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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

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 (10⁹ organisms/ml), and the IP group (40 chicks) was infected intraperitoneally with *Salmonella* Gallinarum (10⁹ 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.

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


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Experimental fowl typhoid in broiler chicken

**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 (21). 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 uninjected 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.

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**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 10⁸ 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.

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**Bacteriological and serological examination**

showing that particular intensity of lesion and then by dividing the total number of birds (sacrificed + dead) examined for lesions.

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**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 (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 for inducing 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.
expérimenter. Les lésions graves ont été détectées pour la première fois dans les organes viscéraux des CR à 1 DPI et ne furent pas détectées dans les OR à 3 DPI.

Les changements initiaux dans les OR incluaient la congestion des organes viscéraux, l’œdème de la rate et de la splénome. Les foyers nécrotiques sur la surface de la rate et de la splénome furent observés à 9 et 14 DPI. D’autres changements incluaient une discoloration de la rate et des nodules grisâtre en forme de nodules sur la paroi cardiaque.

Dans les IP, les changements initiaux, en plus de la congestion des organes viscéraux, incluaient l’œdème de la rate et de la splénome, et la distension de la vésicule biliaire. Les foyers nécrotiques sur la rate furent observés à 5 DPI. Des foyers nécrotiques plus petits furent observés sur la rate dès 5 DPI, mais des foyers nécrotiques plus grands étaient observés dans un oiseau qui est mort à 10 DPI (Figure 1). Des dégénérations congestionnelles et œdémateuses des reins furent observées dans les IP. Dans ce groupe, de visibles nodules grisâtre de différentes tailles étaient observés à 21 DPI projetant au-dessus de la surface du cœur (Figure 2). En général, les changements macroscopiques dans les IP ont été similaires à ceux observés dans les OR, mais les lésions étaient plus sévères et observées à des stades plus précoce et dans un nombre plus élevé d’oiseaux.

Tableau II montre l’intensité des lésions histopathologiques recensées dans différents organes des oiseaux expérimenter. Les lésions histopathologiques dans la rate de CR à 3 DPI étaient caractérisées par la congestion des vaisseaux sanguins, les hémorragies et l’infiltration mononucléaire autour des vaisseaux, ainsi que des foyers isolés de nécrose observés à 14 DPI. Les IP ont montré des lésions similaires dans la rate à 1 DPI, mais à 3 DPI, des agrégats de polynucléaires étaient observés dans le parenchyme (Figure 3). Il y avait de nombreuses zones nécrotiques plus grandes en IP à 7 DPI et 10 DPI (Figure 4).

Des zones nécrotiques importantes ont causé des dépletions sévères des lymphoides ainsi que la hyperplasie endothéliale du rein à partir de 5 DPI dans les IP (Figure 5). Le coeur a montré une dégénérescence du muscle myocardique à partir de 14 DPI et 21 DPI due à l’infiltration mononucléaire, qui était modérée à modéré dans les OR (Figure 7) et étendue en IP entraînant une atrophie, une nécrose et une remplacement des muscles cardiaques (Figure 8). Les poumons montraient une congestion interlobulaire et les hémorragies dans les parabronchi de deux groupes infectés. Les interlobulars étaient infiltrés avec des mononucléaires mélangés avec des polynucléaires. Les reins montraient une congestion, des hémorragies interstitielles, l’infiltration mononucléaire dans l’interstitium, ainsi que des modifications dégénératives modérées dans l’épithélium tubule à partir de 3 à 21 DPI en OR. Ces changements étaient accompagnés de modifications dégénératives sévères dans l’épithélium tubule à partir de 5 et 7 DPI en IP. Dans la rate de Fabricius, une dépletion sévère des lymphoides dans les follicules avec infiltration de lymphocytes dans les espaces interfolliculaires étaient notées.

<table>
<thead>
<tr>
<th>Jours postinfection</th>
<th>CR</th>
<th>OR</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
<td>4</td>
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<td>5</td>
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<td>1</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>19</td>
<td>19</td>
</tr>
</tbody>
</table>

Mortalité (%) 22.50 47.50

CR : groupe contrôle ; OR : poulets inoculés oralement avec Salmo. Gallinarum ; IP : poulets inoculés intraperitonealement avec S. Gallinarum
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 II

<table>
<thead>
<tr>
<th>Intensity score</th>
<th>Gross lesion score</th>
<th>Histopathological score</th>
<th>Mean lesion score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Spleen</td>
<td>Heart</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

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

Note: no lesions were observed in any of the organs of the control birds throughout the experiment.

### Table III

<table>
<thead>
<tr>
<th>Positive samples</th>
<th>OR</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 DPI</td>
<td>Liver 3/3</td>
<td>2/3</td>
</tr>
<tr>
<td>Spleen</td>
<td>2/3</td>
<td>1/3</td>
</tr>
<tr>
<td>Heart blood</td>
<td>2/3</td>
<td>1/3</td>
</tr>
<tr>
<td>Ceca</td>
<td>2/3</td>
<td>1/3</td>
</tr>
<tr>
<td>Feces</td>
<td>2/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

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).
Experimental fowl typhoid in broiler chicken

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PATHOLOGIE INFECTIEUSE

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 10^7 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


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 expérientalement 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 (10⁹ organismes/ml), et le groupe IP (40 poussins) a été infecté par voie intrapéritonéale (10⁹ organismes/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.


Resumen


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 intrapéritoneal. 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 (10⁹ organismos/ml) y grupo IP (40 pollos) infectados intraperitonealmente con Salmonella Gallinarum (10⁹ 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 los 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.

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-phototrophic 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

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. fercinogenes (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.

Keywords
Cattle – Mycobacterium tuberculosis infection – Slaughterhouse – Sudan.

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

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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). Sulaiman 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. paratuberculosis*, 1 (7.7%) *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-carboxylic 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. farrinogenes*.

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>Number (%)</th>
<th>Direct microscopy</th>
<th>Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>Negative (%)</td>
</tr>
<tr>
<td>Lungs</td>
<td>30 (18.4)</td>
<td>24 (80.0)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Liver</td>
<td>17 (10.4)</td>
<td>12 (70.6)</td>
<td>5 (29.4)</td>
</tr>
<tr>
<td>Spleen</td>
<td>9 (5.5)</td>
<td>8 (88.9)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>5 (3.0)</td>
<td>3 (60.0)</td>
<td>2 (40.0)</td>
</tr>
<tr>
<td>Pleura</td>
<td>6 (3.7)</td>
<td>4 (66.7)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>4 (2.5)</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>92 (56.4)</td>
<td>70 (76.1)</td>
<td>22 (23.9)</td>
</tr>
<tr>
<td>Total</td>
<td>163</td>
<td>124 (76.1)</td>
<td>39 (23.9)</td>
</tr>
</tbody>
</table>

**Table I**

Direct microscopy and isolation of *Mycobacterium* sp.
Tuberculose bovine dans les abattoirs de Nyala au Soudan

**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.

**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.

Acknowledgments

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REFERENCES


Résumé


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-alcool-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.


Resumen

Aljameel M.A., Abdel Wahab M.B., Fayza A.O., El Tigani A.E., Abdellatif M.M. Ocurrencia de tuberculosis bovina en ganado 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 inspeccionados 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, bazo, riñones y linfonodos mesentéricos. Se colectaron muestras de tejido, sea en 10% formol salina para histopatología de rutina o en paquetes de hielo para microscopía directa y cultivo. La microscopía directa mostró que 124 (76,1%) de las lesiones tuberculosas albergaron bacilos acidos-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.
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 qualité 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 de Dakar, des mesures adéquates doivent être prises afin d’orienter les moyens de prévention contre la brucellose chez les habitants.

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

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.

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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 soumis à 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 chez les bovins 12.6 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 Éthiopie (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 autochtones 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 par 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’accès 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 :

\[ P_a = P_\text{réelle} x (Se + Sp - 1) + (1 - Sp) \] et ainsi

\[ P_\text{réelle} = P_a - (1 - Sp) / (Se + Sp - 1) \]

où \( P_a \) est la prévalence apparente mesurée dans l’échantillon initial, \( P_\text{réelle} \) 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 : \( Se = Se_1 \times Se_2 \), soit \( Se = 85 \text{ p. 100} \) ; et la spécificité finale a été : \( Sp = 1 - (1 - Sp_1) \times (1 - Sp_2) \), soit \( Sp = 98,75 \text{ p. 100} \).

Analyse statistique


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...
Bovine brucellosis in suburban Dakar, Senegal

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PATHOLOGIE INFECTIEUSE

Les deux tests rose Bengale et de FC état 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).

Tableau III

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>OR (IC : 95%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2,181</td>
<td>1,08 – 4,60</td>
<td>0,031</td>
</tr>
<tr>
<td>Race</td>
<td>1,620</td>
<td>1,07 – 2,45</td>
<td>0,022</td>
</tr>
<tr>
<td>Avortement</td>
<td>1,578</td>
<td>1,04 – 2,37</td>
<td>0,028</td>
</tr>
<tr>
<td>Hygroma</td>
<td>1,271</td>
<td>1,11 – 1,45</td>
<td>0,011</td>
</tr>
</tbody>
</table>

Tableau IV

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

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

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.

Remerciements


BIBLIOGRAPHIE


Bovine brucellosis in suburban 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.
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 endemically present 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), several sporadic outbreaks of AHS have been reported in Nigeria in recent years (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 (RT-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 AHSV 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.

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

D.O. Ehizibolo1* E.C. Nwokike2 Y. Wungak1 C.A. Meseko1

Keywords

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.

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Detection of African horse sickness virus in 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.

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 1

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

<table>
<thead>
<tr>
<th>Stable (years)</th>
<th>Num. samples</th>
<th>Breed</th>
<th>Male</th>
<th>Female</th>
<th>Age range</th>
<th>ELISA positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17</td>
<td>Mixed*</td>
<td>10</td>
<td>7</td>
<td>4–10</td>
<td>17 (100)</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>Exotic</td>
<td>12</td>
<td>16</td>
<td>2–9</td>
<td>24 (85.7)</td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>Local</td>
<td>15</td>
<td>7</td>
<td>6–22</td>
<td>21 (95.4)</td>
</tr>
<tr>
<td>D</td>
<td>93</td>
<td>Exotic</td>
<td>22</td>
<td>71</td>
<td>3–13</td>
<td>80 (86.0)</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>Exotic</td>
<td>3</td>
<td>12</td>
<td>3–11</td>
<td>11 (73.3)</td>
</tr>
<tr>
<td>F</td>
<td>17</td>
<td>Local</td>
<td>16</td>
<td>1</td>
<td>4–15</td>
<td>14 (82.4)</td>
</tr>
<tr>
<td>G</td>
<td>92</td>
<td>Local</td>
<td>22</td>
<td>70</td>
<td>2–17</td>
<td>79 (85.9)</td>
</tr>
<tr>
<td>Total</td>
<td>284</td>
<td></td>
<td>537</td>
<td>404</td>
<td></td>
<td>246 (86.6)</td>
</tr>
</tbody>
</table>

* 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.

Acknowledgments

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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 équine 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 %) étaient positifs, 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.


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 África sub-sahariana 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. Seros 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 inmunabsorción ligado a enzimas (ELISA). Un alto porcentaje de los sueros (86,6%) fueron positivos, indicando una exposición continua de los caballos nigerianos a PEA. 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.
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).

Summary

A national survey on bovine demodicosis was conducted among 48,000 cattle in Sudan during vaccination campaigns (44,800), and at ante- 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.

Keywords

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 ante-mortem and post-mortem 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 ante-mortem 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, preauricular, pre-crural, 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 -20°C 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.
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

Table I

<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>Num. bacteria isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin lesions</strong></td>
<td><strong>Meibomian gland lesions</strong></td>
</tr>
<tr>
<td>Moraxella bovis</td>
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</tr>
<tr>
<td>Proteus vulgaris</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>48</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>80</td>
</tr>
<tr>
<td>Sta. epidermidis</td>
<td>34</td>
</tr>
<tr>
<td>Streptococcus pyogenes (group A)</td>
<td>22</td>
</tr>
<tr>
<td>Trueperella pyogenes**</td>
<td>10</td>
</tr>
<tr>
<td>No bacterium isolated</td>
<td>48</td>
</tr>
</tbody>
</table>

Total 300 218

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

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.

Figure 5: Section from the skin of a cow showing Demodex bovis mites in the hair bulbs (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).

![Figure 10: Typical granuloma in skin section from a cow infected with demodicectic 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.](image)

**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 demodicectic mange. This finding is contrary to that of others 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-time 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 demodicectic 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 demodicectic 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 demodicectic 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 suppurtative lesions in any organ or tissue in farm animals. Some workers (17, 26) report that these organisms produce a suppurtative 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 follicularum* 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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Bovine skin and meibomian gland demodicosis


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Démodécie bovine cutanée et de la glande de Meibomius

Résumé

Abu-Samra M.T., Shuaib Y.A. Pathologie et pathogénèse 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* (groupe A) et *Trueperella pyogenes* furent aïsiés 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 aggraveraient 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.


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 los 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 endoparasitaria. Los cambios histopatológicos observados fueron correspondientes con la inmunidad mediada por células. La liberación del contenido de las colonias de sarna demodecética hacia las capas sub epidermicas 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 folicúlos 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 demodécica 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.


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