Pathological and Molecular Investigations on Foot and Mouth Virus Outbreaks Among Cattle Herds in Dakahlia Governorate, Egypt

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Abstract

The present study represents an extensive field survey of the pathological affections caused by local Food and Mouth Disease (FMD) virus throughout Dakahlia governorate, North Egypt. The study included 670 cattle belonging to local and Frisian breeds. The age of the examined animals ranged from ten days to four years and they were of both sexes. The morbidity rates among the investigated cattle herds of either Frisian or native origin were 100% and 5% respectively. We observed early mortality in infected calves aging ten days old and older calves. This was concurrently associated with lesions of severe myocarditis which appeared to be responsible for the death. On the other hand, adult cattle showed vesicular lesions, erosions and ulcers on the mucous membrane of the mouth and skin on the feet and udder. In addition, fever, anorexia, and pstyalism were observed. Histopathological examination of both young calves and adult cattle revealed multitude of inflammatory and necrotic lesions in the myocardium, liver, lung, intestine and udder. Molecular examination and gene sequencing revealed the presence of RNA belonging to FMD virus ‘type A’ in the affected tissues. The nucleotide sequence of the isolated virus strain was submitted to the gene bank (accession number: BankIt1911105FMD/A/EGY/Dakahlia/KX083565). In conclusion, the study emphasized the importance of FMD as a viral disease induced relatively high mortality and morbidity especially in young calves and gives an account on the associated pathological lesions.

Keywords: FMD, Pathology, Dakahlia, Egypt

Introduction

Foot and mouth disease (FMD) is one of the most contagious viral diseases affecting cloven-hoofed animals [1]. The disease is characterized by the formation of vesicles in the mouth, at the coronary band of the hoof and in the skin of the interdigital cleft, accompanied by fever and lameness [2]. The virus belongs to the genus Aphthovirus of the family Picornaviridae; Order Picornavirales [3]. There are seven major FMD viral serotypes: O, A, C, SAT-1, SAT-2, SAT-3 and Asia-1.

The disease is considered enzootic in Egypt and the main prevalent serotypes are O1 and A [4,5]. FDM is notorious due to high morbidity, however, the mortality rate is usually low except in suckling animals. The mortality rate is about 5% in adult ruminants but the rate can be boosted up to 50% as a result of myocardial damage in young animals [6]. In calves, myocarditis is considered a fatal lesion of FMD- usually occurring without developing the characteristic blister lesions noted in adult cattle [7]. The transmission of FMD virus commonly occurs during direct physical contact between acutely infected and susceptible animals. It is generally accepted that the primary infection occurs via the respiratory route [7]. However, in FMD endemic countries, both the respiratory and oral routes are equally important. Cattle may carry the virus for up to five days before they develop clinical signs. However, small
ruminants mostly develop silent or clinically inapparent infection, which plays an important role in the epidemiology or spread of FMD to cattle [7].

Outbreaks of FMD cause major economic losses in animal production, such as decrease in milk production and weight gain, reproductive inefficiencies and death in young animals [8]. Additionally, the cost of prevention and control and the restrictions in both local and international trade is high. Studies contributing to a better understanding of the FMD pathogenesis and pathology are of great value for designing efficient prevention and control strategies. Therefore, the present study aimed to evaluate the pathology of the local FMD virus strains in Dakahlia governorate, Egypt, where a considerable population of cattle is reared by farmers for both milk and meat production.

**Material and methods**

**Study area and samples**

A total of 670 clinically suspected cattle with FMD virus infection were examined during disease outbreaks that occurred in Dakahlia governorate, Egypt, during the period from October 2012 to October 2015 (Table 1).

<table>
<thead>
<tr>
<th>Localities</th>
<th>Number</th>
<th>Age</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Calves</td>
</tr>
<tr>
<td>Alsemblawen</td>
<td>70</td>
<td>100</td>
<td>10 d-2m</td>
</tr>
<tr>
<td>Almanzalah</td>
<td>150</td>
<td>200</td>
<td>10 d-2m</td>
</tr>
<tr>
<td>Dekernes</td>
<td>50</td>
<td>100</td>
<td>10 d-2m</td>
</tr>
<tr>
<td>Total</td>
<td>270</td>
<td>400</td>
<td>-</td>
</tr>
</tbody>
</table>

Samples including vesicular fluid and oropharyngeal swabs were obtained from emergency slaughtered or freshly dead animals (n=70), together with tissues specimens from the heart, lungs, liver, kidneys, spleen, lymph nodes, gastrointestinal tract, digital skin and the buccal cavity mucous membrane. Tissue specimens from animals assigned for PCR detection and gene sequencing were collected in phosphate buffered saline (PBS) supplemented with viral transport medium, while the oropharyngeal swabs from the same animals were collected in PBS. The virus transport medium composed of sterile PBS, 10% glycerol and antibiotic mixture (1000 IU/mL penicillin, 100 µg/ml streptomycin and 250 µg/mL gentamycin (Gibco Invetrogen, USA). Specimens and swabs were frozen at -20°C until use.

**Virus identification by PCR and gene sequencing**

Swabs and tissue specimens were admitted to Genome Unit of the Animal Health Research Institute, Giza, Egypt, for viral identification by PCR and nucleotide sequencing. Viral RNA was extracted using QIAmp Viral RNA Mini Kit (Qiagen, USA) according to the manufacturer’s instructions. The extracted RNA was subjected to reverse transcriptase PCR using sequence-specific primers targeting the VP1 gene encoding a common capsid protein of the virus [9,10]. The VP1-specific primers were synthesized by (Metabion International AG, Germany). The amplification was carried out using the One-Step RT-PCR kit (Qiagen, USA) in Eppendorff thermal cycler (Eppendorf, Germany). The reaction conditions consisted of cDNA synthesis at 50°C for 30 min, an initial denaturation at 95°C for 15 min, followed by 50 cycles of amplification each consisted of a denaturation step at 95°C for 20 sec, an annealing step at 50°C for 50sec an extension at 72°C for 2.5 min, and a final extension at 72°C for 10 min. The PCR products were electrophoresed in 1.5% agarose gel and visualized with ethidium bromide staining.
Nucleotide sequence of the PCR-amplified VP1 gene was determined using a BigDye Terminator Kit (Applied Biosystems, Canada) on a 3130 Genetic Analyzer (Applied Biosystems, Canada). The sequence was identified by nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and submitted to GenBank with accession number: BankIt1911105FMD/A/EGY/Dakahlia/ KX083565.

**Histopathology**

Tissues specimens were fixed immediately in 10% neutral buffered formalin for histological examination. After routine processing, samples were embedded in paraffin wax blocks, from which five µm sections were made and mounted on glass microscopic slides.Slides were thereafter stained with the routine haemotoxylin and eosin (H&E) for microscopic examination [11].

**Results**

**Clinical signs**

The examined animals suffered from fever, drooling saliva, vesicular lesions, ulcer and erosions of the buccal cavity, interdigital spaces, coronary bands, udder, vulva, prepuce and lameness. Young calves showed only sudden death. The morbidity and mortality rates among the examined animals are outlined in Table (2).

**Table 2: Morbidity and mortality within the demographic groups of the examined animals**

<table>
<thead>
<tr>
<th>Age of Affected animals</th>
<th>Frisian breed</th>
<th>Mortality (number)</th>
<th>Mortality %</th>
<th>Native breed</th>
<th>Mortality (number)</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂♂ ♂♀</td>
<td>♂♂ ♂♀</td>
<td>♂♂ ♂♀</td>
<td>♂♂ ♂♀</td>
<td>♂♂ ♂♀</td>
<td>♂♂ ♂♀</td>
</tr>
<tr>
<td>Up to 2 months</td>
<td>50 80</td>
<td>40 75</td>
<td>80</td>
<td>93.7</td>
<td>25 15</td>
<td>25 15</td>
</tr>
<tr>
<td>1-2 years</td>
<td>20 100</td>
<td>5 45</td>
<td>25</td>
<td>45</td>
<td>10 20</td>
<td>5 15</td>
</tr>
<tr>
<td>3-4 years</td>
<td>10 180</td>
<td>2 9</td>
<td>20</td>
<td>5</td>
<td>20 30</td>
<td>2 2</td>
</tr>
<tr>
<td>Up to 4 years</td>
<td>- 60</td>
<td>- 3</td>
<td>-</td>
<td>5</td>
<td>10 40</td>
<td>1 8</td>
</tr>
<tr>
<td>Total</td>
<td>80 420</td>
<td>47 123</td>
<td>58.7</td>
<td>29.3</td>
<td>65 105</td>
<td>33 40</td>
</tr>
</tbody>
</table>

**Virus identification**

The results of RT-PCR for VP1 gene amplification revealed the presence of FMD virus nucleic acid in all the examined samples. Sequencing of the amplified VP1 gene identified the virus isolates as FMD virus type A. The gene sequences of the virus isolates shared 95% and 92% identity with isolates FMD/A/1D/Egypt/Al-fayoum/2013 and PAK5/2006 on the GenBank, respectively. Sequencing data obtained for one of the identified isolates was submitted to NCBI GenBank with the following accession number: BankIt1911105FMD/A/EGY/Dakahlia/KX083565.

**Gross pathology**

The adult cattle showed several gross lesions as follows: vesicles, erosions and ulcerations were observed on the mouth with ptylaism. Severe ulceration and erosions were also observed in the coronary band and heels of the digits (Figure 1A and B). Similar lesions of erosion and ulceration were also found on
the mammary glands, teat, and prepuce (Data not shown).

However, those of 2 months old showed yellowish to grayish streaking bands of myocardial necrosis, forming the characteristic tiger heart appearance (Figure 1C). In adult cattle, cardiac lesions included pericarditis with the presence of petechiae on myocardium. The lungs showed grayish, firm patches on the surface, while, the liver was severely enlarged and congested. The spleen was enlarged and showed multifocal irregular grayish white necrotic areas on the surface. The ruminal pillar showed hemorrhagic and eroded areas, besides clear ulcers (Figure 1D).

![Figure 1: Gross lesions in animals naturally infected with FMD. (A, B) Sole and coronary bands of infected adult cattle (2 years old) showing necrotizing dermatitis with erosions and ulceration (arrow). (C) A heart of young calve (2 months old) showing alternating yellowish to grayish streaks giving rise to the characteristic tiger heart appearance (arrow). (D) The rumen of adult cattle (3 years old) showing hemorrhage and congestion on the ruminal pillar (arrow).](image)

**Histopathology**

Severe myocardial infarction (coagulative necrosis) was observed in young calves with the presence of edema and multifocal infiltration by lymphocytes and neutrophils (Figure 2A and B). The tongue showed lesions of coagulative necrosis in the superficial epithelial lining, tongue papillae and taste buds. Some cases involved necrosis in the basal cell layers with exposure of the sub epithelial structures. Vesicle formation containing edema, fibrin and little inflammatory cells was also observed (Figure 2C). The coronary bands and interdigital spaces showed focal damage of the cornified epithelium with exposure of the sub epithelial connective tissue (necrotic dermatitis). The latter contained living and dead neutrophils with edema besides partial necrosis. In addition, partial necrosis of muscles and the presence of dark brown pigment engulfed by macrophages were noticed.
Lesions of severe pneumonia were observed in the lungs with the presence of serofibrinous exudates and leukocytic infiltration within the alveoli. Alternating areas of emphysema and atelectasis were also observed (Figure 3A). Thrombosis and edema in the pulmonary blood vessels of alveolar septa with the presence of exudates inside the alveoli with necrotic debris were noticed (Figure 3B).

The liver revealed thickening of the hepatic capsule by collagen and later on undergone necrosis (Figure 3C). In addition, degenerative change and necrosis in hepatic cells portal fibrosis were observed (Figure 3D).

Diffuse areas of coagulative necrosis, leukocytic infiltration, and edema were observed in the intestinal mucosa and extended deeper to submucosal glands and sometimes the inner muscular coat. Discontinuation of the intestinal epithelium was also observed with the presence of desquamated villi in the lumen (Figure 3E and F).

The udder showed mild subacute mastitis, while some acini were encircled by edematous fluid containing few lymphocytes. The interstitial connective tissue contained focal aggregation by lymphocytes, fibroblasts and collagen fibers, which sometimes replaced the acini indicating regenerative attempts. The secretory ducts showed fibrous thickening of their wall with the presence of inflammatory cells (Figure 3G and H).
Discussion

In the present study, 670 cattle from various farms located at Dakahlia governorate, Egypt, were examined for the pathology caused by the local FMD virus isolates during outbreaks (Table 1). The investigation based on clinical signs and gross pathology and was then confirmed using PCR detection and gene sequencing of FMD virus VP1 gene. The results revealed the identification of FMD virus isolates serotype A, which has been responsible for many disease outbreaks in Egypt recently [4,5]. However, due to the limited number of the sequenced samples, the involvement of other virus strains such as SAT2 and O that were previously reported in Egypt cannot be precluded [13].

Diseased calves, aging below six months old, showed 100% mortality and showed signs of sudden death with no apparent gross lesions such as the vesicle formation in the mouth and the feet. However, the majority of them showed signs of myocardial necrosis, which was manifested as yellowish to grayish streaking that are commonly described as the ‘tiger heart’. This characteristic lesion has
been typically observed in FMD virus infection in young calves in other studies [14]. The lesion of myocardial infarction can be correlated to the sudden death and high mortality observed in calves. Therefore, in agreement with Thomson et al. [14], heart failure as the primary cause of death is expected.

Regarding the microscopic examination, the heart of a few adults showed patches of myocardial necrosis, infiltration with few lymphocytes, macrophages and fatty infiltration between necrotic cardiac muscle fibers with the presence of lipofuscin pigment within degenerative and necrotic myocarditis. These lesions are in agreement with those previously reported by Oem et al. [15]. Moreover, the fore mentioned cardiac lesions or gastroenteritis could be responsible for the recorded deaths in adult animals our study.

The tongue showed coagulative necrosis, which involved epithelial and subepithelial structures and vesicle formation containing edema with little inflammatory cells, which are consistent with the findings obtained by Lee et al. [16]. Also, the obtained results coincide with those reported by Jonthan et al. [17] who described extra- and intra-cellular edema mixed with leucocytes infiltration with the presence of vesicular cavities containing variable quantities of fibrin and necrotic depress. These lesions consider the primary target sites for viral infection and replication [18].

Adult cattle showed oral vesicles and erosions surrounded by hyperemic zone. Similar lesions were also observed at the coronary bands, skin of the interdigital space, and the udder. These lesions are typical for FMD virus infection in adult cattle. In agreement with previous reports, other less common lesions such as hemorrhage and ulceration of the rumen and abomasum and necrosis and congestion of the gastrointestinal tract were observed [18, 19]. In our opinions the latter lesions may be due to secondary bacterial infection or follow systemic spread or new targets for viral replications.

Microscopically, skin of the coronary bands revealed focal destruction epithelial and subepithelial structures with the presence of inflammatory cells, which reveals lesions of necrotic and ulcerative dermatitis. This finding is in complete agreement with those previously reported by Oem et al. [15]. The skin interdigital space and muscles showed coagulative necrosis with inflammatory cell infiltration, which is consistent with the findings of Rhyan et al. [20] and Oem et al. [15] who described necrosis in the interdigital space besides losses of basal epithelium with the marked mixed inflammatory cells which were infiltrated in skin and muscles. The formentioned changes in skin and muscles of hooves are responsible for lameness.

The lungs showed variable types of pneumonia with neutrophilic and lymphocytic infiltrations, alternating areas of alveloar atelectasis and emphysema, interstitial edema and presence of pulmonary thrombi. These results coincide with those obtained by Brown et al. [21]. The pulmonary lesions in our study usually follow systemic infection and thrombosis.

Examination of the liver revealed thickening of the hepatic capsule by collagen and incidence of necrosis. Hepatic cells showed degenerative changes, while, the portal areas showed infiltration by lymphocytes and macrophages as well as fibrosis. This comes in agreement with the results reported by Brown et al. [21] who recorded venous congestion in addition to centrolobular coagulative necrosis, fatty changes and some portal area infiltrated by few lymphocytes. Moreover, the lesion in the hepatic capsule and parenchyma (fibrosis) due to concomitant infection by other pathogens.

The intestine revealed diffuse coagulative necrosis in mucosa, while the necrotic process extended to the submucosal glands and sometimes the inner muscular coat. The latter is in complete agreement with the findings of Brown et al. [21]. Our work added that lesions in fore stomach abomasum and intestine may be considered another target sites for viral
replications by the virus. The present study revealed that the mammary glands showed sub-acute mastitis the later lesions may be due to secondary bacterial infections or loss of udder and teat skin vitality by FMD isolate.

Conclusion

The present study highlights the importance of FMD as a viral disease causing high mortality and morbidity, especially in young calves, and gives an account on the associated pathological lesions. It seems that myocardial necrosis is the cause of death in young calves and adult animals, since it is the main systemic pathological lesion beside teat and GIT observed in these animals. Moreover, repeat outbreaks among different farms declared no vaccination or failure of vaccination.

Conflict of interest

None of the authors have any conflict of interest to declare.

Acknowledgement

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References


الملخص العربي

دراسات باناثولوجي وجزيئي على تفشي فيروس الحمى القلاعية في قطعان الماشية بمحافظة الدقهلية في مصر

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قدمت هذه الدراسة مشاكل واسعة من الإصابات المرتجلة التي تسببها سلالات فيروس مرض الحمى القلاعية المحلية (FMD) في أنحاء محافظة الدقهلية في شمال مصر. وشملت الدراسة 350 من الماشية التي تتنوع إلى السلالات المحلية أو الفريزين. وتراوح عدد الإصابات من 10 أيام إلى أربع سنوات، وشملت كلا الجنسين. وكانت معدلات الإصابات والوفيات بين الماشية المصرية 100% و 5% على التوالي. لاحظنا نفوذ مبكر في العجول المصابة في عمر عشرة أيام وارتفاع هذا النفوذ مع الحفاظ على مرض القلاعية لمدة طويلة. نظرًا للإصابة، أظهرت الدراسات الماشية البالغة أساتز حوضيالية، تقلبات وترسوت جلد في القدم والقدمين والضرع، بالإضافة إلى مرض القلب والكبد والرئة والأعصاب، والعيون، واللثاء. وكشف فحص الأنسجة العديد من الأفات الالتهابية، وتشمل في عضلة القلب والكبد والأوعية والضرع. تشمل الفحص الجزيئي والتحليل الجيني وجود الحمض النووي التابع لفيروس الحمى القلاعية نوع A في الأنسجة المصابة. تشمل النوكليوتيدات من سلالات الفيروس المعزول داخل عين الحانين وحصل على الرقم المرجعي (BankIt1911105FMD/A/EGY/Dakahlia/KX083565) في الاستنتاج الدراسة أدت على أهمية مرض الحمى القلاعية كمرض فيروسي مع ارتفاع معدلات الإصابة والوفيات وخصوصا في العجول الصغيرة المصاحبة باقات باناثولوجية.