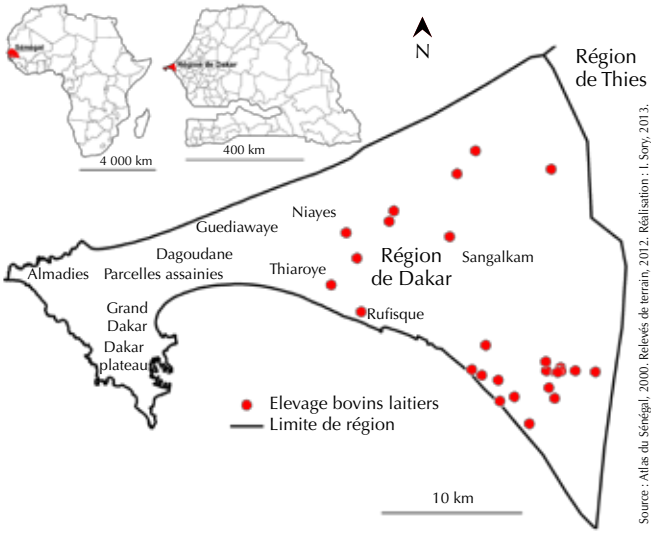

Figure 2 **:** *répartition des élevages bovins enquêtés dans la région de Dakar au Sénégal.*

Tableau I

Variables explicatives de la régression logistique multivariée des bovins prélevé dans la région de Dakar au Sénégal en 2012

Variable	Total (n = 300)	%
Sexe		
Femelle	269	89,7
Classe d'âge		
1 à 4 ans	90	30
5 à 12 ans	210	70
Race		
Exotique	98	32,7
Locale	202	67,3
Vaccin brucellose		
Non vacciné	300	100
Antécédent avortement		
Oui	117	39
Présence d'hygroma		
Oui	73	24,3

Tableau II

Diagnostics sérologiques de la brucellose des trois cent bovins prélevés dans la région de Dakar au Sénégal en 2012

	FC positif	FC négatif	Total
Rose Bengale positif	75 (25%)	0 (0%)	75 (25%)
Rose Bengale douteux	20 (6,7%)	15 (5%)	35 (11,7%)
Rose Bengale négatif	0 (0%)	190 (63,3%)	190 (63,3%)
Total	95 (31,7%)	205 (68,3%)	300 (100%)

FC : fixation du complément

été également au TFC, et 20 échantillons sur les 35 échantillons douteux au rose Bengale l'ont été au TFC. Au total, 95 bovins (31,7 p. 100) ont été positifs au TFC. Quant à la corrélation intra-troupeau, 28 troupeaux (93,4 p. 100) sur 30 ont présenté au moins une réaction positive au rose Bengale. Cette prévalence atteint 29 troupeaux sur 30 (96,7 p. 100) si on intègre les réactions douteuses au rose Bengale. Ainsi, 96,7 p. 100 des troupeaux ont présenté au moins un cas de réaction positive au TFC.

Les deux tests rose Bengale et de FC étant en série, en considérant une sensibilité finale égale à 85 p. 100, une spécificité finale égale à 98,75 p. 100 et une valeur prédictive d'un résultat positif égale à 97,48 p. 100, la prévalence réelle a été évaluée à 36,36 p. 100 chez les bovins. Au moins un animal était infecté dans 96,6 p. 100 des troupeaux.

Les facteurs de risque identifiés chez les animaux ont été consignés dans le tableau III. La positivité au TFC a été significativement associée à l'âge, la race, l'avortement et la présence d'hygromas. Ces variables explicatives ont été considérées comme facteurs de risque identifiés chez les bovins.

Les comportements à risque les plus fréquemment observés chez les humains ont été l'assistance aux mises bas et aux avortements, la manipulation de l'avorton sans gant, la consommation de lait cru ou de lait caillé non pasteurisé et de fromage frais (tableau IV).

■ DISCUSSION

L'échantillonnage aléatoire à deux degrés assure généralement la représentativité des échantillons. Dans cet échantillon, seuls les bovins de plus d'un an ont été inclus dans l'enquête. L'accent a été mis sur les femelles en raison du faible impact des mâles dans l'épidémiologie de la brucellose. L'échantillon serait donc représentatif des vaches de plus d'un an dans les élevages bovins laitiers en périphérie de Dakar au Sénégal.

La prévalence réelle de la brucellose bovine dans notre étude a été évaluée à 36,36 p. 100. Cette valeur est supérieure à 1,52 p. 100 obtenu par Kouamo et coll. (20) en 2010 dans la région de Thies au Sénégal. Cette valeur est aussi plus élevée que celles observées par certains auteurs au Togo (9,2 p. 100) (14), au Tchad (2,6 p. 100) (15), en Côte d'Ivoire (8,8 p. 100) (27), au Burkina Faso (13,2 p. 100) (31), au Mali (15 p. 100) (7) et en Ethiopie (1,7 p. 100) (32). La différence entre ces résultats et le nôtre ne signifie pas nécessairement que la brucellose bovine est galopante au Sénégal. En effet, les études citées ont été menées dans des élevages bovins traditionnels utilisant des races locales, alors que notre étude a été effectuée dans des élevages bovins laitiers semi-modernes utilisant parfois des races européennes qui sont plus sensibles à la brucellose.

En revanche, en élevage extensif ou traditionnel, la séroprévalence de la brucellose bovine reste relativement faible (20, 21, 22). Vingt-neuf troupeaux sur trente (96,7 p. 100) ont présenté au moins un cas de réaction positive au TFC, ce qui signifie que la corrélation intratroupeau a été faible. Ce résultat concorde avec celui de Delafosse et coll. (15) dont les travaux ont été réalisés en zone périurbaine d'Abéché au Tchad. La prévalence réelle à l'échelle du troupeau a été estimée à 96,6 p. 100.

Une grande différence a été observée entre la prévalence réelle au niveau bovin et celle à l'échelle du troupeau. Ce résultat est semblable à celui obtenu par Omer et coll. (26) en 2000. Ce constat pourrait s'expliquer par la faiblesse de la corrélation intra-troupeau. Cela signifie que la transmission intertroupeau et intra-troupeau est identique. Le fait qu'un animal soit infecté dans un troupeau n'augmente pas la probabilité qu'un autre animal appartenant au même troupeau soit infecté.

La positivité au TFC a été significativement associée à l'âge, la race, l'avortement et la présence d'hygroma. Ceci corrobore les résultats obtenus par Delafosse et coll. (15) en 2002, Omer et coll. (25) en 2002, et Bonfoh et coll. (9) en 2011.

Les comportements à risque les plus fréquemment observés chez les humains ont été l'assistance aux mises bas et aux avortements, la manipulation de l'avorton sans gant, et la consommation de lait cru ou caillé non pasteurisés et de fromage frais. En effet, ces comportements à risque avaient déjà été soulignés par Calvet et coll. (10), Dao et coll. (13) au Mali, et Dean et coll. (14) au Togo.

Tableau III

Régression logistique multivariée des facteurs de risque identifiés chez les bovins prélevés dans la région de Dakar au Sénégal en 2012

Variable	OR	OR (IC : 95%)	P
Age	2,181	1,08 – 4,60	0,031
Race	1,620	1,07 – 2,45	0,022
Avortement	1,578	1,04 – 2,37	0,028
Hygroma	1,271	1,11 – 1,45	0,011

OR : *odds ratio* ; IC : intervalle de confiance

Tableau IV

Régression logistique multivariée des comportements à risque observés chez les humains dans la région de Dakar au Sénégal en 2012

Comportement à risque	OR	IC : 95%	P
Assistance aux mises bas	1,78	1,15 – 2,74	0,04
Assistance aux avortements	1,95	1,05 – 4,59	0,01
Manipulation de l'avorton sans gant	2,04	1,67 – 4,38	0,03
Consommation de lait cru non pasteurisé	1,90	1,03 – 3,92	0,02
Consommation de lait caillé non pasteurisé	1,98	1,22 – 4,06	0,03
Consommation de fromage frais	1,85	1,07 – 5,90	0,01

OR : *odds ratio* ; IC : intervalle de confiance

■ CONCLUSION

Cette étude montre que la brucellose est bien présente dans les élevages bovins laitiers en périphérie de Dakar au Sénégal, avec une prévalence réelle évaluée à 36,36 p. 100 au niveau individuel (bovin) et 96,6 p. 100 à l'échelle du troupeau. La positivité au TFC a été significativement associée à l'âge, la race, l'avortement et la présence d'hygroma chez les bovins. Par ailleurs, des comportements à risque ont été relevés chez les éleveurs, notamment lors de la manipulation des animaux et de la consommation de produits laitiers non pasteurisés. La consommation de ces produits provenant de ces élevages n'étant pas sans conséquence pour la santé publique, des mesures adéquates doivent être prises afin de protéger la population contre cette zoonose.

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Summary

Tialla D., Koné P., Kadja M.C., Kamga-Waladjo A., Dieng C.B., Ndoye N., Kouame K.G.G., Bakou S., Akakpo A.J. Prevalence of bovine brucellosis and related risk behavior in the suburban area of Dakar, Senegal

The objective of this study was to evaluate the prevalence and the frequency of risk behaviors in the zoonotic transmission of bovine brucellosis in suburban Dakar. The individual serological status of 300 cattle distributed in thirty farms in this area was determined by the rose Bengal and complement fixation tests. The frequency of risk behaviors toward this zoonosis was determined using two epidemiological surveys that inventoried the known risk factors of brucellosis transmission between animals and humans. Taking into account the sensitivity and specificity of rose Bengal and complement fixation tests used in series, i.e. 85% and 98.75 %, respectively, the true prevalence was estimated to be 36.36%. At least one animal was infected in 96.6% of the herds. Positivity to the complement fixation test was significantly associated with age, breed, abortion and the presence of bursitis in cattle. The risk behaviors the most frequently observed in humans in this area were: assisting during calving and abortion, handling of aborted fetuses without gloves, and consuming unpasteurized raw or curd milk and fresh cheese. These results show that brucellosis is present in dairy cattle farms in suburban Dakar. Since the milk produced in these farms is used to supply the city of Dakar, measures must be developed to promote brucellosis prevention methods aimed at Dakar's population.

Keywords: Dairy cattle – Brucellosis – Morbidity – Zoonosis – Suburban agriculture –Senegal.

Resumen

Tialla D., Koné P., Kadja M.C., Kamga-Waladjo A., Dieng C.B., Ndoye N., Kouame K.G.G., Bakou S., Akakpo A.J. Prevalencia de la brucelosis bovina y comportamientos riesgosos asociadas con esta zoonosis en zona periurbana de Dakar en Senegal

El objetivo del presente estudio fue el de evaluar la prevalencia y la frecuencia de los comportamientos riesgosos en la transmisión zoonótica de la brucelosis bovina en la zona periurbana de Dakar. Se determinó el estatus serológico individual de 300 bovinos, distribuidos en 30 criaderos de esta zona, mediante los tests de rosa de Bengala y de fijación de complemento. La frecuencia de los comportamientos riesgosos de esta zoonosis fue determinada gracias a dos cuestionarios epidemiológicos que permitieron el censo de los factores de riesgo conocidos en la transmisión de la brucelosis entre animales y humanos. Tomando en consideración la sensibilidad y la especificidad del test de rosa de Bengala y el de fijación de complemento utilizados en la serie, 85 y 98,75% respectivamente, la prevalencia real fue evaluada en 36,36%. Al menos un animal estaba infectado en 96,6% de los hatos. La positividad del test de fijación de complemento estuvo significativamente asociada con la edad, la raza, el aborto y la presencia de higromas en los bovinos. Los comportamientos riesgosos más frecuentemente observados en los humanos en esta zona fueron la asistencia a partos y abortos, la manipulación del feto abortado sin guantes, el consumo de leche cruda o de leche cortada no pasteurizada y de queso fresco. Estos resultados muestran que la brucelosis existe en los criaderos bovinos de leche en la periferia de Dakar. Dado que la leche producida en estos criaderos sirve de suministro para la ciudad de Dakar, deben tomarse medidas adecuadas con el fin de orientar los medios de prevención contra la brucelosis en los habitantes.

Palabras clave: Ganado bovino – Ganado de leche – Brucelosis – Morbosidad – Zoonosis – Agricultura peri urbana – Senegal.

Detection of African horse sickness virus antibodies by ELISA in sera collected from unvaccinated horses in Kaduna Metropolis, Nigeria

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Keywords

African horse sickness virus – antibodies – ELISA – Nigeria.

Summary

African horse sickness (AHS) is endemic in sub-Saharan Africa and is recognized as one of the major life-threatening diseases of equids in some parts of the world. Several sporadic outbreaks of AHS have been reported in Nigeria in the past. Sera collected from 284 horses in seven stables in Kaduna Metropolis, Nigeria, were tested for antibodies against AHS virus (AHSV) using a blocking enzyme-linked immunosorbent assay (ELISA). A high percentage of the sera (86.6%) were positive, indicating continual exposure of Nigerian horses to AHSV. Annual vaccination of horses and vector control to minimize incidence in the region is advocated.

■ INTRODUCTION

African horse sickness (AHS) is endemic in sub-Saharan Africa and is still recognized as one of the major life-threatening diseases of equids in Africa, the Middle East, the Eastern Mediterranean and some parts of Europe (10) because of its high mortality rate (up to 90% in epidemics), particularly in naïve populations (13). AHS is an acute or subacute insect-borne infectious disease of Equidae (horses, mules, donkeys and zebras) caused by African horse sickness virus (AHSV), a double-stranded RNA virus in the genus *orbivirus,* belonging to the Reoviridae family (2, 4). AHSV is a viscerotropic virus found in the blood, tissue fluids, serous exudates and several internal organs of Equidae (11). It is a non-contagious disease known to be transmitted to horses by midges, in particular by *Culicoides imicola*, the main field vector found in abundance in Nigeria during the warm rainy seasons (1, 5). The incubation period ranges from 2 to 14 days and the clinical signs appear 5 to 7 days after infection, associated with respiratory and circulatory impairment (11, 13).

AHSV exists as nine immunologically distinct serotypes, all of which have been identified and are considered to be enzootic in sub-Saharan Africa (7). Since the first documented outbreak and subsequent isolation of the virus from a dead horse in Nigeria in 1970 (9), sporadic outbreaks of AHS have occurred in different regions of the country (3, 8, 16). Furthermore, Lazarus et al. (12) reported in 2010 AHSV in a captive zebra that died in a game reserve in Bauchi, Nigeria. AHSV was detected from tissue samples collected from the dead zebra at postmortem by real-time reverse-transcription polymerase chain reaction (rRT-PCR). Recently (in 2014), outbreaks of AHS have been reported in South Africa and Mozambique resulting in the death of several horses (15, 19). Historically, only serotypes 4 and 9 AHS viruses have been found in West Africa. In recent past, other types of AHSV have been reported for the first time in sub-Saharan Africa. AHSV serotype 6 was identified in Ethiopia in 2003 and AHSV serotype 2 was also confirmed in Senegal and Nigeria in 2007 (17). So far, only AHSV serotypes 2 and 9 have been confirmed in Nigeria (3, 8, 9).

The population of horses in Nigeria has been estimated at over 1.2 million (18) consisting of both a local breed (Arewa), which is popular with traditional institutions, and exotic breeds (particularly Argentine and Sudanese) mostly kept under intensive management by a few elites for racing and polo games. This study shows the presence of antibodies against AHSV in horses in Kaduna, Nigeria.

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■ MATERIALS AND METHODS

Study area and stable management

Kaduna State is located in the Northwestern region of Nigeria, in the Northern Guinea savannah zone. Horses in seven different stables, designated A through G (Figure 1), belonging to government security institutions (n = 2), private organizations (n = 4) and a traditional institution (n = 1) located in Kaduna Metropolis, Nigeria, were used for the study. These stables were kept under intensive to semi-intensive management and each accommodated 30 to 200 horses. The horses were primarily kept for special ceremonial activities, training, crowd control, race competition and polo games. Routine veterinary care was provided for all the stables except stable G. No preventive vaccination is routinely applied against AHSV in Nigeria.

Sample collection

Using the principles of convenient sampling, about 50% of the total number of horses in each stable was selected for blood sampling. A total of 284 horses of all ages, different breeds and both sexes were sampled under proper restraint. Approximately ten milliliters of blood was collected from each horse into properly labeled Vacutainer tubes. The blood samples were allowed to clot and were then centrifuged in the laboratory at 1500 *g* for 10 min. Sera were separated into cryovials and stored at -20°C until tested.

Test procedure

An African horse sickness virus blocking enzyme-linked immunosorbent assay (ELISA) kit (INGENASA, Madrid, Spain) was used for the detection of group-specific antibodies to AHSV in equine (i.e. the test detects all nine serotypes [6]) according to the manufacturer's instruction. Briefly, the antigen was fixed in a solid support (polystyrene plate). After incubation with serum samples, an AHSV specific monoclonal antibody (Mab peroxidase conjugate) was added. If the sample contains specific antibodies to the virus, they will not allow the binding of labeled Mab to the antigen, whereas if the sample does not contain specific antibodies, Mab will bind to the antigen coating the plate. After washing the plate with a multichannel pipetting device suitable for dispensing 300 ml on each well to eliminate all non-fixed materials, presence or absence of labeled Mab can be detected by adding the substrate which, in the presence of peroxidase, will develop a colorimetric reaction. The optical density reading was performed with a spectrophotometer at 405 nm. Samples showing blocking percentages higher than 50% were considered positive for antibodies to AHSV.

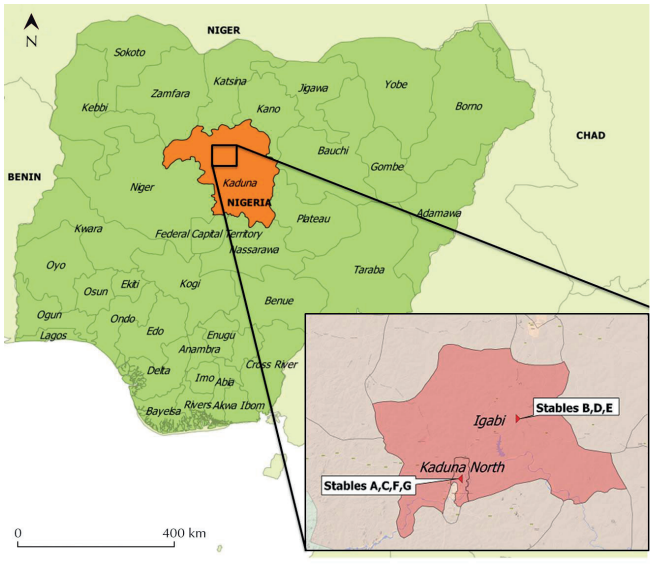

Figure 1: *Location of the seven horse stables of the study in Kaduna Metropolis, Nigeria*

■ RESULTS

The stables sampled, breed, sex and age range of the horses are presented in Table I. Results show that overall there was a high prevalence of antibodies in the sera from all the stables included in the study. In total, 246 (86.6%) of the 284 tested horses were positive for antibody to AHSV by ELISA. Stable A had the highest prevalence (100%), whereas stable E had the lowest (73.3%). There was a high prevalence of antibodies in the sera collected from the local (Arewa breed) horses (87.7%; 121/138), as well as in those collected from exotic (Argentine and Sudanese breeds) horses (85.6%; 125/146). The difference between AHSV antibodies detected in local and exotic horses was not statistically significant ($\chi^2 = 0.113$; $p > 0.05$).

■ DISCUSSION

The prevalence of AHSV antibodies detected by ELISA in the horse stables in Kaduna Metropolis, Northwest Nigeria, was estimated at 86.6%. This high prevalence is consistent with previous serological studies in Nigeria (2, 14, 16), and may be an indication of continual exposure of the horses regardless of age, sex and breed. Our study area (Kaduna) extends from the tropical savannah of Guinea to the savannah of Sudan, with thick vegetation and hot and dry climatic conditions which favor vector propagation and virus transmission. In Nigeria, no preventive vaccination is routinely applied against AHSV, particularly in indigenous and local crossbred horses. There appears to be a form of innate resistance to infection by AHSV as corroborated by Best et al. (3), and Nawathe et al. (14). It could explain the probable absence of reported outbreak of the disease in the region, despite the high prevalence of antibodies. In contrast, newly imported horses, particularly from AHS-free areas, are susceptible to infection and are therefore usually vaccinated before importation. But once established in the country (the animals of exotic breeds examined during the study had been introduced more than ten years ago or were born in the

Table I

Detection of African horse sickness virus antibodies by ELISA in horse sera from Kaduna Metropolis, Nigeria

Stable (years)	Num. samples	Breed	Male	Female	Age range	ELISA positive (%)
A	17	Mixed*	10	7	4–10	17 (100)
B	28	Exotic	12	16	2–9	24 (85.7)
C	22	Local	15	7	6–22	21 (95.4)
D	93	Exotic	22	71	3–13	80 (86.0)
E	15	Exotic	3	12	3–11	11 (73.3)
F	17	Local	16	1	4–15	14 (82.4)
G	92	Local	22	70	2–17	79 (85.9)
Total	284					246 (86.6)

* Ten exotic and seven local breeds

country), they are seldom revaccinated (2) and are therefore vulnerable to AHSV infection.

■ CONCLUSION

This report corroborates previous studies and could suggest a potential threat of AHS to the equine industry in Nigeria, and a continual prevalence of the disease. Annual vaccination of imported horses is advocated. Vector control and good stable management practices may assist in minimizing incidence. Suspected outbreaks should be investigated to ascertain the circulating serotypes in the region.

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Résumé

Ehizibolo D.O., Nwokike E.C., Wungak Y., Meseko C.A. Détection par un test Elisa d'inhibition des anticorps dirigés contre le virus de la peste équine africaine chez des chevaux non vaccinés de la métropole de Kaduna, Nigeria

La peste équipe africaine (PEA), endémique dans les pays subsahariens, est considérée dans certaines parties du monde comme l'une des maladies des équidés les plus mortelles. Plusieurs foyers sporadiques de PEA ont été signalés dans le passé au Nigeria. Des anticorps dirigés contre le virus de la PEA ont été recherchés par un test Elisa d'inhibition dans les sérums de 284 chevaux collectés dans sept écuries de la métropole de Kaduna au Nigeria. Un grand pourcentage de sérums (86,6 p. 100) était positif, témoignant d'une exposition permanente des chevaux nigérians au virus de la PEA. La vaccination annuelle des chevaux et la lutte contre les vecteurs du virus sont recommandées pour limiter l'incidence de la maladie dans la région.

Mots-clés : Virus peste équine africaine – Anticorps – Test Elisa – Nigeria.

Resumen

Ehizibolo D.O., Nwokike E.C., Wungak Y., Meseko C.A. Detección de anticuerpos contra el virus de la peste equina africana mediante ELISA en sueros colectados de caballos no vacunados en Kaduna Metropolis, Nigeria

La peste equina africana (PEA) es endémica en Africa subsahariana y es reconocida como una de las enfermedades que amenazan la vida de los equinos en algunas partes del mundo. En el pasado, se han reportado varios brotes esporádicos de PEA en Nigeria. Sueros colectados de 284 caballos en siete establos en Kaduna metrópolis, Nigeria, fueron examinados para anticuerpos contra el virus de PEA (PEAV), utilizando el ensayo por inmunoabsorción ligado a enzimas (ELISA). Un alto porcentaje de los sueros (86,6%) fueron positivos, indicando una exposición continua de los caballos nigerianos a PEAV. Se recomienda una vacunación anual de los caballos y control de vectores para minimizar la incidencia en la región.

Palabras clave: Virus de la peste equina africana – Anticuerpos – ELISA – Nigeria.

Pathology and pathogenesis of bovine skin and meibomian gland demodicosis

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Keywords

Cattle – *Demodex* – Demodectic mange – Sebaceous gland – Skin – Bacteriosis – Pathogenesis – Sudan.

Summary

A national survey on bovine demodicosis was conducted among 48,000 cattle in Sudan during vaccination campaigns (44,800), and at antemortem and postmortem examination in abattoirs (3200). Among the total surveyed, 44,908 were adult (2-8 years) of which 34.6% were infected, and 3092 were calves (< 2 years) of which 34.6% were infected. Three hundred cattle with severe skin lesions among which 218 also had meibomian gland lesions were selected. The clinical pictures of skin and meibomian gland demodicosis were described. *Proteus vulgaris, Pseudomonas aeruginosa*, *Staphylococcus aureus, Sta. epidermidis, Streptococcus pyogenes* (group A) and *Trueperella pyogenes* were isolated from skin lesions, and *Moraxella bovis* and *Sta. aureus* were isolated from meibomian gland lesions. These bacteria produced deleterious toxins and enzymes aggravating the lesions caused by *Demodex bovis* and *D. ghanensis* mites in skin and meibomian glands, respectively. Neither mite was found in the internal tissues or organs, indicating that they had no endoparasitic phase. The histopathological changes observed were commensurate with cell-mediated immunity. Liberation of the contents of demodectic mange colonies in the subepidermal and dermal layers of the skin, and surrounding connective tissue of the meibomian glands evoked severe histopathological changes characterized by massive high-turnover granulomatous reactions with influx of macrophages and lymphocytes. The pathogenesis of the disease, from the stage of initial invasion of the hair follicles and collecting tubules of the meibomian glands by the mites and associated bacteria, to the stage of regression of the lesions was described. It was concluded that the nature of association between *Demodex* mites and bacteria in demodectic mange lesions was synergistic and of equal significance. The high-turnover granulomatous reactions which characterized the histopathological changes showed that *Demodex* mites and associated bacteria were persistent and immunogenic.

■ INTRODUCTION

Demodex mites live in the hair follicles and sebaceous glands of various mammals including humans, causing demodectic or follicular mange (38, 44). *Demodex* mites are considered to be host specific and designated after the name of the host they infest (38, 39). Demodectic mange in cattle is caused by *Demodex bovis* Stiles 1892 (27, 44). Transmission usually occurs by direct contact from the dam to her offspring during nursing in the neonatal period and never between host animals of different species (23, 31).

The cutaneous disease is characterized by the formation of papules, nodules, pustules and cysts of varying sizes (4, 5, 39). The predilection sites of the lesions seem to be the neck, withers, shoulders and forequarters (3, 5, 39). As the disease progresses, the lesions spread from their original site to the rest of the body and, in severe infections, most of the skin becomes involved (5, 31, 39). Many cattle with demodectic mange might have no visible cutaneous lesions and the disease might pass unnoticed. A satisfactory diagnosis of demodicosis can only be made by the demonstration of *Demodex* mites in the infected purulent material extracted from nodules and pustules (4, 32).

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Meibomian glands are also infested by *Demodex* mites but this demodicosis received the attention of only a few workers. In a recent paper we characterized the ocular lesions (5). Briefly, the disease was characterized by lacrimation, hyperemia and congestion of the mucous membranes, and in extreme cases by purulent exudation, swelling and closure of the eyelids. *Demodex ghanensis* and the primary pathogenic bacteria – *Moraxella bovis* and *Staphylococcus aureus* – were isolated from the infected material extracted from the meibomian gland lesions.

The disease pathology was described in different animals, for example in cattle (14, 30, 41), dogs (6, 8, 43), and also in humans (7, 11). Demodectic mange is cosmopolitan and has been reported by many workers in different parts of the world (38, 39). However, most of the published works reported the disease in a very limited number of cases. Moreover, most of the authors who studied the pathology of the disease had ignored or undermined the role played by the bacteria associated with the mites in the pathogenesis of the disease. The nature of the association between *Demodex* mites and the bacteria involved in demodectic mange lesions has not yet been ascertained. For some authors, *Demodex* mites cause dilatation of the hair follicle and pave the way for secondary bacterial invasion (15, 31, 39). For others the bacteria are actively introduced in the hair follicles on the exoskeleton or in the gut of the mite (20, 36).

In the pathogenesis of a disease, it is interesting to study the fate of both host and pathogen. In the present work, the host-parasite interactions were studied by investigating the pathogen, the clinical manifestation of the disease, host tissue reactions and defensive mechanisms against infection. In this study severe and extensive lesions of demodectic mange were described in a large number of cattle, and most of the parameters concerning the disease have been investigated.

■ MATERIALS AND METHODS

Survey

A national survey of bovine demodicosis was conducted over three years in five states of Sudan during vaccination campaigns or in abattoirs. The total number of cattle surveyed was 48,000 (5) belonging to 189 herds, among which 44,800 cattle were surveyed during vaccination campaigns, and 3200 cattle were examined antemortem and postmortem in abattoirs (1242 infected and 1958 non-infected). Three hundred cattle with severe skin lesions, among which 218 had simultaneously meibomian gland lesions, and 50 non-infected control animals were selected from abattoirs for this study. Non-infected cattle were selected after verifying that they were free of demodectic mange lesions at antemortem examination. This was achieved by running the hand over the shoulders, axillae, brisket and neck, and by rolling the loose skin in the axillae and brisket between the thumb and other fingers as suggested by other authors (33, 39). Skin brushings were collected in sterile screw-capped plastic containers from the 50 non-infected control animals using a coarse brush, and two sets of swabs and two impression smears were also collected from the eyes of these animals after examination under a magnifying lens.

Purulent infected material was extracted from the 300 skin and 218 meibomian gland lesions of the infected cattle using sterile techniques. Each specimen of the infected material was divided into two parts. The first part was kept in sterile bijou bottles and refrigerated for bacteriological investigation. The second part was kept in bijou bottles containing equal volumes of glycerol and ethanol for parasitological investigation. The skin brushings, impression smears and swabs from the eyes of the 50 non-infected cattle were refrigerated for both bacteriological and parasitological investigations. Skin biopsy specimens were collected from the 300 infected and 50 non-infected animals (1). After slaughter, the eyelids of the right eye from each of 25 infected and ten non-infected animals were also excised and collected (5). The biopsy and necropsy specimens were fixed in 10% formal saline for histopathological examination.

A critical postmortem examination was also conducted on the 25 infected and 10 non-infected animals. The following necropsy specimens were collected from each animal: skin, upper and lower eyelids of the left eye, left eyeball, brain, spinal cord, tongue, esophagus, trachea, lung, pleura, heart, pericardium, aorta, diaphragm, spleen, liver, gall bladder, rumen, reticulum, omasum, abomasum, pancreas, omentum, duodenum, small and large intestines, mesentery, caecum, rectum, kidneys, urinary bladder, testes, penis, ovaries, uterus, vagina, salivary glands, muscles (masseter, neck, shoulder, intercostal, belly, hindquarters), lymph nodes (parotid, mandibular, bronchial, mediastinal, mesenteric, prescapular, precrural, popliteal, inguinal and supramammary). To avoid contamination, all necropsy specimens were collected from non-infected animals before infected ones, and necropsy specimens from internal tissues, glands, organs and muscles were collected before sampling the skin, eyelids and eyeballs. The necropsy specimens were rinsed in sterile distilled water and the water was left to drain. The respective glands, tissues, organs and muscles were pooled together and kept in labeled plastic bags at -20ºC until investigation.

Parasitological examination

A small piece from each specimen of infected purulent material from skin and meibomian gland lesions was crushed between two microscope slides and examined. Another piece of the infected material, skin brushings from non-infected control animals and one set of eye impression smear were examined in 20% potassium hydroxide. Individual mites were isolated (2) and identified. Confirmation of the identification was conducted in the Department of Veterinary Parasitology, Liverpool School of Tropical Medicine, UK, and the Department of Zoology, University of Massachusetts, USA.

Necropsy specimens

The respective pooled necropsy specimens from the 25 infected and 10 non-infected cattle were digested in 20% potassium hydroxide solution. Before digestion was performed, the specimens were chopped into small pieces, thoroughly mixed and 25 g were transferred to 200 ml flasks containing 100 ml of 20% potassium hydroxide solution. The flasks were placed in a boiling water bath and continuously shaken until the necropsy specimens were completely digested. A drop of the digested material was placed in the middle of a microscope slide, covered with a coverslip and examined for *Demodex* mites. Ten milliliters of the digested material of each organ was transferred to sterile test tubes and centrifuged at 3000 rpm for three minutes and a drop from the supernatant fluid was also placed in the middle of a microscope slide, covered with a coverslip and examined for mites.

Bacteriological investigations

Purulent infected material from skin and meibomian gland lesions were cultured. Two milliliters of sterile nutrient broth were added to the purulent infected material in each bottle, and the contents of the bottles were thoroughly mixed using a mechanical shaker. Culture media and media for biochemical tests were prepared according to standard methods and techniques (10). Moreover,

β-hemolytic streptococci were subjected to the Lancefield's grouping by the acid extraction technique (10).

Each specimen was cultured under aerobic, anaerobic and increased carbon dioxide conditions at 37°C for 24-48 hours on the following media: nutrient agar, 5% sheep, bovine or horse blood enriched agar prepared from blood agar base, McConkey's agar and nutrient broth (Oxoid). Moreover, one set of the seeded blood-enriched agar was incubated at 33°C in a humid chamber. Pure cultures were obtained through serial subcultures. The pure isolates were biochemically tested according to standard methods and techniques (10). The second set of eye impression smears were stained by Gram's stain and examined.

Histopathological investigations

A skin biopsy specimen from each of the 300 infected animals and 25 necropsy specimens from the upper and lower eyelids of the right eye of severely infected cattle and 10 non-infected animals were processed, embedded in paraffin wax and sectioned at 5 μm before staining with hematoxylin and eosin, and examined following standard methods and techniques (9).

■ RESULTS

Survey

Among the total number of cattle surveyed (48,000), 16,608 had skin lesions of demodectic mange (34.6%), among which 8012 cattle (48.2%) also had skin and meibomian gland demodicosis (5). Among the total surveyed, 44,908 were adult (2-8 years) male and female cattle of which 15,537 were infected (34.6%), and 3092 were calves (up to 2 years) of which 1071 were infected (34.64%). The unaffected animals were grazing side-by-side with the infected ones.

Cutaneous and ocular bovine demodicosis in cattle is locally known by cattle owners in Sudan as *Um-Krush*. According to the history given by the owners, the disease in some adult cattle persisted for 2-3 years, but none of the owners was able to identify these animals or give information regarding when the lesions initially appeared. The disease was not fatal and was observed in emaciated cattle as well as in animals in good bodily condition. All animals with a light or moderate infection showed no change in feeding, drinking or sexual behavior. However, all animals with severe infection and some with moderate infection preferred shaded areas, showed a marked reduction in food intake and milk yield, and had severe pruritus. Most of the animals with severe infection and some with moderate infection had fair to poor bodily condition.

Among the 300 cattle examined during the present study, 218 had simultaneously skin and eye infection whereas none of the infected cattle only had meibomian gland lesions (5). The skin and ocular symptoms observed in the examined animals have been previously described (5). The various classical forms of lesions were observed in skin (Figures 1 and 2) and meibomian glands.

Parasitological findings

Examination of crushed infected material from skin and meibomian gland lesions in all animals revealed an uncountable number of adult mites, eggs and molting stages of *Demodex* mites (Figure 3), pus and cell debris. The mites were successfully isolated and identified as *Demodex bovis* from skin (Figure 4) and *D. ghanensis* from meibomian gland lesions (5). Examination of eye impression smears and skin brushings from control non-infected cattle was negative for *Demodex* mites.

Necropsy specimens

Examination of the digested material of internal tissues, organs, glands and muscles was negative for *Demodex* mites. However, *Demodex bovis* and *D. ghanensis* mites were identified in the digested specimens of the skin and eyelids of the left eyes of infected cattle, respectively. All digested material including the skin and eyelids from non-infected cattle was negative for both mites.

Bacteriological findings

The culture of 300 specimens of infected material extracted from skin lesions revealed bacteria growth in 252 specimens although no bacterium was isolated from the remaining 48 specimens. Culture of 218 specimens of infected material expressed from meibomian gland lesions revealed growth in 128 specimens and no bacterium in the remaining 90 specimens (Table I). No bacterium was isolated from swab cultures of the eyes of non-infected cattle, and Gram-stained impression smears from the eyes of non-infected cattle showed insignificant numbers of microorganisms.

Figure 1: *Cow showing numerous demodectic mange nodules mainly involving the lower half of the body.*

Figure 2: *Demodectic mange pustules covered with thin white crusts involving the head neck and dewlap of a bull with generalized skin lesions of demodectic mange. Note: wrinkling and folding of the skin.*

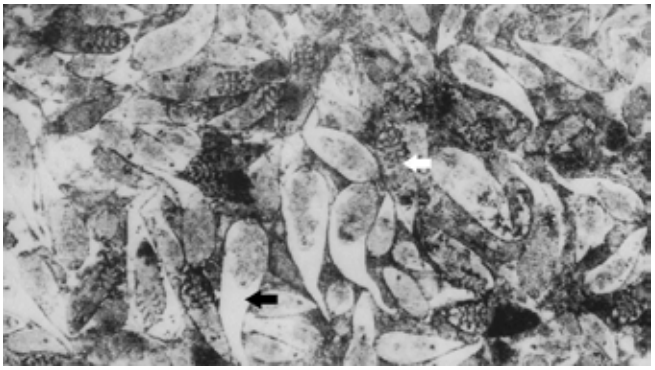

Figure 3: *Numerous* Demodex bovis *mites (white arrow) showing different molting stages (black arrow) in a crushed specimen of infected purulent material extracted from skin lesions of demodectic mange. Scale bar: 70 µm.*

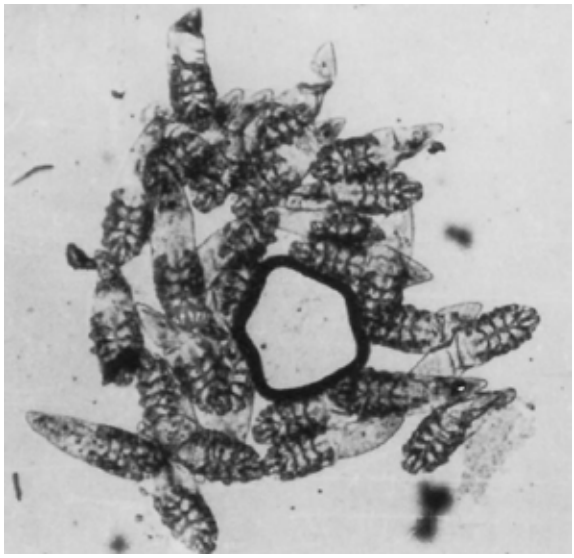

Figure 4: *Cigar-shaped* Demodex bovis *mites isolated from infected material extracted from skin lesions of demodectic mange in cattle. Scale bar: 140 µm.*

Table I

Bacteria isolated from skin and meibomian gland lesions of bovine demodicosis

Bacteria isolated	Num. bacteria isolated	
	Skin lesions	Meibomian gland lesions
Moraxella bovis	–	128
Proteus vulgaris	58	–
Pseudomonas aeruginosa	48	–
Staphylococcus aureus	80	26*
Sta. epidermidis	34	–
Streptococcus pyogenes (group A)	22	–
*Trueperella pyogenes***	10	–
No bacterium isolated	48	90
Total	300	218

* Always present with *Moraxella bovis*; ** Formerly *Arcanobacterium pyogenes*, *Actinomyces pyogenes*, *Corynebacterium pyogenes*

Pathological findings

Skin lesions

The mites reached the hair bulb (Figure 5) by passing between the hair and the inner root sheath. They caused inflammation and dilatation of the orifices of the hair follicles and paved the way for active and/or passive introduction of pathogenic, commensal and opportunistic bacteria (Table I) in the hair follicles and sebaceous glands (pilosebaceous unit). The epithelial lining of the hair follicles became atrophied and the hair broke and fell out. The follicles became enlarged with replication of the mites, the blood vessels were dilated and the surrounding tissue was slightly infiltrated by lymphocytes and eosinophils (Figure 5).

Maximum distension of the hair follicles with mites, bacteria, pus, secretions and excretions resulted in the transformation of the pilosebaceous units to very enlarged cylindrical or saccular bladder-like cysts (colonies of demodectic mange), with one layer of an intact but jagged and extremely stretched epithelial lining. These cysts (colonies) were seen in the subepidermal and dermal layers. In many sections the cysts in the dermal layer had long ducts (sinus tracts) directed toward the surface of the skin (Figure 6), and on reaching the stratum papillare, the sinus tracts became shorter and broader and the colonies became extremely enlarged, utilizing the extra length of the sinus tract (Figure 7). The wall of these cysts showed moderate hyperplasia and was extremely jagged throughout its length (circumference). In many sections, the cysts communicated with the superficial epidermal layers which also showed marked hyperplasia and keratinization occluding the orifices of these colonies (bladder cysts) by a plug of epithelial cells and keratin (Figure 8). Liquefaction or breaking of the sealing plug of the cysts causes discharge of their contents onto the surface of the skin. In those areas there was acanthosis and scab formation. Under the scab, the epidermal layer showed severe degenerative and necrotic changes, and the subepidermal layer and dermal papillae were infiltrated by lymphocytes, macrophages, neutrophils and eosinophils.

More replication of the mites and associated bacteria resulted in much enlarged colonies with one layer of jagged and extremely stretched epithelial lining, surrounded by a thin layer of connective tissue. Partial or complete rupture of these cysts (colonies of demodectic mange) occurred discharging their contents in the

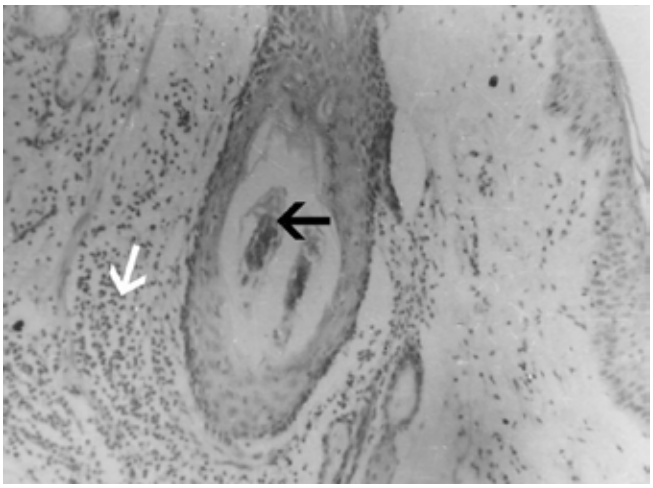

Figure 5: *Section from the skin of a cow showing* Demodex bovis *mites in the hair bulb (black arrow). Note: dilatation of the hair bulb and infiltration by mononuclear cells in close proximity of the hair follicle (white arrow). Stained with hematoxylin and eosin. Scale bar: 150 µm.*

subepidermal and dermal layers. This produced exudative and productive lesions in areas where the contents of the colonies had come in direct contact with the elements of the surrounding connective tissue and evolved high-turnover granulomas with influx of macrophages and lymphocytes.

In areas where the contents of the ruptured cysts congregated (mites, pus and cell debris), the exudative lesions showed hemorrhage, marked infiltration by macrophages, lymphocytes, plasma cells, some eosinophils, epithelioid and few multinucleated giant cells showing degenerate mites (Figure 9). The productive lesions

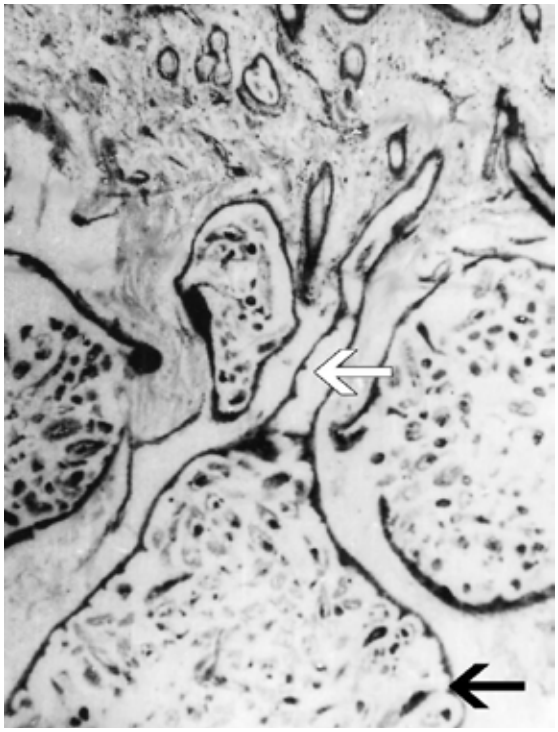

Figure 6: *Enlarged saccular bladder-like cysts (colony of demodectic mange) in the dermal layer (black arrow) showing a thin jagged wall and a long duct (sinus tract) directed toward the surface of the skin (white arrow). Stained with hematoxylin. Scale bar: 300 µm.*

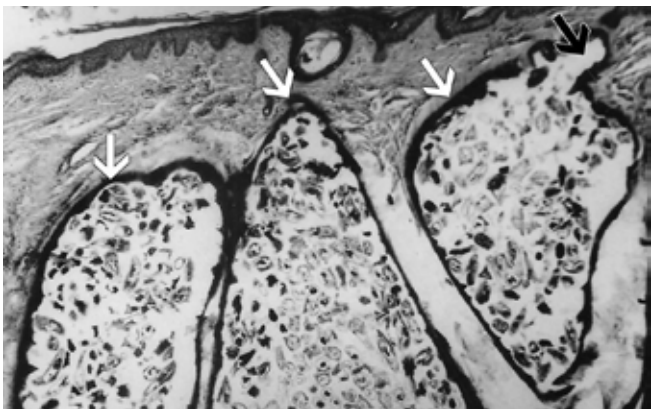

Figure 7: *Skin sections from an infected cow showing pilosebaceous units transformed to enlarged cylindrical or saccular bladder-like cysts (colonies of demodectic mange) in the subepidermal layer (white arrows). Note: hyperplasia and jagging of the walls of the colonies and broad channel-like communication with the surface epidermal layers of the skin (black arrow). Stained with hematoxylin and eosin. Scale bar: 250 µm.*

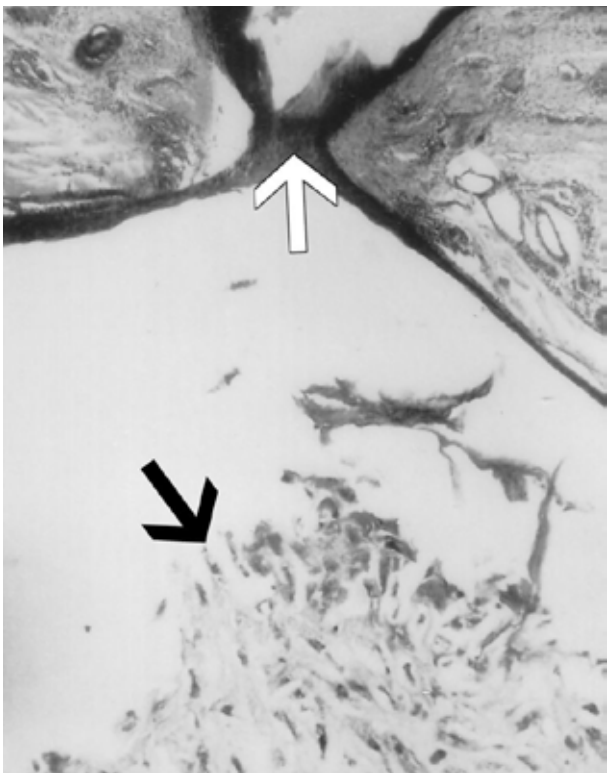

Figure 8: *Skin section from a cow infected with demodectic mange showing enlarged bladder-like cysts distended with mites, pus and cell debris (black arrow) and communicating with the superficial epidermal layers. Note: occlusion of the orifice of the bladder cyst by a plug of epithelial cells and keratin (white arrow). Stained with hematoxylin. Scale bar: 250 µm.*

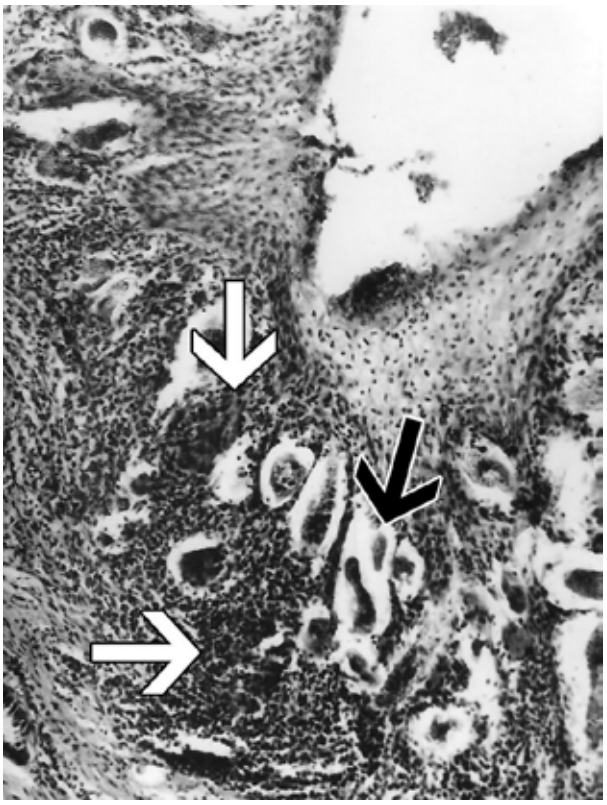

Figure 9: *Exudative lesion of demodectic mange in the subepidermal and dermal layers of the skin of a cow showing a granulomatous reaction (white arrows) in the area where the contents of the ruptured cyst congregated (black arrow). Stained with hematoxylin and eosin. Scale bar: 250 µm.*

were exemplified by typical granulomas (Figure 10) in which the mites, bacteria, and purulent exudate of the ruptured cysts were surrounded by dominant proliferation of connective tissue, giant and epithelioid cells in the inner layers, and macrophages, lymphocytes, plasma cells, and few eosinophils in the outer layers. Remnants of mites were seen in the multinucleated giant cells. In other areas of the same section or in different sections there was regression and/or early healing of the lesions as inferred by the diffuse proliferation of connective tissue and degeneration of the granulomatous reaction. Skin sections from non-infected cattle showed no histopathological changes.

Meibomian gland lesions

The histopathological observations regarding the eyelids of cattle with meibomian gland lesions have been previously detailed (5). The lesions of meibomian gland demodicosis produced by *Demodex ghanensis* were aggravated by *Moraxella bovis* and *Sta. aureus*. The pathogenesis and progress of the lesions from initial invasion of the main collecting tubules of the meibomian gland by the mite and bacteria to the stage of regression of the lesions have also been described (5).

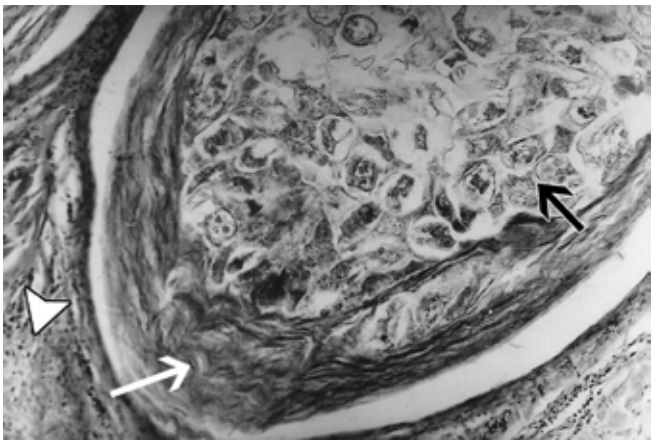

Figure10: *Typical granuloma in skin section from a cow infected with demodectic mange showing dominant proliferation of connective tissue (white arrow), epithelioid and giant cells in the inner layer with degenerate and fragments of* Demodex *mites (black arrow), and macrophages, lymphocytes, plasma cells and eosinophils in the outer layers (white arrow head). Stained with hematoxylin. Scale bar: 250 µm.*

■ DISCUSSION

The current survey is probably the first report of a severe skin and simultaneous skin and meibomian gland demodicosis observed in a large number of cattle (16,608). Previous workers only described skin (3, 35, 42) or meibomian gland lesions (21, 24, 37). The great majority of infected cattle (87.7%) had visible clinical lesions which were highly suggestive of the disease. Accordingly, the disease was tentatively diagnosed from the clinical appearance of the lesions as demodectic mange. This finding is contrary to that of other authors who report that animals with numerous large lesions are uncommon, and the condition might pass without being suspected or diagnosed (34). It is also contrary to the findings of other authors (28, 34, 35) who report that when the disease is mild it is unlikely to be diagnosed under ordinary circumstances, and the lesions are most readily seen in the dehaired-lime sulphide treated hides. In this study, only 12.3% of the infected animals had palpable lesions in the form of papules that were detected by palpation of the skin between the thumb and other fingers.

Calves and adult cattle of both sexes were found to be equally susceptible to the disease and the incidence in calves and adult cattle was similar. Calves had probably acquired infection from dams harboring persistent lesions of the disease for 2-3 years as gathered from the history given by the owners. This finding agrees with those of other authors (23, 29, 31) who report that transmission of the mite usually occurs by direct contact from the dam to her offspring during nursing in the neonatal period, which explains why many cattle in contact or grazing side-by-side with infected ones were not infected. The mite acquired during the nursing period exists in harmony with the host, and it is only when equilibrium between the host and parasite is altered in favor of the mite that excessive proliferation occurs and lesions of demodectic mange are produced. Other authors (6, 7, 22) also mention that rupture of the harmony between the host and the mite, which is part of the commensal flora of the skin, results in proliferation of the mite, and the appearance of lesions due to hereditary predisposition, stress, poor nutrition, concurrent diseases and any other factors that suppress immune function.

Although *Demodex bovis* and *D. ghanensis* were isolated from skin and meibomian gland lesions, respectively, in 218 cattle, it was interesting to report that none of the infected animals examined during the present study, as well as during the whole survey, had only meibomian gland demodicosis. This finding agrees with that of Fantahun et al. (22).

Demodex bovis was only found in the digested skin, and *D. ghanensis* was only found in the digested eyelids of infected cattle, but none of the mites was encountered in the digested internal tissues, organs, glands or muscles of the infected animals, indicating that neither mite had an endoparasitic phase. This finding agrees with those of other authors (35, 36, 37, 42), who examined the lymph nodes and internal organs of infected cattle, and report that *D. bovis* has no endoparasitic phase even in the most serious cases. However, some workers (8, 16) report the occurrence of *D. canis* in lymph nodes, internal organs and body fluids of dogs, and others (46, 47) found *D. caprae* in internal tissues, organs and body fluids of adult goats and their fetuses. In the authors' opinion, the mites observed were most probably dead and considered as foreign bodies transported to lymph nodes by the lymph.

Proteus vulgaris, *Sta. aureus*, *Sta. epidermidis*, and *Streptococcus pyogenes* (group A) were isolated from the infected purulent material extracted from skin lesions of infected cattle. The skin surface is probably the natural habitat of these bacteria which may alternatively belong to the intestinal flora existing in the surroundings of the animals as reported by some workers (39). These workers along with others (30, 31, 39) report that the bacteria originate from bladder-like cysts (colonies of demodectic mange), which open toward the exterior; they liberate their contents onto the skin surface of infected animals and thus spread the infection as well as contaminate their surroundings. *Pseudomonas aeruginosa*, and *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes, Actinomyces pyogenes, Corynebacterium pyogenes*) were also isolated from skin lesions of demodectic mange. *Pseudomonas aeruginosa* usually infects damaged tissues or tissues with reduced immunity, whereas *T. pyogenes* is one of the most common opportunistic pathogens of domestic ruminants capable of producing suppurative lesions in any organ or tissue in farm animals. Some workers (17, 26) report that these organisms produce a suppurative reaction and possess multiple virulence factors that cause serious damage resulting in marked deterioration of tissues.

Moraxella bovis and *Sta. aureus* were isolated from the infected material extracted from meibomian gland lesions (5). *M. bovis* is an opportunistic pathogen and might have been acquired from surroundings as the animals might have been contaminated by ocular discharges from cattle infected with infectious keratoconjunctivitis. Morbidity from this organism is high, reaching epizootic proportions when transmission agents from infected cattle become available as reported by Radostits et al. (39), whereas *Sta. aureus* might have been acquired from the skin when the animals scratched or rubbed their irritated eyes against their bodies (5).

Histopathological examination of skin biopsy and eyelid samples from infected and non-infected cattle was conducted on qualitative rather than quantitative criteria. Variable histopathological changes were observed in different areas of the same section or in different sections. This enabled a detailed study of the pathology and pathogenesis of the disease from the stage of initial infection to that of regression and lesion healing.

Demodex bovis invaded the corium through the orifices of hair follicles and *D. ghanensis* invaded the meibomian glands through the orifices of main collecting tubules as previously observed (5, 35, 36, 37). The mites caused severe irritation by their movement and continuous gnawing and feeding on the follicular epithelium and/or glandular tissue (5). The secretions, excretions and somatic debris of the mites might have caused allergic and/or immunologic responses. The lesions were aggravated by pathogenic bacteria, including *Sta. aureus* and *Str. pyogenes* group A in skin lesions, and *M. bovis* and *Sta. aureus* in meibomian gland lesions. These bacteria produced various enzymes and toxins which exacerbated the lesions causing severe pruritus, resulting in scratching, rubbing, licking and gnawing at the affected areas. This resulted in more inflammation, damage of the infected areas and facilitated the invasion of further secondary and opportunistic bacteria (*Proteus vulgaris, Pseudomonas aeruginosa*, *Sta. epidermidis*, and *Trueperella pyogenes*) which produced a suppurative reaction complicating the lesions.

The occlusion of the orifices of hair follicles and main collecting tubules of the meibomian glands created conditions highly conducive to mite and bacterial multiplication. The severe inflammatory response caused distortion of the meibomian glands (5) and hair follicles which were transformed to enlarged bladder-like cysts (demodectic mange colonies), and resulted in marked dilatation and damage to the main collecting tubules and ducts of the meibomian glands (5). The breaking or liquefaction of the sealing plug of demodectic mange colonies which opened onto the skin surface might be caused by the increased pressure within the distended colonies and/or by the action of hyaluronidase enzymes produced by pathogenic bacteria. Damage of weak spots in the wall of the colonies or their complete destruction and liberation of their contents in surrounding tissues, and seeping-out contents from main collecting tubules and ducts of meibomian glands in surrounding tissues of the eyelids (5) resulted in a severe inflammatory response. The histopathological changes observed in skin sections were more severe than those previously described (31, 35), and the changes observed in meibomian gland sections were similar (5) but also more severe than reported by other workers (22, 24, 37).

As mentioned above, *Sta. aureus* and *Str. pyogenes* group A, *Proteus vulgaris*, *Pseudomonas aeruginosa Sta. epidermidis* and *T. pyogenes* were isolated from skin lesions of demodectic mange, and *M. bovis* and *Sta. aureus* were isolated from meibomian gland lesions. These bacteria produce toxins and enzymes (12, 13, 17, 18, 25, 26) that aggravate lesions caused by *Demodex* mites, resulting in marked deterioration of the skin and meibomian glands (5). They also produce severe histopathological changes which are highly compatible with cell-mediated immunity. Authors report that on the basis of histopathological investigations an immunological response to the parasite seems to be involved (40). In this study, the liberation of the contents of bladder-like cysts in the subepidermal and dermal layers of the skin and main collecting tubules of the meibomian glands (5) in the surrounding connective tissue evoked severe histopathological changes characterized by massive high-turnover granulomatous reaction with influx of macrophages and lymphocytes, proving that *Demodex* mites and associated bacteria were both persistent and immunogenic, as previously mentioned (5, 19, 45). This resulted in the severe and progressive disease that has been encountered in natural field cases.

The histopathological changes recorded in this study were comparable and similar to those described by other workers in cattle, dogs and humans. Authors report that the granulomatous reaction observed in cattle suggests a progressive disease (5, 22). In dogs dilatation of the hair follicles with mites, folliculitis, perifolliculitis furunculosis, and granulomas resulting from damage to hair follicles and liberation of *D. canis* in the extra-follicular space have been observed (6, 43). In humans *Demodex folliculorum* and *D. brevis* infections cause hyperkeratinization and epithelial hyperplasia resulting in follicle blockage, induce a foreign body granulomatous reaction, and stimulate host humoral and cell-mediated immune reactions (7).

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Résumé

Abu-Samra M.T., Shuaib Y.A. Pathologie et pathogenèse de la démodécie cutanée et de la glande de Meibomius chez des bovins

Une enquête nationale menée au Soudan sur la démodécie bovine a concerné 48 000 animaux examinés soit au cours de campagnes de vaccination (44 800), soit lors d'inspections *ante* et *post mortem* dans des abattoirs (3 200). Sur l'ensemble des animaux enquêtés, 44 908 étaient des adultes (2-8 ans) dont 34,6 p. 100 étaient infectés, et 3 092 étaient des veaux (< 2 ans) dont 34,6 p. 100 étaient infectés. Trois cents bovins affectés par des lésions cutanées graves, dont 218 présentaient également des lésions de la glande de Meibomius, ont été sélectionnés. Les tableaux cliniques de la démodécie cutanée et de la démodécie de la glande de Meibomius ont été décrits. *Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Sta. epidermidis, Streptococcus pyogenes* (groupe A) et *Trueperella pyogenes* ont été isolés dans les lésions cutanées, et *Moraxella bovis* et *Sta. aureus* l'ont été dans les lésions de la glande de Meibomius. Ces bactéries produisaient des toxines et des enzymes qui aggravaient les lésions causées par les acariens *Demodex bovis* et *D. ghanensis*, respectivement dans la peau et dans les glandes de Meibomius. Aucun de ces acariens n'a été retrouvé dans les tissus internes ni dans les organes, indiquant qu'il n'y a pas eu de phase endoparasitaire. Les modifications histopathologiques observées étaient liées à l'immunité à médiation cellulaire. L'écoulement du contenu des colonies de *Demodex* dans les couches sous-épidermique et dermique, et dans les tissus environnant les glandes de Meibomius a entraîné des modifications histopathologiques sévères, caractérisées par le renouvellement continu des réactions granulomateuses avec afflux de macrophages et de lymphocytes. La pathogenèse de la maladie a été décrite du stade d'invasion initiale des follicules pileux et des tubes collecteurs des glandes de Meibomius par les acariens et les bactéries associées, au stade de régression des lésions. Il a été conclu que la nature de l'association entre les acariens *Demodex* et les bactéries dans les lésions démodéciques était synergique et que les deux agents pathogènes avaient la même importance. Le renouvellement continu des réactions granulomateuses qui caractérisaient les modifications histopathologiques a montré que les *Demodex* et les bactéries associées étaient persistants et immunogènes.

Mots-clés : Bovin – *Demodex* – Gale démodécique – Glande sébacée – Peau – Bactériose – Pathogenèse – Soudan.

Resumen

Abu-Samra M.T., Shuaib Y.A. Patología y patogénesis de la demodicosis bovina en piel y glándulas de Meibomio

Se llevó a cabo una encuesta nacional de la demodicosis bovina en 48,000 cabezas de ganado en Sudan durante campañas de vacunación (44,800) y durante exámenes ante mortem y post mortem en mataderos (3200). Entre el total encuestado, 44,908 fueron adultos (2-8 años) de los cuales 34,6% estaban infectados y 3092 fueron terneros (< 2 años) de los cuales 34,6% estaban infectados. Se seleccionaron trescientas cabezas con lesiones de piel severas, entre los cuales 218 también presentaban lesiones de la glándula de Meibomio. Se describieron las fotos clínicas de demodicosis en piel y de glándula de Meibomio. *Proteus vulgaris, Pseudomonas aeruginosa*, *Staphylococcus aureus, Sta. epidermidis, Streptococcus pyogenes* (grupo A) y *Trueperella pyogenes* fueron aislados en lesiones de piel y *Moraxella bovis* y *Sta. aureus* fueron aislados en lesiones de glándulas de Meibomio. Estas bacterias produjeron toxinas y enzimas dañinas, agravando las lesiones causadas por ácaros de *Demodex bovis* and *D. ghanensis* en piel y glándulas de Meibomio respectivamente. Ninguno de los ácaros se encontró en órganos o tejidos internos, indicando que no había fase endo parasita. Los cambios histopatológicos observados fueron correspondientes con la inmunidad mediada por células. La liberación del contenido de las colonias de sarna demodéctica hacia las capas sub epidérmicas y dérmicas de la piel y el tejido conectivo adyacente de las glándulas de Meibomio, evocaron cambios histopatológicos severos, caracterizados por reacciones granulomatosas masivas y de alta rotación con flujo de macrófagos y linfocitos. Se describe la patogénesis de la enfermedad, desde el estadio de invasión inicial por los ácaros y bacterias asociadas de los folículos pilosos y túbulos colectores de las glándulas de Meibomio hasta el estadio de regresión de las lesiones. Se concluye que la naturaleza de la asociación entre los ácaros de *Demodex* y las bacterias en las lesiones de sarna demodectica fue sinergística y de igual importancia. Las lesiones granulomatosas de alta rotación que caracterizaron los cambios histopatológicos mostraron que los ácaros de *Demodex* y las bacterias asociadas fueron persistentes e inmunogénicos

Palabras clave: Ganado bovino – *Demodex* – Sarna – Glándula sebácea – Piel – Bacteriosis – Patogénesis – Sudan.