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SEROPREVALENCE OF MALIGNANT CATARRHAL FEVER-RELATED OVINE GAMMAHERPES VIRUS IN CATTLE

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ABSTRACT

Malignant catarrhal fever (MCF) is a worldwide viral, non-contagious disease that is transmitted to cattle. It is determined to be a significant source of economic loss for several ruminant species. It is mainly caused by ovine herpesvirus-2 (OvHV-2), which affects the epithelial and lymphoid tissues of the respiratory and digestive tracts. There are limited data on MCF in Egypt, and there is no epidemiological investigation of the clinical prevalence of MCF in Assiut governorate. So the aim of this work is to study the clinical occurrence of MCF at Manfalut center in Assiut governorate, and serological detection of MCF infection in cattle. A total of 30 cows suspected infected with MCF were screened using enzyme-linked immunosorbent assay (ELISA) test. Each investigated cow's age, sex, breed, admission time, and contact with sheep were recorded in order to determine the prevalence of MCF. Records included fever, lymphadenitis, corneal opacity, erosions in the buccal cavity, abnormal breathing, purulent nasal discharge with the nasal ulcer, and diarrhea. Overall, 10% of the examined cows were affected. The seroprevalence of MCF infection was higher in native female cows aged 1-3 years, which had previously interacted with sheep, particularly in April and May, although statistical analysis did not reveal a significant difference. Cattle are more likely to get infected if raised in the same grazing area as sheep and goats. As a result, we advise keeping cattle grazing areas distinct from those used for sheep and goats.

Keywords: *MCF, Manfalut, ELISA, ovine herpesvirus-2.*

INTRODUCTION

Malignant catarrhal fever (MCF) is a globally distributed viral disease (Plowright, 1986). currently emerging as a significant source of economic loss for several ruminant species. The disease was initially discovered in Europe, but it has since spread throughout the world. Contact with reservoir species that release virus particles in nasal and ocular discharge is the most common method for spreading the disease. Sheep-associated MCF virus (SA-MCF) is a Maca virus of the subfamily Gamma herpes virinae and is known as ovine herpesvirus-2 (OvHV-2) (Kumar *et al.,* 2021). The majority of clinical cases have been classified as acute or severely fatal illnesses, with a fatality rate of up to 90% in most reports (O'Toole *et al.,* 1997). Numerous

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species, including domestic cattle, buffalo, pigs, horses, and several species of deer, have been shown to experience intermittent, lymphoproliferative, and multisystemic effects from MCF (Metzler, 1991). High fever, frequent nasal discharge, ocular opacity, widespread lymphadenopathy, leukopenia, severe inflammation of the nasal, oral, and conjunctival mucosa, and necrosis extending to the trachea and esophagus are among the clinical signs. There may occasionally be skin rashes, diarrhea, non-suppurative arthritis, and CNS symptoms (Schock and Reid, 1996; Pardon *et al.,* 2009; O'Toole and Li, 2014 and Çitil, M. and Uzlu, E., 2017). The term MCF refers to a couple of diseases that are not distinguishable clinically or pathologically, but are associated with two different pathogens. Neither of these viruses affects the primary hosts (wildebeests and sheep) or spreads from one animal to another. The first disease is called wildebeest-associated MCF virus (WD-MCF), also referred to as Alcelaphine herpesvirus-1 (AlHV1), and it is a new genus of Macavirus (previously known as Rhadinovirus) of the subfamily Gamma herpesvirinae in the family Herpesviridae. The second disease is called sheep-associated MCF virus (SA-MCF), and it is also a Macavirus of the subfamily Gamma herpesvirinae known as Ovine herpesvirus-2 (OvHV2), which is transmitted to cattle from sheep. (Nelson *et al.,* 2013 and Li *et al.,* 2014). Although MCF-like signs have long been observed in Egypt, the disease has never been officially confirmed, and the causative agent wasn't identified until Bastawecy and Abd El-Samee (2012) isolated it from water buffaloes and calves. The geographical distribution of SA-MCF animal cases is approximately equal in Upper and Lower Egypt. This can be explained by the fact that sheep in Damietta, El-Gharbia, El-Sharkia, Dakahlia, Kafr El-Shiekh, Giza, Fayoum, and El-Menia had the virus in 2013–2014 and that infected animals (cattle and buffaloes) had MCF clinical signs (Zaki *et al.,* 2016). Currently, few data on MCF are available in Egypt, and there is no epidemiological investigation of the clinical prevalence of MCF in the Assiut governorate. The goal of the work is the serological detection of MCF in cattle and the epidemiological study of the clinical occurrence of MCF in Manfalut Center at Assiut Governorate.

MATERIALS AND METHODS

Ethical approval

All procedures were carried out following the ethical regulations established by Assiut University's institutional ethics committee. Thirty cattle of different breeds, ages, and sexes belonging to Manfalut Center in Assiut Governorate were admitted to the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine at Assiut University between April and October 2023. The cattle under examination had corneal opacity, purulent nasal discharge, bloody diarrhea, enlarged superficial lymph nodes, fever, and mouth lesions (erosions and ulcers). All cows were treated in accordance with the guidelines established by Assiut University on animal research, and samples were taken only with the owners' permission.

Study area

The current study was carried out in Assiut Governorate between April 2023 and October 2023. The animals under investigation came from Manfalut Center in Assiut Governorate and were admitted to the Veterinary Teaching Hospital at Assiut University Faculty of Veterinary Medicine.

Animals and samples.

This study included a total of 30 cows of different ages, sexes, and breeds. Only cows exhibiting one or more clinical signs of malignant catarrhal fever infection (high fever, corneal opacity, lymphadenitis, erosions of the oral and nasal mucosa, abnormal respiration, nasal discharge, and bloody diarrhea) were included. Serum samples were drawn directly from the jugular vein of diseased cattle by using

clean, dry, sterile syringes, put in vacutainer tubes without anticoagulant, and then used for ELISA test.

Data collection and clinical examination

Data obtained from each cow included the age (less than one year, <1-3years, and <3-5years), sex (male or female), breed (native, Friesian), time of admission (April-May, June-July, and August-September), and contact with sheep. Physical examination included body temperature measurement, visual examination of mucous membranes, and checking for signs of respiratory illness and digestive disturbances. Cattle with high fever, profuse nasal discharge, ocular opacity, lymphadenitis, erosions of the oral and nasal mucosa, which may spread to the esophagus and trachea, central nervous system symptoms, and blood-stained feces will be included in the study.

ELISA test

The ELISA test kit (Chongqing Biospes Co., Ltd., BZEK 1949) was used to detect OvHV-2 antibodies in the serum of suspected cows according to the manufacturer`s manual.

Statistical analysis

To measure the impact of each factor individually on the prevalence of the disease in cattle (i.e., age, sex, breed, admission time, and contact with sheep) and the association of clinical signs with the occurrence of the disease (i.e., temperature, corneal opacity, lymphadenitis, erosions of the oral and nasal mucosa, abnormal respiration, nasal discharge, and diarrhea), relative risk was calculated and chi-square tests were performed using SPSS statistics software (IBM Corp, USA, Version 29). A probability value (P-value) less than 0.05 was considered statistically significant.

RESULTS

Clinical findings.

The thirty cows in this investigation displayed one or more of the usual clinical symptoms of MCF disease. A wide variety of clinical symptoms were recorded, which may vary from case to case. 26 cows were suffering from high fever, 24 of them with enlargement of superficial lymph nodes (Figure 1). 20 cows showed erosions and ulcers in the oral mucosa (Figure 3). Respiratory distress was seen in 8 cases, and 10 cases showed mucoid to mucopurulent nasal discharge, and only one case showed an ulcer in the nasal mucosa (Figure 4). There were 13 cases of diarrhea, 7 of which had bloody diarrhea. Four cases had corneal opacity (Figure 2), and it was discovered that 17 of the cases had previously come into contact with sheep. The clinical signs seen in cows of different breeds, ages, and sexes are shown in Table 1.

Results of ELISA test.

The overall seroprevalence of MCF infection in examined cows was 10% (3/30) using the ELISA test. 12.5% of 24 cases showed enlarged lymph nodes, especially the prescapular and prefemoral lymph nodes, and 11.3% of 26 cases had a persistent high fever. 10 % of the 20 cases had oral lesions, including ulcers and erosions. 10 % out of the 10 cases exhibited purulent nasal discharge. Only one case with a nasal ulcer had a positive result, and none of the patients with irregular breathing did. 10% out of 20 cases with oral lesions, such as erosions and ulcers. Of the six cases of diarrhea, 33.3% had positive results. However, none of the cases involving bloody diarrhea had positive results. In all cases with bilateral corneal opacity, the result was negative (Table 2).

Risk factors associated with MCF prevalence.

Compared to other age groups, cows between the ages of one and three years had a higher infection rate (13.3%). Our results also showed that the infection rate in females (12.5 %) was higher than in male cows (9.1%). Two of the three positive cases (14.3%) were admitted to the clinic in April and May, and we discovered that all three instances (15%) were of the native breed. It

was reported that two cases of the positive cases (11.8%) had previously come into contact with sheep. According to Table 3, there was no significant variance found in the statistical analysis of these risk factors.

Table 1: The reported clinical signs in examined cattle in each age, sex, and breed.

Cases		breed		Sex		Signs									
age	no	native	Friesian	male	female	Fever	Respiratory signs	Nasal discharge	Enlarged lymph nodes	Corneal opacity	Ulcer oral mucosa	Ulcer nasal mucosal	diarrhea	Bloody diarrhea	Contact With sheep
Less than year	12	8	4	11	1	9	4	4	8	0	10	0	2	3	6
>1 year-3year	15	11	4	10	5	14	2	4	13	1	9	1	2	4	9
>3year-5year	3	1	2	1	2	3	2	2	3	3	1	0	2	0	2
total	30	20	10	22	8	26	8	10	24	4	20	1	6	7	17

Table 2: Association of clinical signs of MCF infection with the results of ELISA test in examined cattle.

Factor	No. tested	ELISA test		Odds ratio	95% CI	*P*-value
		Positive n (%)	Negative n (%)			
Temperature						
Normothermic	4	0 (0)	4 (100)			
Hyperthermic	26	3 (11.5)	23 (88.5)	1.143	0.770 – 1.016	0.474
Total	30	3 (10)	27 (90)			
Lymph nodes						
Enlarged	6	0 (0)	6 (100)			
Normal	24	3 (12.5)	21 (87.5)	1.130	0.982 – 1.329	0.361
Total	30	3 (10)	27 (90)			
Corneal opacity						
Present	4	0 (0)	4 (100)			
Absent	26	3 (11.5)	23 (88.5)	1.000	0.984 – 1.299	0.474
Total	30	3 (10)	27 (90)			
Oral lesions						
Present	20	2 (10)	18 (90)			
Absent	10	1 (10)	9 (90)	1.000	0.080 – 12.557	1.000
Total	30	3 (10)	27 (90)			
Nasal ulcer						
Present	1	1 (100)	0 (0)			
Absent	29	2 (6.9)	27 (93.1)	14.500	3.807 – 55.225	**0.002**
Total	30	3 (10)	27 (90)			
Fecal consistency						
Non-diarrheic	17	1 (5.9)	16 (94.1)			
Diarrheic	6	2 (33.3)	4 (66.7)	8.000	0.572 – 111.958	
Bloody diarrhea	7	0 (0)	7 (100)	-	-	0.086
Total	30	3 (10)	27 (90)	-	-	
Respiration						
Normal	22	3 (13.6)	19 (86.4)			
Abnormal	8	0 (0)	8 (100)	0.864	0.732 – 1.020	0.217
Total	30	3 (10)	27 (90)			
Nasal discharge						
Present	10	1 (10)	9 (90)			
Absent	20	2 (10)	18 (90)	1.000	0.080 – 12.557	1.00
Total	30	3 (10)	27 (90)			

Table 3: Factors associated with MCF seroprevalence among the examined cases.

Factor	No. tested	ELISA test		Odds ratio	95% CI	*P*-value
		Positive n (%)	Negative n (%)			
Age						
Less than one year	12	1 (8.3)	11 (91.7)	1.692	0.135 – 21.270	0.681
> 1-3 years	15	2 (13.3)	13 (86.7)			
> 3-5 years	3	0 (0)	3 (100)	-	-	
total	30	3 (10)	27 (90)	-	-	
Sex						
Males	22	2 (9.1)	20 (90.9)	1.429	0.112 – 18.298	0.783
Females	8	1 (12.5)	7 (87.5)			
Total	30	3 (10)	27 (90)			
Breed						
Native	20	3 (15)	17 (85)	0.850	0.707 – 1.022	0.197
Friesian	10	0 (0)	10 (100)			
Total	30	3 (10)	27 (90)			
Time						
April-May	14	2 (14.3)	12 (85.7)	0.667	0.052 – 8.549	0.754
June-July	10	1 (10)	9 (90)			
August-September	6	0 (0)	6 (100)	-	-	
Total	30	3 (10)	27 (90)	-	-	
Contact with sheep						
present	17	2 (11.8)	15 (88.2)	0.625	0.050 – 7.749	0.713
absent	13	1 (7.7)	12 (92.3)			
total	30	3 (10)	27 (90)			

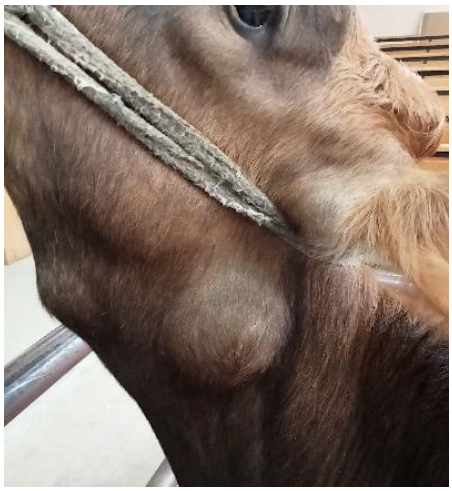

Figure 1: 2-years native female cow showing enlarged parotid and submandibular lymph nodes.

Figure 2: 3-years native cow with corneal opacity

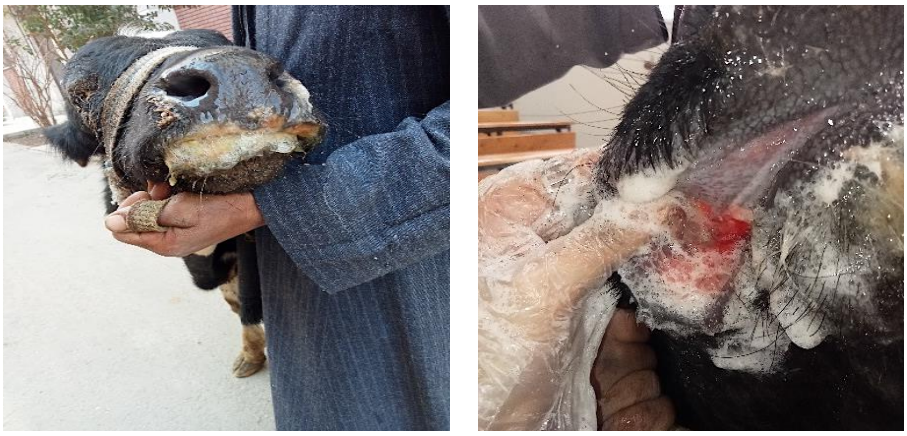

Figure 3:4-years native female cow showing salivation with erosions and ulcers in the gum.

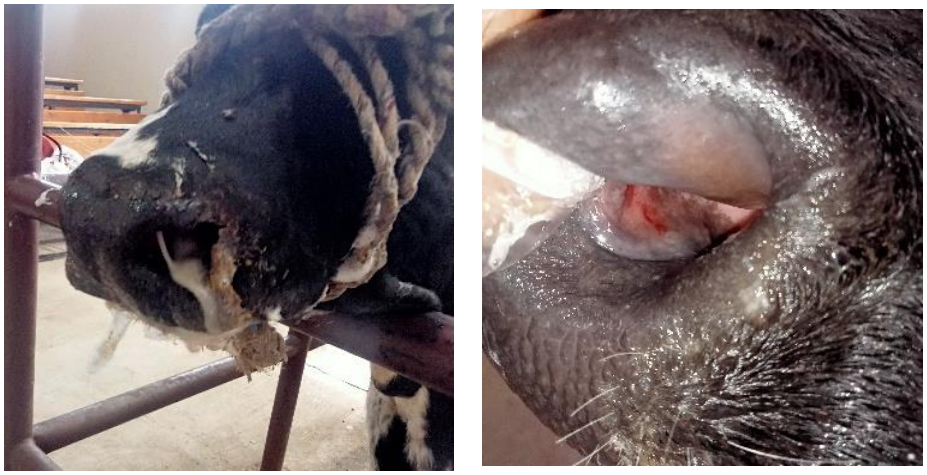

Figure 4: 4-years native cow with purulent nasal discharge and nasal ulcer.

DISCUSSION

Although MCF is a sporadic disease, outbreaks due to both sheep- and wildebeest-associated types have been documented (Schultheiss *et al.,* 2000, and Holliman *et al.,* 2007). Based on clinical signs and a history of contact with sheep and goats, particularly during parturition in these species, a preliminary diagnosis of MCF can be made. Thirty cows from Manfalut Center at Assiut Governorate were admitted to the infectious disease clinic of the veterinary teaching hospital at Assiut University, and we noted the most prevalent clinical symptoms related to MCF disease. High fever, enlarged lymph nodes, purulent nasal discharge, bilateral corneal opacity, erosions and ulcers of the oral mucosa, which may extend to the esophagus and trachea, respiratory distress, nasal ulcers, and severe diarrhea with dysentery were the most common clinical signs. These results were in line with earlier research published by Zaki *et al.* (2016) and Abd El Rahman *et al.* (2020). Additionally, fever, oral lesions such as erosions and ulcers, and nasal discharge were the most common clinical forms of MCF by O'Toole *et al.* (1997), Brenner *et al.* (2002), and Constable *et al.* (2017). Furthermore, Murray and Blood (1961) reported that no disease other than MCF frequently exhibits the combination of mucosal lesions, corneal opacity, and persistent fever.

The severity of the disease varies according to immunological status, age, and co-infections in cattle, and not all affected animals show clinical symptoms. Clinical findings associated with MCF infection can be explained by lymphoid proliferation and infiltration, widespread vascular epithelial lesions, and involvement of the vascular

adventitia. These factors also explain the development of gross lesions, such as epithelial erosions, that are linked to lymphoid cells like CD8+ T lymphocytes, which are the predominant cells linked to the vascular lesions, and OvHV-2 replication in lung tissue. Because sinusoidal cells are the preferred sites for gamma herpesvirus replication in lymphoid tissue, such as OvHV-2, the enlargement of the lymph nodes is caused by the abnormal proliferation of these cells (Zaki *et al.,* 2016, and Constable *et al.,* 2017).

Using the ELISA test, our study revealed that 10% of cattle in Manfalut City, the center of Assiut Governorate tested positive for MCF. This outcome was remarkably similar to the one that Yeşilbag (2007) reported (15%). Higher infection rates (62%, 56%, 26.6%, and 97.3%) were reported by Li *et al.* (2001), Abu Elzein *et al.* (2003), Powers *et al.* (2005), and Dabak and Bulut (2015), respectively. These differences could be caused by variations in the timing and number of collected samples, hygienic conditions, environmental factors, and the diagnostic techniques used. Serology detection of MCF infection using ELISA is less sensitive than either histopathology or PCR. A portion of the limited sensitivity of ELISA in clinically MCF-affected cattle may be attributed to an individual's lack of response, as in some cases of chronic or recovered MCF, as well as the rapid clinical progression that leads to death before antibodies reach a detectable level. Certain cattle with MCF disease which were positive for the PCR yet tested negative for antibodies by ELISA. It is unknown if these individuals do not respond humorally to the viral antigens in general or to the specific 15A epitope, but the fact that certain animals are genetically incapable of responding to this epitope may indicate the assay's limits. Thus, if the assay is used for the routine diagnosis of acute clinical cases, more sensitivity improvement of ELISA would be required (O'Toole *et al.,* 1997, and Li *et al.,* 1998).

The seroprevalence of MCF infection and disease symptoms did not differ statistically, except for one case with a nasal ulcer that had very high statistical significance (Table 2). The severity of the clinical manifestations and the stage of the disease may help to explain this, as nasal ulcers usually occur in the later stages of the disease. This case was in the last stage of the disease, showing all its other severe signs, and died shortly after the collection of the sample.

Table 3 proved that there was no statistically significant difference in the seroprevalence of MCF infection between the sex, breed, and age of the studied cattle. These findings are in line with those of Constable *et al.* (2017), who stated that all cattle, irrespective of breed, age, or sex, are equally susceptible to SA-MCF. However, a number of environmental and management factors, including the amount of virus exposure, stress exposure, and the presence of other infections, can influence the rate and severity of MCF infection. The highest rate of MCF infection was found in 1-3 years age groups. According to Abu Elzein *et al.* (2003), this could be explained by 1- to 2-year-old cows contracting the virus and losing their maternal immunity. Additionally, we found that April and May had the highest infection rates of the year. This is consistent with the findings of Blood *et al.* (1983), who discovered that the late winter and early spring months have the highest disease incidence. Since the owners of Manfalut Center typically keep native breeds indoors and in contact with sheep, higher MCF seroprevalence was observed in native cows. On the other hand, Friesian cows are typically kept on farms apart from small ruminants. It is also possible that the higher seroprevalence of the disease in females than in males is due to the fact that local owners usually keep female cows for milk production and reproduction.

The majority of positive cases in our study had previously been raised with lambing

ewes and recently weaned lambs, and these findings are consistent with those of Li *et al.* (2004). Most cases of SA-MCF are caused by cattle coming into contact with lambing ewes and freshly weaned lamb. Cattle can contract MCF from sheep infected with OvHV-2, and the incidence of infection rises when sheep and cattle are housed together (Plowright, 1990 and Fenner *et al.,* 1993).

CONCLUSIONS

In conclusion, raising goats and sheep in the same grazing area as the susceptible species can increase the risk of infection. Thus, we suggest separating grazing areas for sheep and goats from those used for cattle and buffalo. Additionally, extensive research on the ages of sheep and goats is necessary to determine the suitable age at which to infect susceptible species with MCF.

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مدي الانتشارالمصلي لفيروس جاما هربس الأغنام الخبيث المرتبط بالحمى الرشحية الخبيثة في الماشية

غادة بدر محمد مريد ، أسامة عبد الحكيم على ، مها ابراهيم حامد

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الحمى الرشحية الخبيثة هي مرض فيروسي معدي ينتشر في جميع أنحاء العالم وينتقل إلى الماشية. وهو مصدرهام للخسارة الاقتصادية في العديد من المجترات. وسببه هو فيروس الهربس الغنمي-٢ (OvHV-2)، الذي يؤثر على الجهاز التنفسي والجهاز الهضمي. هناك دراسات محدودة عن الفيرس في مصر، ولا يوجد تسجيل وبائي لانتشار الحمي الرشحية الخبيثة في محافظة أسيوط، لذا فإن الهدف من هذا العمل هو دراسة مدي تواجد الحمي الرشحية الخبيثة في مركز منفلوط بمحافظة أسيوط وذالك باالكشف عن الاجسام المضادة لهذا المرض في الماشية. تم فحص ٣٠ بقرة يشتبه في إصابتها بمرض الحمي الرشحية الخبيثة باستخدام اختبار الإليزا. تم تسجيل عمر كل بقرة، وجنسها، وسلالتها، واتصالها بالأغنام من أجل تحديد مدى انتشار المرض. وشملت الاعراض الحمى، والتهاب العقد اللمفاوية، وعتامة القرنية، وقرح فموية، والتنفس غير الطبيعي، وإفرازات أنفية قيحية مع قرحة الأنف، والإسهال. بشكل عام، تواجد المرض في ١٠% من الأبقار التي تم فحصها. كان الانتشار المصلي للعدوى أعلى في إناث الأبقار المحلية التي تراوحت أعمارها بين ١-٣ سنوات والتي سبق لها الاحتكاك مع الأغنام، خاصة في شهري أبريل ومايو، على الرغم من أن التحليل الإحصائي لم يكشف عن اختلاف معنوي. من المرجح أن تصاب الماشية بالعدوى إذا تمت تربيتها في نفس منطقة رعي الأغنام والماعز. ونتيجة لذلك، ننصح بإبقاء مناطق رعي الماشية منفصلة عن تلك المستخدمة لرعي الأغنام والماعز.

الكلمات المفتاحية: الحمى الرشحية الخبيثة، منفلوط، اختبار الإليزا، فيروس الهربس الغنمي-٢.