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Epidemiological investigation of foot-and-mouth disease outbreak in a vaccinated Egyptian dairy herd with analysis of associated risk factors

Omnia Hamdy Muhammad Refaei [1], Ausama Abdelraouf Abdelmoneim Yousif [2,*], Yamen Mohammed Hegazy [3], Soliman Mohammed Soliman [1], Sayed Ahmed Hassan Salem [4] and Adel Abdel-Azim Mahmoud Fayed [1]

[1] Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt
[2] Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt
[3] Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Kafr El-Sheikh University, Kafr El-Sheikh, 33516, Egypt
[4] Arab Organization for Agricultural Development, Giza, Egypt

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Abstract

This study was conducted to investigate an outbreak of foot-and-mouth disease (FMD) in a vaccinated dairy herd (n = 4,145) and to identify the associated risk factors. Foot-and-mouth disease virus (FMDV) A Asia Iran-05 and SAT2 Libya 12-like viral RNAs were detected in clinical samples. Our data indicates that the outbreak occurred due to introduction of a field virus into cattle with minimal matching protective immune response. Previous vaccination with a multivalent vaccine did not prevent replication of a field virus that is an antigenic match to one of the vaccine seed viruses; with subsequent development of a mixed infection. The total cumulative incidence for the 31-day follow up period was 49.8% and the total mortality rate was 0.8%. The total incidence rate was 21 cases/1,000 cows/day, with confidence interval (CI) 20.32, 22.15. Analysis of epidemiological data revealed that lactation is the primary factor in disease development and mortalities in dairy herds ($P < 0.005$), possibly due to increased frequency of exposure and higher virus loads. Within this group, cows with 1 parity are more vulnerable in terms of disease development (relative risk 1.2, CI 1.121, 1.285) but not mortalities ($P = 0.359$). Correlations between FMD development and age should only be considered in the context of the reproductive state. Our analysis revealed that a reduction of the overall disease impact can be achieved by reduction of virus burdens in farms during outbreaks.

Key Words: Dairy cattle, Dual infection, Epidemiology, Foot and Mouth Disease, Risk factors

Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-footed domestic animals and more than 70 wild animal species. The disease is caused by foot-and-mouth disease virus (FMDV), a naked icosahedral virus with a linear, positive-sense, single-stranded RNA genome that belongs to *Aphthovirus* of family *Picornaviridae* [31]. The virus exhibits a high degree of genetic and antigenic diversity, therefore seven immunologically distinct FMDV serotypes (A, O, C, SAT1, SAT2, SAT3 and Asia 1) were identified with multiple subtypes within each serotype [27]. There is only partial or no protection between serotypes and subtypes of the same FMDV

* Corresponding author: Ausama Abdelraouf Abdelmoneim Yousif
Department of Virology, Faculty of Veterinary Medicine, Cairo University, 12211, Egypt
E-mail: ausama_yousif@yahoo.com; ausama_yousif@cu.edu.eg
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serotype [33]. FMD causes heavy economic losses particularly in dairy farms due to loss of milk production, deaths, lower fertility, abortions, and diagnosis, treatment and vaccination costs [22].

In Egypt, three FMDV serotypes (A, O and SAT2) have established an enzootic state. The national FMD control program relies on mass vaccination of all cattle, buffalo, sheep and goat populations [2]. Multivalent vaccines are used in control programs to circumvent the limited cross-protection between circulating serotypes and subtypes of FMDV [5]. Government-sponsored vaccination campaigns use an inactivated local trivalent vaccine (containing O Pan-Asia 2, A Iran/05, and SAT2 Ghb-12 lineage seeds) in combination with a monovalent vaccine containing SAT2 Lib-12 lineage seed [26]. Alternatively, some farms rely on an imported hexavalent vaccine (containing O Manisa, O-3039, A Iran 05, A Saudi 95, SAT2 Eritrea, and Asia1 Shamir seeds) [24].

Aside from the introduction of new viruses by transboundary events, vaccination coverage is suboptimal, particularly among sheep and goats [2]. Furthermore, FMDV mutation may be contributing to the emergence of new viruses that can replicate in apparently protected vaccinated animals [4]. The co-circulation of FMDV strains from different pools in Egypt paves the way for mixed infections of animals with different FMDV serotypes [3]. The continuous viral evolution, the variability of trading patterns [25], and the suboptimal vaccine coverage lead to the dynamic and complicated FMD epidemiological situation that results in the yearly recurrence of FMD outbreaks in Egypt.

Various risk factors associated with FMD were investigated, such as age, sex, breed, history of contact with wild animals, mixing of different species, farming system, seasonal influences and animal movement [13, 18, 23, 29]. However, very little information exists about the risk factors associated with FMD in Egyptian herds. A study conducted in Egypt demonstrated the significant impact of breeding purpose and locality on FMDV infection [11]. No such studies were conducted in Egyptian dairy herds, where the production life is much longer than fattening herds and the stressors are diverse.

This study was conducted to investigate an outbreak of FMD in a large vaccinated dairy herd in order to identify the epidemiological determinants of such an outbreak and the risk factors associated with mortalities and morbidities, and to suggest enhancements of current practices to reduce the overall impact of FMD in dairy herds.

Materials and Methods

Study population and case definition

An epidemiological study was conducted on the FMD outbreak in a cattle dairy farm located on Cairo Alexandria desert road. The farm population was about 4,145 female cattle allocated as 2050 heifers (female cattle that have never calved), 1798 milking cows, and 297 dry cows. The index case was reported on December 1st, 2018, and the follow-up continued for 30 additional days. Male calves were excluded because they were sold shortly after birth.

Animals were considered clinically infected according to FAO guidelines [12]. A clinical FMD case was the one showing two or more of the following signs: salivation, smacking of lips, vesicular lesions in the mouth, lameness, and sharp decline in milk production in lactating cows. Dead animals were considered FMD-related mortalities if the following was observed: 1) vesicles and/or blisters in the mouth, muzzle, nostrils, teats, udder, or coronary bands; and/or 2) erosions on rumen pillars and/or myocardial necrosis. Dead animals showing signs of any other cause of death without FMD lesions were not considered FMD-related mortalities.

Five samples were collected from vesicular lesions of five randomly selected cattle on the 14th day of the outbreak and sent to the National Foot-and-mouth disease Reference Laboratory, Dokki, Giza, for nucleic acid detection using RT-PCR, and sequencing (Supplemental File S1). All procedures were done in accordance with Cairo

University Institutional Animal Care and Use Committee (Approval # VetCU11112018012).

Farm description

The farm consisted of 2 blocks about 2 km away from each other. The first contains pens housing growing, insemination, and pregnant heifers. The second contains pens housing milking cow, dry cows, close-up heifers, and weaned calves, in addition to hutches of new-born calves. The farm contains two milking parlours located close to lactating cows pens. Cows are milked three times per day. The farm applied standard biosecurity measures; including disinfection of vehicles and restriction of visitors. No new animals were introduced into the farm prior to the outbreak. Special attention was paid to the management of calves. The milk given to calves was pasteurized, nipples were sterilized before feeding calves, sufficient quantity and quality of colostrum was given to calves within the first hour after birth, calves were housed in hutches managed by dedicated farm workers, and calf enclosures were frequently cleaned and disinfected.

The farm kept regular FMD vaccination programs using an imported hexavalent vaccine containing O Manisa, O-3039, A Iran 05, A Saudi 95, SAT2 Eritrea and Asia1 Shamir seeds. Calves were vaccinated at 2 months of age and given a booster vaccination 21 days later. Annual herd vaccination was performed 3 times a year. All cattle scheduled to be vaccinated had received vaccination according to standard procedures.

Data collection and statistical analysis

Data on animal age, reproductive state, number of parities, vaccination history, FMD infection history, farm biosecurity measures, and management procedures were collected. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY). The OpenEpi online software, Version 3.0, was used to estimate the 95% confidence interval values [30].

The total and daily cumulative incidence and the incidence rates for the FMD outbreak in the examined farm were calculated according to the following equations [9]:

$$\text{Cumulative Incidence} = \frac{\text{Number of new cases of disease during specified period}}{\text{Size of population at risk at start of period}} \times 100$$

While

$$\text{Incidence rate} = \frac{\text{number of new cases observed over the follow-up period}}{\text{the accumulated sum of all individuals time at risk}}$$

The denominator represents the sum of the time that each animal stayed under observation from the beginning of follow-up period until become clinically diseased or died or removed from the herd.

The influence of age, reproductive state (heifers, lactating, or dry cows), and number of parities on the clinical FMD and mortalities were analysed by the Chi-square test of independence. The relative risk (RR) estimates of morbidity and mortality among cows of different age groups, reproductive states and parities were calculated according to the following equation [16]:

$$(D1/N1)/(D2/N2)$$

Where D1/N1 is the cumulative incidence of FMD morbidity/deaths within an age, reproductive, or parity group. While D2/N2 is the cumulative incidence of FMD morbidity/deaths in the baseline groups. Cattle < 2 years, heifers, and cows with 3 parities or more were used as the baseline groups for age, reproductive state, and parity variables, respectively.

Kaplan-Meier curves for mortality among cattle with different age groups, reproductive states and number of parities were generated to demonstrate the pattern of infected animal survival during the 31 days follow-up period. The difference between survival curves patterns of different groups over time was compared using the log-rank test. Pairwise groups for each variable were compared and the threshold *P*-value was adjusted using Bonferroni correction method according to the following equation

$$P_i \leq \alpha / n$$

Where P_i is the adjusted *P*-value, n is the number of comparison groups and $\alpha = 0.05$ [32].

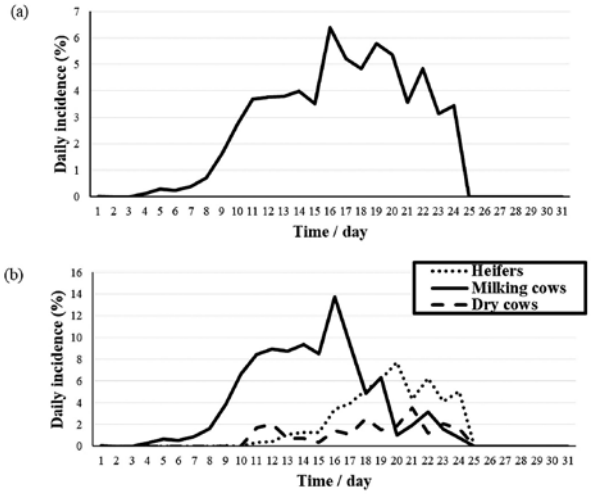

Fig. 1.
Epidemic curves for the FMD outbreak along the follow-up period (31days). The FMD new clinical cases presented as daily incidence. The numerator is the new cases appeared in specific day and the denominator is the animal at risk in the same day. (a) Epidemic curve for all cattle in the farm. (b) Comparing epidemic curves of cattle with different reproductive states.

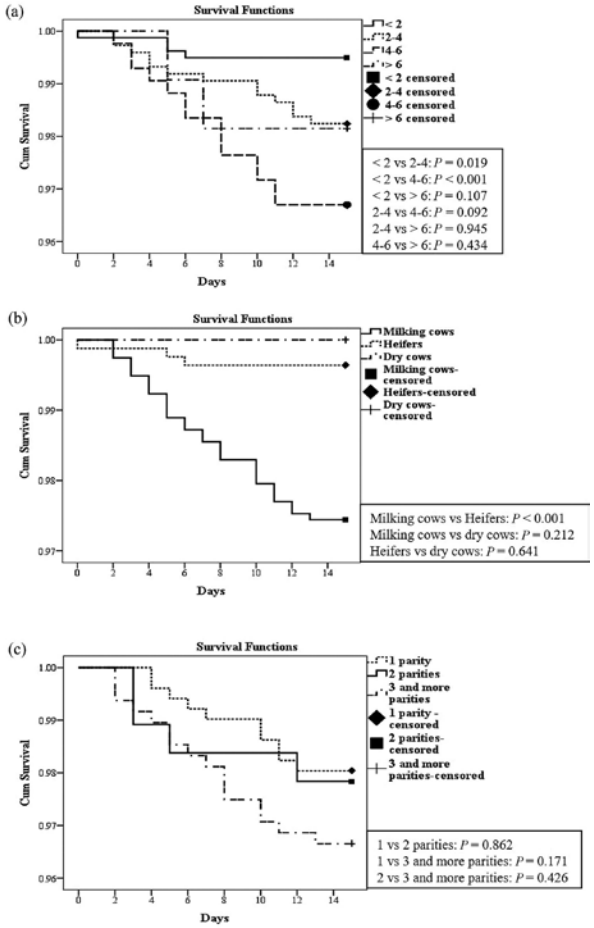

Fig. 2.
Kaplan-Meier survival curves and log-rank test *P*-values for the pairwise comparison groups of each variable. (a) Survival curves for groups with different ages. The Bonferroni-adjusted *P*-value ≤ 0.008 (b) Survival curves for groups with different reproductive states. The Bonferroni-adjusted *P*-value ≤ 0.017. (c) Survival curves for groups with different number of parities. The Bonferroni-adjusted *P*-value ≤ 0.017.

Results

Clinical signs and post-mortem findings

FMD cases showed a variety of clinical symptoms including fever, drooling of saliva, lameness, and vesicular lesions and erosions on the oral mucosa, nasal mucosa, muzzle, and inter-digital skin. Lactating cows showed vesicles, erosions and ulcerations of the teats. A marked decrease in milk production was evident, especially in cows with foot and/or udder lesions.

Thirty-three animals (3 heifers and 30 lactating cows) died after showing clinical FMD. The index case and one of the cases detected on the 4th day of the outbreak died shortly (within 2 to 3 days) after serious clinical illness. Post-mortem examination revealed myocarditis and necrosis of the cardiac muscles (tiger heart), pulmonary congestion, and ulcers on the pillars of the rumen.

Outbreak description

The index case was reported on the 1st of December 2018 (2 months after the last farm FMD vaccination campaign), and the last clinical cases were reported on the 24th of the same month. FMDV A Asia Iran-05-like and SAT2 Libya 12-like viral RNAs were detected in clinical samples (GenBank Acc. Numbers MT508912 and MN889525, respectively). A/EGY/2018 (MT508912) had 96.9%, 96.3%, 96.1%, 95.9%, 93.9% and 93.1% nucleotide identity with A/LIB/2009 (KF112913), A/IRN/2009 (KF112908), A/EGY/2012 (KC440882), A/BAR/2008 (FJ755010), A/EGY/2018 (MH732982) and A/IRN/2005 (EF208769), respectively. SAT2/EGY/Alexandria/2018 (MN889525) had 97.6%, 97.2%, 95.6%, 95.6% and 90.9% nucleotide identity with SAT2/EGY/Beni-Suef/2017 (MK975972),

Table 1. Relative risk (RR) estimates of morbidity in cattle with different age groups, reproductive states and number of parities. The significantly higher groups are bold. Frequencies of FMD clinical cases were used for the Chi-square test of independence (χ^2).

Variable	Total number	FMD clinically diseased cattle (%)	RR (95% CI)
Age/year [a]			
<2	1,699	792 (46.6)	1(baseline group)
2 - 4	1,244	739 (59.4)	**1.27 (1.19, 1.365)**
4 - 6	829	424 (51.2)	**1.1 (1.009, 1.193)**
> 6	373	108 (29)	0.62 (0.5257, 0.734)
Total	4,145	2,063 (49.8)	
Reproductive state [b]			
Heifers	2,050	830 (40.5)	1(baseline group)
Milking cows	1,798	1,173 (65.2)	**1.61 (1.514, 1.715)**
Dry cows	297	60 (20.2)	0.5 (0.3956, 0.6293)
Total	4,145	2,063 (49.8)	
Number of parities [c]			
1	665	510 (76.7)	**1.2 (1.121, 1.285)**
2	385	185 (48.1)	0.75 (0.6689, 0.8453)
3 and more	748	478 (63.9)	1(baseline group)
Total	1,798	1,173 (65.2)	

[a] $\chi2 = 118.234$, $P < 0.001$
[b] $\chi2 = 346.621$, $P < 0.001$
[c] $\chi2 = 89.200$, $P < 0.001$

SAT2.EGY/Alexandria/2018 (MK493347), SAT2/ETH/2018 (MN987552), SAT2/LIB/2012 (MH814723) and SAT2/LIB/2012 (JX570633), respectively. (Supplemental File S2)

The total cumulative incidence of FMD in the infected farm was 49.8% (n=2,063). The first day incidence (FDI) of FMD clinical infection was 0.024% (n = 1). No clinical cases were reported afterwards until the 4th day of the outbreak, then the daily incidence continued to increase until it reached its peak (6.37 %, n=205) on the 16th day (Fig. 1. a).

The outbreak started in pens housing lactating cows. The index case was a 3.5-years old cow with two parities. The initial clinical cases were clustered in the pens housing the lactating cows around the index case. Cases in heifers and dry cows commenced on the 9th and 11th day of the outbreak, respectively (Fig. 1. b). By the 19th day, cases started in pens holding calves less than 2 months old. The incidence rate was 21 FMD cases/1000 cows/day, 95% confidence interval (CI) 20.32, 22.15.

Risk factors identification and survival analysis

The association between clinical FMD infection and different risk factors related to dairy herds, including age, reproductive state and number of parities, was found to be statistically significant ($P < 0.05$). The older animal groups (2-4 and 4-6 years) were found to be at higher risk of clinical FMD infection than those of less than 2 years. Lactating cows were more likely to be affected by FMD than heifers and dry cows. On the other hand, the morbidity of FMD among cows with one parity was significantly higher than that with two or more parities (Table 1).

The total mortality was 0.8% (n =33). RR estimates showed that the mortality among cattle aged 2-4 and 4-6 years was significantly higher than those under 2 years of age (Table 2). Moreover, infected cows aged 4-6 years were associated with poorer survival (log-Rank test: $P < 0.001$) than those under 2 years of age. (Fig. 2. a). There were no deaths in dry cows. Milking cows were associated with significantly higher mortality (Table 2) and poorer survival rate (Fig. 2. b) compared to heifers

Table 2. Relative risk (RR) estimates of mortality in cattle with different age groups, reproductive states and number of parities. The significantly higher groups are bold. Frequencies of FMD deaths were used for the Chi-square test of independence (χ^2).

Variable	Total number	FMD deaths (%)	R.R.(95% CI)
Age/year [a]			
<2	1,699	4 (0.24)	1(baseline group)
2 - 4	1,244	13 (1.05)	**4.439 (1.451, 13.58)**
4 - 6	829	14 (1.69)	**7.173 (2.369, 21.72)**
>6	373	2 (0.54)	2.28 (0.4187, 12.39)
Reproductive state [b]			
Heifers	2,050	3 (0.15)	1(baseline group)
Milking cows	1,798	30 (1.67)	**11.40 (3.486, 37.29)**
Dry cows	297.5	0.5[d]	0.98 (0.05092, 18.98)
Number of parities [c]			
1	665	10 (1.50)	0.7 (0.3213, 1.538)
2	385	4 (1.04)	0.49 (0.1635, 1.443)
3 and more	748	16 (2.14)	1(baseline group)

[a] $\chi 2 = 16.421$, $P = 0.001$
[b] $\chi 2 = 26.104$, $P < 0.001$
[c] $\chi 2 = 2.049$, $P = 0.359$
[d] no reported deaths among dry cows so 0.5 was added to enable calculation 8).

($P < 0.001$). There was no significant relationship ($P = 0.359$) between the number of parities and FMD mortality (Table 2) or survival rate (Fig. 2. c).

Discussion

The aim of the current work was to conduct an epidemiological investigation of a confirmed FMD outbreak in an organized dairy farm with analysis of associated risk factors. The outbreak under investigation occurred in a farm that adopted regular FMD vaccination using an imported vaccine known to have high potency (6 PD_{50} of each strain used in vaccine formulation). However, the calculated incidence proportion (49.8%) indicated a clear case of vaccination failure as defined by the world organisation for animal health (OIE) [19].

Another indication of the absence of proper protection in the herd is the low FDI value. According to a previous report [17], FMD infected herds with low FDI and severe clinical disease are expected to have no subclinical disease before the first clinical diagnosis and have low or no vaccinal protection. Our FDI was the lowest possible (only one animal was infected), and the daily incidence started to increase dramatically after three days. Moreover, cases in the first 4 days of the outbreak, including the index case, showed severe clinical signs. Hence, we can conclude that vaccine-induced response was inappropriate, and that there was probably no subclinical circulation of the FMDV responsible for the outbreak prior to the appearance of clinical cases.

A mixed infection with SAT2 Lib-12-like and A Iran-05-like FMDV was demonstrated. Outbreaks caused by SAT2 Lib-12 lineage were reported in many parts of Egypt in the same year [26]. However, the seed strain (SAT2 Eritrea) included in the vaccine used in the farm had an r1 value of 0.8 with SAT2 Lib-12 Egyptian strains [34]. Such a vaccine would then be expected to provide protection against the circulating SAT2 strain. A vaccine seed with r1 value ≥ 0.3 is expected to protect against challenge with the strain that is antigenically homologous to the circulating strain used in r1 value calculation; determination of antigenic relationship between the field strain and the vaccine strain using two-dimensional virus neutralization tests can indicate the potential for

protection against challenge following vaccination [19]. On the other hand, vaccine matching strain-differentiation reports show little matching with the Asian serotype A FMDV strains circulating in Egypt since 2010 [34]. Therefore, vaccination failure is likely a result of mixed infection rather than poor vaccine storage, or improper administration. The latter two possibilities are unlikely because of the management system in place.

The actual route of FMDV introduction into the farm was unknown. However, airborne infection was initially excluded because only one case was observed for the first three days; infection of multiple animals would be expected if the farm was exposed to plumes carrying infectious viruses [28]. Even though this was the conclusion of the research team, an argument might be made that one animal was the index case, but other animals might have gotten exposed around the same time without showing clinical disease. If the latter argument were true, then the number of cases reported on day 4 would have been bigger, especially that at the end of the outbreak about 50% of the animals turned out to be susceptible to clinical disease. Another evidence to support the exclusion of airborne introduction is the clustering of clinical cases in the early phase of the outbreak in lactating cow pens.

The scenario by which farm workers introduced the virus is more probable. Farm workers can transmit FMDV indirectly to susceptible animals if proper personal hygiene is not followed prior to work with animals. FMDV particles can survive on clothing materials, then become suspended as aerosols and act as a source for infection. Additionally, FMDV may be obtained from the human respiratory passages for up to 48 hours after exposure to infected animals [6].

As the index case remained the sole case for 3 days, it would be reasonable to conclude that shedding occurred for almost 4 days before disease development [10]. Subsequently, it took around 5-7 days from the first infection to the appearance of clinical disease in the first cluster of lactating animals (the cases that were recorded on day 4). This falls within the range of days reported for FMDV incubation [1]. Moreover, some farm workers were allowed to go home after work and come back every day. Their exposure to their own livestock may have facilitated the primary introduction since their animals are usually less protected by routine vaccination, they use manual milking techniques, and they usually slaughter animals at home; all are risk factors for infection of humans

In our study, lactating cows were the first animals to be infected, and the primary source of infection to other parts of the farm; it took 8 days for FMD to appear in other groups. By that time, the virus would have spread to most groups of lactating animals within the farm due to routine visits to milking parlours. On the 9th day, the disease appeared in the heifer yards about 2 Km away and not in other pens closer to pens holding lactating cows. The reason for this might be the sharing of workers and farm equipment between different yards, except for calving units.

There was a strong correlation between the reproductive state and clinical disease development. Lactating cows are exposed to udder injuries during milking [10]. Moreover, claw injuries, laminitis, and lameness are commonly described problems in lactating cows [15]. These injuries are additional ports for FMDV entry to milking cows, compared to heifers and dry cows. Mechanical transmission of FMDV via cutaneous and mucosal abrasions has been documented [1].

It is also worth noting that milking cows are potent virus shedders, and milk from infected cows is an important source for FMDV. Lactating cows excrete FMDV in milk for up to 4 days with a concentration of up to $10^{6.6}$ $TCID_{50}$/ml before the appearance of vesicular lesions [10]. Experimental evidence showed that the FMDV infective dose was only 10 $TCID_{50}$ for the airborne infection and 100 $TCID_{50}$ for the mucosal or cutaneous infection [1]. The possible contamination of the milking machine cups and people handling infected milk, hand stripping before milking, and dropping of infected milk from the udder of the infected cow after milking support rapid and high transmissibility

of FMDV between milking cows within a milking station. The aggregation of lactating cows three times/day in the milking parlour and waiting area before milking also increases virus transmission between lactating cows. In addition, farm policy allowed regrouping of milking cows according to their productivity despite recommendations for strict animal movement [13]. It is worth mentioning that routine interventions applied at the beginning of the outbreak, including disinfection, multivitamin supplementation, and antibiotic treatment appear to have failed to curb virus spread, probably because widespread infection had already occurred prior to the implementation of the interventions. No other FMD-specific interventions were applied in the farm.

Interestingly, RR analysis showed that cattle aged 2-6 years old were at higher risk of clinical disease development and mortality than young cattle (<2 years). However, data from dry cows point to another direction. Dry cows were the least group affected by clinical FMD, and no mortalities were recorded within this group. Dry cows are most certainly above 2 years, therefore, reliance on age-related statistics could be misleading without consideration of the production state.

Our results showed a significant increase in FMD frequency, but not mortality, in cows with one parity. These cows are unfamiliar with milking parlours, dairy workers, and the milking process [7]. They also must overcome the change in their routine and compete with older experienced cows to eat and lie. This stress results in higher adrenal activity following their introduction to the lactating herd, especially in the first month after parturition [14]. Thus, this category of animals would also be subject to increased risk of disease development when challenged with a virus that it has little or no immunity to.

Since younger animals are classically more susceptible to sever disease than older animals, it is reasonable to conclude that good management practices succeeded in alleviation of the disease outcome despite exposure to the virus. We believe that the primary mechanism must have been reducing the virus load and/or changing the method of exposure. This means that good farm management during FMD outbreaks will reduce disease outcome.

An overall view of the epidemic curve will show that it has the classical characteristics of a new virus introduction into a susceptible herd [20]. The earlier peaking of clinical cases in lactating animals is consistent with the first case being in this category, and with a particularly heavy virus load and a higher rate of exposure. A flatter disease curve in other animal groups reflects a reduced stress level, and a different exposure mechanism/level, especially since all animals were vaccinated equally around the same time before the outbreak.

What appears to be a sudden disappearance of new cases at 24th day of outbreak is not surprizing. It is consistent with the drop in virus shedding pattern reported by other research groups in controlled challenge experiments [20]. The sudden disappearance of new cases in all animal categories also means that exposure was widespread in all animal categories following virus introduction in lactating animals. Hence, all animals were infected, and the pool of susceptible animals was depleted.

Taken together, it is evident that the rapid and high-level of exposure of animals carries dire consequences for dairy herds. It increases incidence, disease severity, and dilutes the veterinary care for animals. Therefore, it is essential to adopt modified routine and emergency (immediately after FMD detection in farms) management approaches for lactating animals. Examples of the suggested modifications include: 1. Enhancement of the disinfection routines between animals to reduce virus loads in the environment, especially around viral portals of entry. 2. The use of portable milking machines in isolation spaces to prevent contamination of milking parlours and to reduce spread within the farm. 3. Application of early disease detection technologies in farms, including milk tank testing, and general animal health monitoring, to provide enough time for application of emergency measures and reduction

of the total disease impact on animals. Last, but not least, it may be necessary to change vaccine technology to enhance dairy animal mucosal immunity especially in endemic areas, and to revise the criteria of vaccine efficacy evaluation to include young lactating animals.

The current study has two seemingly apparent limitations, sample size and potential disease reporting bias. While it is always beneficial to increase sample size, the farm is a large farm, made up of different yards, which gives us the opportunity to draw conclusions with confidence. Furthermore, focus on the analysis of data from a single large farm reduces the number of variables that have to be included in the analysis. The potential disease reporting bias for Day 0 in survival analysis on the other hand could not have been avoided. FMD in individual animals is mostly diagnosed based on clinical manifestations once the presence of the virus in the farm has been confirmed in the laboratory [21]. In addition, field veterinarians can easily identify FMD because no other vesicular diseases exist in Egypt and the cost of laboratory confirmation of individual animal infections is prohibitive.

In conclusion, our data indicates that the outbreak occurred due to introduction of a field virus into cattle with minimal matching protective immune response. Previous vaccination with a multivalent vaccine did not prevent replication of a field virus that is an antigenic match to one of the vaccine seed viruses; with subsequent development of a mixed infection. Analysis of epidemiological data revealed that lactation is the primary factor in disease development and mortalities in dairy herds, possibly due to increased frequency of exposure and higher virus loads. The analysis also shows that cows with 1 parity are more vulnerable than other parity groups in terms of disease development but not mortalities. Correlations between FMD development and age can be misleading when new viruses have been introduced into vaccinated herds and should only be considered in the context of the reproductive state. Our work highlighted the need to make specific modifications to the disinfection routines, the management of infected lactating animals, and the early disease detection systems to reduce animal exposure frequency, environmental virus loads, and the overall impact.

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Supplemental data

Supplemental data associated with this article can be found, in the online version, at http://dx.doi.org/10.14943/jjvr.68.4.237

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