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FOOT AND MOUTH DISEASE IN CAMELS : ROLE OF CAMELS IN THE EPIZOOTIOLOGY AND TRANSMISSION OF FOOT AND MOUTH DISEASE IN EGYPT

(With 2 Tables and One Figure)

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مرض الحمى القلاعية فى الجمال
دور الجمال فى وبائية وانتقال المرض فى مصر

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لدراسة مرض الحمى القلاعية فى الجمال ومعرفة الدور الذى تلعبه الجمال فى وبائية ونقل هذا المرض للأبقار فى مصر تم استخدام ٥٠ جمل و٥٠ بقرة على احتكاك بهذه الجمال. تم فحص هذه الحيوانات من الناحية الإكلينيكية والفيروسية بالإضافة إلى استخدام البيولوجيا الجزيئية (التفاعل التبلمرى المتسلسل). تم إجراء العزل الفيروسى واختبار التفاعل التبلمرى المتسلسل مرتين متتاليتين يفصل بينهما شهر. الفحص الإكلينيكى فى بداية التجربة أوضح ظهور أعراض فى صورة إفرازات لعابية على ٣ جمال بينما كانت باقى الجمال وكل الأبقار سليمة ظاهريا. بعد ٣٠ يوم من احتكاك الأبقار مع الجمال ظهرت بعض الأعراض المرضية المتمثلة فى إفرازات لعابية وأنفيةمع ارتفاع فى درجة الحرارة وعرج فى عدد ٧ أبقار بينما كانت باقى الحيوانات سليمة ظاهريا. فى العزل الفيروسى الأول، تم عزل فيروس مرض الحمى القلاعية من عدد ١٤ جمل وكانت كل الأبقار سلبية للعزل الفيروسى بينما تم عزل فيروس مرض الحمى القلاعية من ١٧ جمل بالإضافة إلى ٢٥ بقرة فى العزل الفيروسى الثانى. تم التعرف على الحامض النووى لفيروس مرض الحمى القلاعية فى ١٩ جمل فى الوقت الذى كانت كل الأبقار سلبية فى اختبار التفاعل التبلمرى المتسلسل الأول. بعد إجراء اختبار التفاعل التبلمرى المتسلسل الثانى، تم التعرف على الحامض النووى لفيروس مرض الحمى القلاعية فى ٢٤ جمل و٢٧ بقرة. أوضح التفاعل التبلمرى المتسلسل وجود عترتين (A وO) من فيروس مرض الحمى القلاعية فى الجمال والأبقار كما أظهر وجود تشابه جينى بين فيروس مرض الحمى القلاعية فى الجمال والأبقار. نتائج هذا العمل أثبتت أن التفاعل التبلمرى المتسلسل أشد حساسية من العزل الفيروسى فى تشخيص مرض الحمى القلاعية فى الجمال والأبقار فى مصر بالإضافة إلى أنه أمكن استخدامه فى تصنيف الفيروس وتحديد عترته كما أمكن استخدامه فى دراسة العلاقة الجينية بين فيروس مرض الحمى القلاعية فى الجمال والأبقار مما أعطى الفرصة لتتبع ومعرفة مصدر العدوى فى هذه الدراسة. أوضح

هذا العمل أن مرض الحمى القلاعية موجود فى الجمال فى مصر وينتقل بينها وإن كانت معظم الجمال المصابة تبدو سليمة ظاهريا وهذا عامل خطير جدا من الناحية الوبائية. أثبتت هذه الدراسة الدور الهام الذى تلعبه الجمال فى وبائية ونقل مرض الحمى القلاعية للأبقار فى مصر. هذه أول دراسة تتناول الدور الذى تلعبه الجمال فى نقل مرض الحمى القلاعية للأبقار كما أنها المرة الأولى التى يستخدم فيها التفاعل التبلمرى المتسلسل فى تشخيص وتصنيف فيروس مرض الحمى القلاعية فى الجمال ودراسة العلاقة الجينية بينه وبين الفيروس فى الأبقار بالإضافة إلى أنها المرة الأولى التى يتم فيها تسجيل العترة A لفيروس مرض الحمى القلاعية فى الجمال فى مصر.

SUMMARY

To study FMD in camels and investigate the role played by camels in epizootiology and transmission of the disease to cattle, 50 camel and 50 cattle (in contact with camels) were used. All these animals examined clinically virologicaly (virus isolation, VI) in addition to examination using molecular biology based technique (reverse transcription polymerase chain reaction, RT-PCR). VI and RT-PCR were performed two times one month apart. Clinical examination at the beginning of this work showed 3 camels suffered from excessive salivation while remainmder of camels and all cattle were apparently healthy. After 30 day from contact between camels and cattle, salivation, nasal discharge in addition to rise of body temperature and lameness were recorded in 7 cattle and other animals were apparently healthy. In the 1st VI, foot and mouth disease virus (FMDV) was isolated from 14 camels while all cattle were negative for VI. FMDV was isolated from 17 camel in addition to 25 cattle in the 2nd VI. FMD viral RNA was identified in 19 camel while all examined cattle were negative for 1st RT-PCR. After performing 2nd RT-PCR, FMD viral RNA was observed in 24 camel and 27 cattle. 2 serotypes (O and A) of FMDV were detected in camels and cattle and genetic relationship between FMDV in camels and cattle has been proved after using RT-PCR. Results of this work proved that RT-PCR is more sensitive than VI in diagnosis of FMD in camels and cattle in Egypt, in addition, this technique could be used in serotyping of FMDV and could be used in studying the genetic relationship between FMDV in camels and cattle. Therefore, RT-PCR enabled us to fellow up and know the source of infection in this study. This work proved that FMDV is present and transmite between camels in Egypt inspite of absence of any clinical signs in most infected camels and this is very dangerous factor from epizootiological point of view. Important role played by camels in epizootiology and transmission of FMD to cattle in

Egypt has been proved in this study. This is the 1st study concerning the role of camels in transmission of FMD to cattle and the 1st to use RT-PC R in diagnosis, typing of FMDV in camels and study its genetic relationship with FMDV in cattle, in addition to the 1st recording of FMDV serotype A in camels in Egypt.

Key words: *Foot and mouth disease (FMD), foot and mouth disease virus (FMDV), camels, cattle, Virus isolation (VI), Reverse transcription polymerase chain reaction (RT-PCR), Egypt*

INTRODUCTION

Foot and mouth disease virus (FMDV) causes a highly contagious viral disease of even-toed ungulates and is one of the most important economic disease of livestock. The disease was reported in more than 33 species of a domesticated or wild species including camels (Fondevila *et al.*, 1995; Barnett and Cox, 1999; Bronsvoort *et al.*, 2004; Deem *et al.*, 2004; Ishimaru *et al.*, 2004).It has been reported in many Arabic counteries, still present in Africa and considered endemic disease in the Middle East (Hafez *et al.*, 1993; Hafez *et al.*, 1994; Callens *et al.*, 1998; Marquardt and Haas.,1998). The disease was initially described in the 16th century and was the first animal pathogen identified as a virus which is RNA virus belongs Picornaviridae family, genus Aphthovirus, 7 immunologically distinct serotypes of the virus have been identified (Callens and De Clerck, 1997; Grubman and Baxt, 2004; Musser, 2004).

Since the clinical signs can be very mild or absent, Callens *et al.*, (1998) and Wee *et al.* (2004) emphasized the danger of carrier animals acting as a maintenance host and asymptomatic transmitter. It is clear that once an individual in a herd become infected with FMDV, herd infectivity is not static (Jones *et al.*, 2004). Therefore, early diagnosis of FMD rely on early virus detection from animals in the preclinical phase of infection or in carrier animals will be critical in limiting the number of infectious animals capable of transmitting the virus to other herds (Carpenter *et al.*, 2004). FMDV spreads extremely fast and therefore there is a need for rapid and robust diagnostic system that is crucial for disease control (Marquardt and Haas,1998; Rasmussen *et al.*, 2003). An important FMD risk reduction factor is early recognition of the disease at the source of the commodity (Sutmoller and Casas Olascoaha, 2003).

At present, identification and typing of FMDV largely relies on serological tests. However, these tests have many disadvantages and do not provide complete information on the epizootiological tracing of

viruses (Nunez *et al.*, 1998; Lomakina *et al.*, 2004). At the same time, positive serology dose not indicate that the virus is still present. Therefore, reverse transcription polymerase chain reaction (RT-PCR) or virus isolation (VI) are necessary to demonstrate the presence of the virus (Callens *et al.*, 1998).

Isolation of FMDV has to be attempted, but usually take several days. RT-PCR ia an additional method that can be used to diagnose FMD (Marquardt and Hass 1998).

The application of molecular diagnostic methods offers good alternative procedure for developing and optimizing a sensitive method for the detection of FMDV (Barlic-Maganja *et al.*, 2004; Sangare *et al.*, 2004). RT-PCR specifically detected FMDV, provides fast results and can handle large number of samples. Therefore, it is seen as a valuable tool for FMDV diagnosis (Reid *et al.*, 2002). RT-PCR is being 500 fold more sensitive than conventional indirect ELISA and constitutes a simple, rapid and efficient alternative method for diagnosis of FMD (Rodriguez *et al.*, 1994 Pattnaik *et al.*, 1997). This assay also exceeded sensitivity of viral isolation. In many instances the assay detected viral RNA in the mouth and nose 24 to 96 hours before the onset of clinical disease and it could be detected within 24 hours post infection (Callahan *et al.*, 2002; Zhang *et al.*, 2004).

The increased international commercialization of camels has led to a need to obtain more information on the epidemiology of various disease agents that may affect these animals. Although some studies have been conducted with regard to their role in the epidemiology of many animal infectious conditions, the epidemiology of FMDV in camels has not been extensively studied. Unlike cattle, which are known to carry FMDV to extended period of time, little is known about the carrier abbility, if any, of camels (Fondevila *et al.*, 1995).

In Egypt, Little is known about FMD in camels and there is no data concerned role of camels in epizotiology and trasmission of FMD to cattle. Therefore this study was planned to study FMD among camels and to study the role of camels, if any, in epizootiology and transmission of FMD to cattle. Selection of the most sensitive and reliable technique for detection and typing of FMDV in camels and cattle was another important aim of this work.

MATERIALS and METHODS

Animals: 50 camel and 50 cattle in contact with these camels were used in the study, some camels (3) were suffered from clinical signs of FMD

while other camels and all cattle were apparently healthy at the beginning of the work.

Nasal swabs: 2 nasal swabs were collected one month apart from each examined camel and cattle for extraction of viral RNA.

Mouth swabs: 2 mouth swabs were collected one month apart from each examined animal for extraction of viral RNA.

Probang materials: Probang material was collected twice one month apart from each examined animal for virus isolation.

1-Clinica examination:

Clinical examination of all investigated animals was performed three times weekly, any abnormal clinical signs were reported.

2-RT-PCR:

(a) Sample preparation

Nose and mouth swabs were solubilised in 1ml phosphate buffer saline buffer

(b) Synthetic oligonucleotide primers

The primers for FMD serotypes (Pharmacia Biotech.) were designed by comparison of published sequences of FMDVand based on VPI gene of FMDV (Suryanarayana *et al.,* 1999; Chen *et al.,* 2004; Oem *et al.,* 2004).Four primers were used in the present study. More specifications of these primers are listed in table (1)

(c) RNA extraction

Total RNA was extracted with TRIzol Reagent (Gibco BRL). 100 ul sample (nasal or mouth swab extracted in phosphate buffer saline) was mixed with 900 ul TRIzol Reagent.The samples were incubated for 5 minutes at room temperature, addition of equal amount of chloroform, precipitation of RNA with isopropanol and washing of RNA with 75% ethanol. The purified RNA was resuspended in 20ul Rnase free-water and redissolved by incubating for 10 min at 55 °C.

(d) Reverse transcription

First strand cDNA synthesis was performed for 15 min at 37°C in a 10ul reaction mix containing the followings:- (1)50mM Tris-Hcl (pH 8.3), (2)75mM KCl, (3)3mM MgCl2, (4)10mM dithiothreitol, 0.125 mM (each) dNTP, (5)5uM (each) downstream primer, (6) 100 units Moloney murine leukemia virus reverse transcriptase (GibcoBRL) and (7)0.5 ug RNA. This was followed by heating for 5min at 95°C, in order to denature RNA-cDNA hybrid. Samples were chilled and stored at -20°C.

(e) PCR amplification

A PCR mix (10ul) consists of the following: 10mMTris-Hcl (pH8.8), 50mM KCl, 1.5mM MgCl2, 0.1% Triton X-100, 0.05mM (each) dNTP, 0.6um (each) primer, 0.25 units Taq polymerase (Promega) and 2% cDNA reaction mix. Amplification and reverse transcription was performed on a gene Amp CR system 9600 (Perkin Elmer) using program as follow: (1) 1 min at 95°C (2) 15 sec at 95°C (3) 1 min at 60°C (4) 6 min at 60°C, repeating steps (2) and (3) for 35 cycles.

(f) Gel electrophoresis

The samples were electrophoresed at 100 V for 30 min in TAE buffer on 1.5% agarose gels, stained with ethidium bromide (0.6ug/ml). DNA molecular weight marker type 100bp DNA ladder (Gibco BRL) was applied to identify the size of the PCR products.Negative control for RT-PCR were performed by adding of distilled water instead of the primers. RT-PCR was performed as described by Vangrysperre and De Clercq (1996); Marquardt *et al.*, 1996 and Marquardt and Haas (1998).

Table 1: Specification of used primers.

Primer	Sequence 5` - 3`	Expected serotype	Position
P33	AGCTTGTACCAGGGTTTGGC	FMDV	3832-3851
P38	GCTGCCTACCTCCTTCAA	FMDV-O	3450-3467
P87	GTCATTGACCTCATGCAGACCCAC	FMDV-A	3124-3147
P40	GTTTCTGCACTTGACAACACA	FMDV-C	3259-3279

3-Virus isolation

Virus isolation was performed according to Sakamoto *et al.*, (2002). In brief, the probang materials were inoculated to bovine kidney (BK) and bovine thyroid cell cultures. Cytopathic effect (CPE) was observed at two days post inoculation.

RESULTS

1-Clinical examination:

(a) At the beginning of the study: 3 camels showed salivation only while other camels and all cattle were clinicaly healthy.

(b) After contact between camels and cattle: salivation, nasal discharge in addition to rise of body temperature and lameness were recorded in 7 cattle and other animals were clinicaly healthy.

2-RT-PCR:

(a) First examination: FMD viral nucleic acid (RNA) was detected in 19 camel while no positive result was reported among examined cattle with using of mouth swabs for extraction of viral RNA while 18 camel and no cattle were positive for RT-PCR when nasal swabs used for extraction of viral RNA.

(b) Second examination: FMD viral RNA was detected in 24 camel (including all 19 camel that gave positive result in the first examination) and 27 cattle when we used mouth swabs for extraction of viral RNA while 22 camel and 24 cattle were positive for RT-PCR with using of nasal swabs for extraction of viral RNA.

(c) The positive samples in both camels and cattle were belong 2 FMD viral strains (O and C). Strain O was reported in 2o camel and 18 cattle, while strain A was recorded in 4 camels and 9 cattle.

(d) The similarity of size and location of nucleic acid band in both camels and cattle were observed and indicated the genetic identity between FMDV in both animals.

-Results of RT-PCR are showen in Fig.1

3- Virus isolation (VI):

(a) First examination: FMD virus was isolated from 14 camel wherase all probang materials collected from cattle were negative for FMD viral isolation

(b) Second examination: FMD virus was isolated from 17 camel and 25 cattle.

Table 2: Results of laboratory investigation.

	VI		RT-PCR			
	1st	2nd	1st		2nd	
			(O)	(A)	(O)	(A)
Camels	14	17	16	3	20	4
Cattle	0	25	0	0	18	9

VI= Virus isolation RT-PCR= Reverse transcription polymerase chain reaction
(O)= Serotype O of FMDV (A)= Serotype A of FMDV

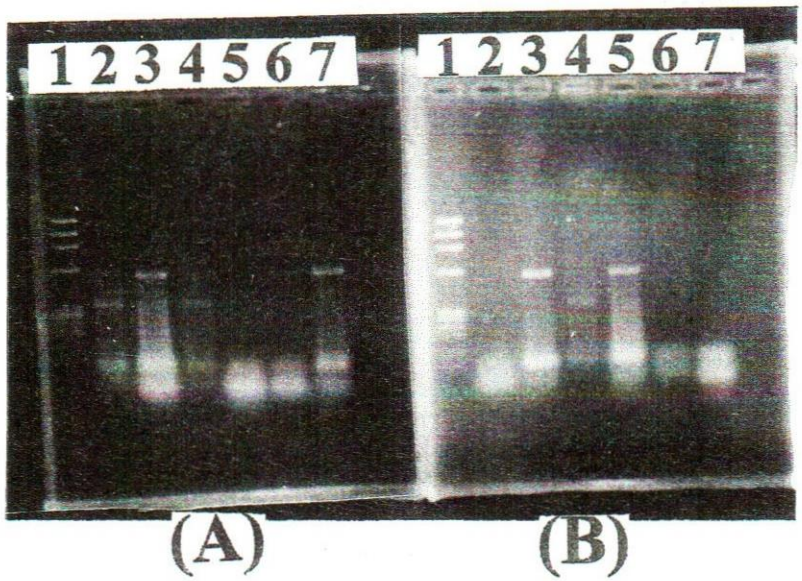

Fig. 1: Results of reverse transcription polymerase chain reaction (RT-PCR) in camels (A) and cattle (B).

(A) Agarose gel electrophoresis of PCR product in camels. Lane 1 (100 base pair DNA ladder), Lane 2 (FMD virus serotype O), Lane 3 (FMD virus srotype A),Lane 4 (FMD virus serotype O), Lane 5 (negative result), Lane 6 (negative control) and lane 7 (FMD virus serotype A).

(B) Agarose gel electrophoresis of PCR product in cattle. Lane 1 (100 base pair DNA ladder), Lane 2 (negative result, Lane 3 (FMD virus serotype A), Lane 4 (FMD virus serotype O), Lane 5 (FMD virus serotype A), Lane 6 (negative control) and lane 7 (negative result).

DISCUSSION

Results of the present work proved that FMD is present and transmitted among camels in Egypt. Hedger (1976); Palling *et al.* (1979) Hedger *et al.* (1980); Hafez *et al.* (1993) reported that camels known to be quite susceptible to FMDV where the virus has been detected for months to years after infection. This contrasts with that of Fondevila *et al.,* (1995) who mentioned that their study clearly indicate that, although camels can be infected with FMDV by direct contact, it is not a very susceptible animal. The results of these authors could be explained by the fact that they study FMD in one species only of camels (Lama glama) that found in North America.

The disease has a wide spectrum of clinical signs. There is, however, general agreement that the disease in naturally acquired infections often takes on a milder form in small ruminants and camels than in cattle, and in many cases may be vague (Barnett and Cox, 1999). In our study, most infected camels were apparently healthy and showed no clinical signs.

Our present investigation proved that camels (even apparently healthy one) plays an important role in transmission of FMD to contact cattle. It is clear that serologically positive contact animal without clinical signs present a danger for the transmission of FMDV (Callens *et al.*, 1998; Carpenter *et al.*, 2004)

Mouth and nasal swabs were used in extraction of FMD viral RNA in our work. Callens *et al.* (1998) and Zhang *et al.* (2004) concluded that FMDV was most often found in saliva followed by nasal secretion and sera. Nasal swabs are suitable samples to detect viral genomes at asymptomatic stage of the disease (Marquardt and Hass, 1998). Results of the present study proved that mouth swabs are more suitable for extraction of FMD viral RNA. Most success was obtained with the saliva samples for detection of FMD viral RNA. In subclinically infected, like in carriers, FMDV was only intermittently recovered. It is therefore essential to take several different kinds of samples such as mouth swabs and nose swabs (Callens *et al.*, 1998).

The results of this study clearly indicate that RT-PCR assay is more sensitive than VI in diagnosis of FMD in both camels and cattle as FMD viral RNA was detected in 51 animal (24 camel and 27 cattle) while FMDV was isolated from 41 animal (17 camel and 25 cattle) only. RT-PCR is a robust, reliable and sensitive test in diagnosis of FMD. Its sensitivity is 500-1000 times higher than VI and serological tests so, it is efficient alternative for the diagnosis and characterization of FMD and it is presented for highly sensitive and specific detection of FMDV (Rodriguez *et al.*, 1992; Rodriguez *et al.*, 1994; Suryanarayana *et al.*, 1999; Callahan *et al.*, 2003; Moonen *et al.*, 2003 and Saiz *et al.*, 2003).

In our study we used RT-PCR in diagnosis and serotyping of FMDV. By using this technique, 2 FMDV serotypes (O and A) were detected. RT-PCR had been shown to identify and differentiate all seven serotypes of FMDV because it can be used for the specific detection and identification of viral sequences that correlate with established FMDV serotype (Rodriguez *et al.*, 1992; Callens and De Clerck., 1997; Reid *et al.*, 1999; Reid *et al.*, 2000; Callahan *et al.*, 2002; Reid *et al.*, 2002; Rasmussen *et al.*, 2003 and Barlic-Maganja *et al.*, 2004).

Persistence and the carrier state of FMD have been reported (Barnett *et al.*, 2004).Only three camels (out of 24 camels proved to be infected with FMDV) showed clinical signs while the rest of animals (21) were apparently healthy. These animals could be classified as persistently infected or carrier camels as the FMDV was detected in these animals two successive times one month apart. No clinical signs reported in animals infected with FMDV in the study of Bouma et al (2004) who considered these animals persistently infected with FMDV without suffering from any clinical signs. The same observation has been rercorded by Musser (2004) who concluded that FMD infected animals can become inapparent carriers of FMDV.

The detection of FMDV in persistently infected or carriers among exposed ruminats is of great importance (Zhang and Alexandersen, 2003). In the present work, RT-PCR was used in detection of apparently healthy persistently infected or carrier animals. RT PCR assay may be suitable for detection of FMDV carrier animals (Zhang and Alexandersen, 2003; Rasmussen *et al.*, 2003).

RT-PCR provides fast results. Therefore it is seen as a valuable tool for diagnosis of FMDV (Marquardt *et al.*, 1995; Marquardt *et al.*, 1996; Reid *et al.*, 2001; Reid *et al.*, 2002). In the present study we obtained results of RT-PCR within sex hours from collection of the samples. The same time was recorded in the study of Marquardt and Haas (1998) and Zhang *et al.* (2004) who added that the rapidity by which results are obtained and sensitivity by which viral genomes are detected are the greatest advantages of this technique. Therefore, a RT-PCR was set up with the aim of being able to detect all virus isolates irrespective of their antigenic characteristics.

In the present investigation we did not use RT-PCR in detection and serotyping FMDV only but also we used it in establishment a genetic relationship between FMDV in infected or persistently infected carrier camels and FMDV in contact cattle. Our results reported the genetic similarity between FMDV in camels and cattle. Therefore, this study proved the role of camels in transmission of FMDV to cattle. RT-PCR enables fast determination of genetic character of FMDV and could be used in detection of probable origin of the causative virus and the source of infection (Nunez *et al.*, 1998; Scherbakov *et al.*, 1998)

Results of virological and molecular biology investigations confirmed that RT-PCR more sensitive than virus isolation in diagnosis of FMD, so, we recommend using of this recent technique for diagnosis and typing of FMDV specially in imported camels (even those

apparently healthy) before permission of its enterance into Egypt. These results also proved that camels are susceptible to FMD infection, and that they play a major role in transmitting the virus to domestic livestock. This is the first experiment to clearly show the role of camels in transmission of FMD to cattle and the 1st to use RT-PC R in diagnosis, typing of FMDV in camels and study its genetic relationship with FMDV in cattle, in addition to the 1st recording of FMDV serotype A in camels in Egypt

REFERENCES

Barlic-Maganja, D; Grom, J.; Toplak, I. and Hostnik, P. (2004): Detection of foot and mouth disease virus by RT-PCR and microplate hybridization assay using inactivated viral antigens. Vet. Res.Commun., 28 (2): 149-158.

Barnett, P. and Cox, S. (1999): The role of small ruminants in the epidemiology and transmission of foot and mouth disease. The Veterinary Journal, 158: 6-13.

Barnett, P.; Keel, P.; Reid, S.; Armstrong, R.; Statham, R.; Voyce, C.; Aggarwal, N. and Cox, S. (2004): Evidence that high potency foot and mouth disease vaccine inhibits local virus replication and prevent the carrier state in sheep. Vaccine, 22 (9-10): 1221-1232.

Bouma, A.; Dekker, A. and De Jong, M. (2004): No foot and mouth disease virus transmission between individually housed calves. Vet. Microbiol., 98 (1): 29-36.

Bronsvoort, B.; Radford, A.; Tanya, V.; Nfon, C.; Kitching, R. and Morgan, K. (2004): Molecular epidemiology of foot and mouth disease viruses in the Adamaw a province of Cameron. J. Clin. Microbiol., 42 (5): 2186-2196.

Callahan, J.; Brown, F.; Osorio, F.; Sur, J.; Kramer, E.; Long,Glubroth, J.; Ellis, S.; Shoulars, K.; Gaffney, K.; Rock, D. and Nelson, W. (2002): Use of portable real-time reverse transcriptase polymerase chain reaction assay for rapid detection of foot and mouth disease virus. J.Am.Vet. Med. Assoc., 220 (11): 1636-1642.

Callens, M. and De Clerc, K. (1997): Differentiation of the seven serotypes of foot and mouth disease virus by reverse transcriptase polymerase chain reaction. J.Virol. Methods, 67 (1): 35-44.

Callens, M.; De Clercq, K.; Gruia, M. and Danes, M. (1998): Detection of foot and mouth disease by reverse transcription polymerase chain reaction and virus isolation in contact sheep without clinical signs of foot and mouth disease. The Veterinary Quarterly 20, Suppl. 2: S37-S40.

Carpenter, T.; Thurmond, M. and Bates, T. (2004): A simulation model of intraherd transmission of foot and mouth disease with reference to disease spread before and after clinical diagnosis. J. Vet. Diagn. Invest., 16 (1): 11-16.

Chen, W.; Yan, W.; Du, Q.; Fei, L.; Liu, M.; Ni, Z.; Sheng, Z. and Zheng, Z. (2004): RNA interference targeting VPI inhibits foot and mouth disease virus replication in BHK-21 cells and suckling mice. J. Virol., 78 (13): 6900-6907.

Deem, S.; Noss, A.; Villaroel, R.; Uhart, M. and Karesh, W. (2004): Disease survey of free-ranging grey brocket deer (Mazama gouazoubira) in the Gran Chaco, Bolivia. J. Wildl. Dis., 40 (1): 92-98.

Fondevila, N.; Marcoveccio, F.; BlancoViera, J.; O`Donnell, V.; Carrillo, B.; Schudel, A.; David, M.; Torres, A. and Mebus, C. (1995): Susceptibility of Llamas (Lama glama) to infection with foot and mouth disease virus. J. Vet. Med., B42: 595-599.

Grubman, M. and Box, B. (2004): Foot and mouth dissease. Clin. Microbiol. Rev., 17(2): 465-493.

Hafez, S.M.; Farag, M.A.; Al-Sukayran, A. and Al-Mujalli, D.M. (1993): Epizootiology of foot and mouth disease in Saudi Arabia: I. Analysis of data obtained through district field veterinarians. Rev. Sci. Tech., 12 (3): 807-816.

Hafez, S.; Farag, M. and Al-Sukayran (1994): The impact of live animal importation on the epizootiology of foot and mouth disease in Saudi Arabia. Dtsch. tierarzll. Wschr., 101: 381-420.

Hedger, R. (1976): Foot and mouth disease in wild life with particular reference to the African Buffalo (*Syncerus Caffer*). In Page, L (ed.), Wildlife diseases. Plenum Press, New York, pp. 235-244.

Hedger, R.; Barnett, I. and Gray, D. (1980): Some virus diseases of domestic animals in the Sultanate of Oman. Trop. Anim. Health Prod., 12 (2): 107-114.

Ishimaru, D.; Sa-Carvvalho, D. and Silva, J. (2004): Pressure-inactivated FMDV: a potential vaccine. Vaccine, 22 (17-18): 2334-2339.

Jones, R.; Kelly, L.; French, N.; England, T.; Livesey, C. and Wooldridge, M. (2004): Quantitative estimates of the risk of new outbreak of foot and mouth disease as a result of burning pyres. Vet. Rec., 154 (6): 161-165.

Lomakina, N.; Fallacara, F.; Pacciarini, M.; Amadori, M.; Lomakin, A.; Timina, A.; Shcherbakova, L. and Drygin, V. (2004): Application of universal primers for identification of foot and mouth disease virus and swine vesicular disease virus by PCR and PCR-ELISA. Arch. Virol., 149 (6): 1155-1170.

Marquardt, O. and Haas, B. (1998): Aims of the FMDV-specific RT-PCR as it is performed at the BFAV, Tuebingen laboratory. The Veterinary Quarterly, Suppl. 2: S31-S32.

Marquardt, O.; Srtaub, O.; Ahl, R. and Haas, B. (1995): Detection of foot and mouth disease virus in nasal swabs of asymptomatic cattle by RT-PCR witin 24 hours. J. Virol. Methods, 53: 255-261.

Marquardt, O.; Ahl, R.; Haas, B.; Ohlinger, V. and Strub, O. (1996): Methods for rapid extraction of RNA from clinical samples for use in diagnostic RT-PCR. Med.Microbiol Letters, 5: 55-63.

McGrane, J. and Higginsm, A. (1985): Infectious diseases of the camel: viruses, bacteria and fungi. Br. Vet. J., 141 (5): 529-547.

Moonen, P.; Boonstra, J.; Van Der Honing, R.; Leendertse, C.; Jacobs, L. and Dekker, A. (2003): Validation of a light cycler-based reverse transcription polymerase chain reaction for the detection of foot and mouth disease virus. J. Virol. Methods, 113 (1): 35-41.

Musser, J. (2004): A practitioner`s primer on foot and mouth disease. J. Am. Vet. Med. Assoc., 224 (8): 1261-1268.

Nunez, J.; Blanco, E.; Hernandez, T.; Dopazo, J. and Sobrino, F. (1998): RT-PCR in foot and mouth disease diagnosis. The Veterinary Quarterly, Suppl. 2: S34-S36.

Oem, J.; Lee, K.; Cho, I.; Kye, S.; Park, J. and Joo, Y. (2004): Comparison and analysis of the complete nucleotide sequence of foot and mouth disease viruses from animals in Korea and other pan Asia strains. Virus Genes, 229 (1): 63-71.

Palling, R.; Jessett, D. and Heath, B. (1979): The occurrence of infectious diseases in mixed farming of domesticated wild herbivores and domestic herbivores including camels in Kenya. I. Viral diseases: a serologic survey with special

reference to foot and mouth disease. J. Wildl. Dis., 15 (2): 351-358.

Pattnaik, B.; Sanyal, A.; George, M.; Tosh, C.; Hemardi, D. and Venkataramanan, R. (1997): Evaluation of primers for PCR amplification of RNA polymerase gene sequences of foot and mouth disease virus. Acta Virol., 41 (6): 333-336.

Rasmussen, T.; Uttenthal, A.; De Stricker, K.; Belak, S. and Storgaard, T. (2003): Development of a novel quantitative real-time RT-PCR assay for the simultaneous detection of all serotypes of foot and mouth disease virus. Arch. Virol., 148 (10): 2005-2021.

Reid, S.; Hutchings, G.; Ferris, N. and De Clercq, K. (1999): Diagnosis of foot and mouth disease by RT-PCR: evaluation of primers for serotypic characterisation of viral RNA in clinical samples. J. Virol. Methods, 83 (1-2): 113-123.

Reid, S.; Ferris, N.; Hutchings, G.; Samuel, A. and Knowles, N. (2000): Primary diagnosis of foot and mouth disease by reverse transcription polymerase chain reaction. J. Virol. Methods, 89 (1-2): 167-176.

Reid, S.; Ferris, N.; Hutchings, G.; De Clercq, K.; Newman, B.; Knowle, N. and Samuel, A. (2001): Diagnosis of foot and mouth disease by RT-PCR: use of phylogenetic data to evaluate primers for the typing of viral RNA in clinical samples. Arch. Virol., 146 (12): 2421-2434.

Reid, S.; Ferris, N.; Hutchings, G.; Zhang, Z.; Belsham, G. and Alexendersen, S. (2002): Detection of all seven serotypes of foot and mouth disease virus by real-time fluorogenic reverse transcriptase polymerase chain reaction assay. J. Virol. Methods, 105 (1): 67-80.

Rodriguez, A.; Martinez-Salas, E.; Dopazo, J.; Davila, M.; Saiz, J. and Sobrino, F. (1992): Primer design for specific diagnosis by PCR of highly variable RNA viruses: typing of foot and mouth disease virus. Virology, 189 (1): 363-367.

Rodriguez, A.; Nunez, J.; Nolasco, G.; Ponz, F.; Sobrino, F. and De Blas, C. (1994): Direct PCR detection of foot and mouth disease virus. J. Viro. Methods, 47 (3): 345-349.

Saiz, M.; De La Morena, D.; Blanco, E.; Nunez, J.; Fernandez, R. and Sanchez-Vizcaaino, J. (2003): Detection of foot and mouth disease virus from culture and clinical samples by reverse

transcription-PCR coupled to restriction enzyme and sequence analysis. Vet. Res., 34 (1): 105-117.

Sakamoto, K.; Kanno, T.; Yamakawa, M.; Yoshida, K.; Yamazoe, R. and Murakami, Y. (2002): Isolation of foot and mouth disease virus from japanese black cattle in Miyazaki prefecture ,Japan 2000. J. Vet. Med. Sci., 64 (1): 91-94.

Sangare, O.; Bastos, A. Venter, E. and Vosloo, W. (2004): A first molecular epidemiological study of SAT-2 type foot and mouth disease viruses in West Africa. Epidemiol. Infect., 132 (3): 525-532.

Scherbakov, A.; Lomakina, N.; Drygin, V. and Gusev, A. (1998): Application of RT-PCR and nucleotide sequencing in foot and mouth disease diagnosis. The Veterinary Quarterly, Suppl .2: S32-S34.

Suryanarayana, V.; Madanamohan, B.; Bist, P.; Natarajaan, C. and Tratschin, J. (1999): Serotyping of foot and mouth disease virus by antigen capture reverse transcriptase polymerase chain reaction. J. Virol. Methods, 80 (1): 45-52.

Sutmoller, P. and Casas Olascoaga, R. (2003): The risks posed by the importation of animals vaccinated against foot and mouth disease and products derived from vaccinated animals: a review. Rev. Sci. Tech., 22 (3): 823-835.

Vangrysperre, W. and De Clerck, K. (1996): Rapid and sensitive polymerase chain reaction based detection and typing of foot and mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. Arch. Virol., 141: 331-344.

Wee, S.; Park, J.; Joo, Y.; Lee, J. and An, S. (2004): Control measures implemented during the 2002 foot and mouth disease outbreak in the Republic of Korea. Vet. Rec., 154 (19): 598-600.

Zhang, Z. and Alexendersen, S. (2003): Detection of carrier cattle and sheep persistently infected with foot and mouth disease virus by a rapid real-time RT-PCR assay. J. Virol. Methods, 111 (2): 95-100.

Zhang, Z.; Murphy, C.; Quan, M.; Knight, J. and Alexandersen, S. (2004): Extent of reduction of foot and mouth disease virus RNA load in oesophageal –pharyngeal fluid after peak levels may be a critical determinant of virus persistence in infected cattle. J. Gen. Virol., 85 (Pt 2): 415-421.