9th Scientific Congr. of Egypt. Soc. For Anim. Manag. 12-16 Sept.,2017: 20- 31

STUDIES ON ANTIVIRAL ACTIVITY OF ZEOLITE AGAINST FOOT AND MOUTH DISEASE AND EPHEMERAL FEVER VIRUSES

Assem, A. Mohamed[*] and Mustafa Amin[**]

*Department of FMD, Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia, Cairo, Egypt P.O. Box 131 svri@idsc.gov.eg
** Central Laboratory for Evaluation of Veterinary Biologics, Abassia, Cairo

ABSTRACT

This work was aimed to document the antiviral activates of zeolite against foot and mouth disease virus (FMDV) sero types O/Panasia, A/Iran 05, and SAT2/ Egy 2012 and bovine ephemeral fever virus (BEFV/Abbasia/2000) to evaluate its replication in Baby Hamster Kidney (BHK) and Vero cell culture and in baby mice. zeolite is a natural non-toxic , fine powder of micronized zeolite (MZ) . Cytotoxicity assay studied for zeolite on BHK cells and Vero cell culture to determine the non-toxic dose. The non-toxic dose of zeolite was mixed with each type of FMDV (A, O, SAT2) also BEFV. Different viral suspensions were treated with different concentrations of zeolite ranging from 10 to 50 µg/ml. The viral replication was evaluated by inverted microscope as percentage of cytopathic effect (CPE). Furthermore, old baby Swiss mice were inoculated with 0.1 ml intraperitonially from the mixture of FMDV different types and different concentration of zeolite. After 48 h post inoculation, all the baby mice examined to evaluate the antiviral action of zeolite. The result showed that the concentrations of 10 and 20 µg / ml of zeolite little or no antiviral effect was observed at all, while concentrations of 30 to 50 µg/ ml of zeolite had no cytotoxicity effect on cells also revealed 100 % reduction in the virus infectivity.The antiviral effect of zeolite seems to be non-specific and is more likely based on the incorporation of viral particles into pores of zeolite aggregates than ion exchange properties of zeolite.This study confirmed the biological activity of zeolite against FMDV Types O, A, and SAT2 and BEFV. From the results, zeolite could be useful as antiviral lead to limitation of infection.

Keywords: BEFV , Cytotoxicity , foot and mouth disease virus, zeolite.

INTRODUCTION

Foot-and-Mouth Disease Virus (FMDV) is the etiologic agent of one of the most devastating diseases that can affect cloven-hoofed livestock. It is a small, non-enveloped single stranded, positive sense RNA virus related to family *Picornaviridae* and has seven serotypes: O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3, all of which cause a highly contagious vesicular diseas ***(Alexandersen et al., 2003)***. Within these

serotypes, over 60 subtypes have also been reported. Because of this diversity there are no universal vaccines thus presenting challenges in the selection of vaccine strains ***(Brown, 2003 and Arzt et al., 2011).*** Infection with FMDV causes an acute disease that spreads very rapidly and is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats, with high morbidity but low mortality ***(Grubman and Baxt, 2004).*** Although vaccines have been extensively be used to control FMD, there was no antiviral therapy available to treat ongoing infections with FMD virus (Grubman, 2005).

The most effective FMD vaccines consisted of chemically inactivated FMDV and can only offer complete protection after 7 days of vaccination because of the time needed to trigger an immune Response ***(Pacheco et al., 2015 and Zhang et al., 2015).*** Bovine ephemeral fever virus (BEFV) is an arthropod-borne rhabdovirus which causes a disabling febrile infection of cattle and water buffalo. The disease is characterized by sudden onset of fever, depression, difficult swallowing, serous ocular and nasal discharge, dyspnea, stiffness and lameness ***(Nandi and Negi, 1999)*** .Since the exact vector of BEF has not been identified, prevention efforts are mainly aimed at efficient vaccination of susceptible animals. The earliest BEF vaccines were based on field isolates of BEFV which were attenuated by repeated passages in suckling mice and/or cell cultures ***(Van der Westhuizen, 1967)***.

It has been proposed that a combination of vaccine and antiviral agents can be more efficacious strategy to treat FMD-infected animals, limiting the spread of the disease and reduce the number of animals that need to be slaughtered during outbreaks ***(Lefebvre et al., 2010).*** However, there are currently no approved anti-FMDV drugs for the treatment or prevention of FMD ***(Vagnozzi, et al. 2007).*** It is also clear the use of antivirals could help to reinforce, or maybe to replace, the authorized control measures in the protection of livestock against epizootic diseases ***(Lefebvre et al 2014).*** Virus infections pose significant global health challenges, especially in view of the fact that the emergence of resistant viral strains and the adverse side effects associated with prolonged use continue to slow down the application of effective antiviral therapies (***Galdiero et al., 2011*** and ***Stenfeldt et al., 2015)***. This makes imperative the need for the development of safe and potent alternatives to conventional antiviral drugs

(Upadhyayula and Michaels 2013). Antiviral drugs interfere and interrupt the life cycle of the virus. Different targeted-steps of the antivirals are the viral entry in the host cell, the viral replication in the host cell and the formation and egress of newly produced virus ***(Nayak et al., 2015)***. Moreover, antiviral agents act directly on the virus and do not rely on the host immune system, as is the case with vaccines. By consequence the use of a potent antiviral could provide instant protection of animals and herds ***(Hill and Cowen, 2015)***.

The present work aims to study the antiviral activity of **zeolite** against foot and mouth disease virus (FMDV)sero types O/Panasia, A/Iran 05, and SAT2/ Egy 2012 and bovine ephemeral fever virus (BEFV/Abbasia/2000) . Zeolite is a natural, non-toxic zeolite that has monoclinic crystal structure symmetry and strong adsorptive and ion exchange capacity **(*Breck (1964).*** These properties have been largely exploited in industrial, agricultural, environmental and biological technologies **(Mumpton, F.A. 1999)** . Zeolites also possess biological activities, either positive or negative. The best known and documented positive biological activity of natural zeolite is its action as antidiarrheal drug (**Rodriguez et al.,** 1997) .Furthermore, some of them seem to have antibacterial property **(Maeda, T. Nose, Y. 1999)** . Zeolite administered by gastric intubation to mice injected with melanoma cells significantly reduced the number of melanoma metastases (**Mumpton, F.A. 1999) .**

Zeolite treatment of mice and dogs suffering from a variety of tumor types led to improvement in the overall health status, prolongation of life span, and decrease in tumor size. Local application of zeolite to skin cancers of some dogs effectively reduced tumor formation and growth **(Pavelicet al., 2002) and (Pavelic et al., 2001)**. The major negative biological effect of zeolite could be its toxicity in higher organisms (mammal) if the content of heavy metals (Pb, Cd, Zn, etc.) is high.Therefore, a classic acute, sub-chronic and chronic toxicity study of the zeolite from was performed on mice and rats **(Pavelic et al., 2001)** and **(Martin-Klein et al., 2001)** . The aim of this work is study the effect of a natural zeolite on in vitro viral replication of Foot and mouth disease virus types O/Panasia, A/Iran 05, and SAT2/ Egy 2012 and bovine ephemeral fever virus.

MATERIAL AND METHODS

Foot-and-Mouth Disease Virus (FMDV)

FMD viruses, O PanAsia2, A/Iran 05 and SAT2/Egy 2012, are locally isolated strains of bovine origin. The viruses were typed at Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo and confirmed by Pirbright, International Reference Laboratories,United Kingdom .

Bovine ephemeral fever virus (BEFV)

(BEFV) /Abbasia/2000) **(Azab et al., 2002)** which were isolated in VSVRI during an outbreak in Egypt **2000.**

Baby hamster kidney cell line and Vero cells

Baby Hamster Kidney cell line (BHK21) Clone 13, was maintained in FMD Department, VSVRI, according to the technique described before **(Macpherson and Stocher 1962)** using Eagl's medium with 8-10% sterile new bovine serum, obtained from Sigma,USA, 100 IU /ml penicillin, and 100 µg/ml streptomycin, used for detection of the antiviral and cytotoxic effect of Zeolite . Virus dilutions were performed in Eagle's MEM .

Guinea pigs

Healthy adult albino male Guinea pigs of approximately 400-500 grams body weight were used. Thirty animals were used for preparation of Guinea pig adapted FMD virus. Later thirty six Guinea pigs were used for determination of the effect of different concentrations of zeolite against different types of FMDV.

Guinea pig adapted virus

It was prepared by inoculation of FMD virus strains O PanAsia2, A/Iran 05 and SAT2 / Egy 2012, in Guinea pigs using thirty Guinea pigs (Ten for each virus strain through the intradermoplanter in metatarsal pads. After 24-48 hours , the developed lesions (hotness –redness , swelling and filled with fluid of dermis of limbs guinea pigs) were collected aseptically in glycrine buffer.The lesion extract was reinoculated in other Guinea pigs for 5 passages until the virus became adapted to Ginea pigs.

Unweaned baby mice

According to (13-14) 2-3 day old baby Swiss mice were inoculated with 0.1 ml intraperitonially from the mixture of FMDV different types (10^7 ID_{50}/ml) and different concentration of zeolite (six mice for each dilution). Besides that, a group of baby mice inoculated with FMDV only as a control. After 48 h post inoculation, all the baby mice examined to evaluate the antiviral action of zeolite against FMDV strains Paralysis at hind limps of baby mice or death of infected mice means that these mice infected with FMDV death after 24 h is non-specific. 0.3 ml of BEFV virus and different concentrations 10-50 µg/ml of zeolite was inoculated intracerebral in each of 5 baby mice **(Hamoda et al., 2002)** , inoculated mice were kept under hygienic measures in separate cages with their dams subjecting for daily clinical observations. Healthy baby mice were kept as test control. On the 3^{rd} to 4^{th} day post inoculation, when affected mice showed specific signs of BEFV infection (nervous signs, limb paralysis and cyanosis followed by death), the brains of dead mice were collected and subjected to another 2 viral passages in baby mice brains.

Natural Zeolite

A fine powder of natural zeolite, micronized zeolite, was obtained by mechanical micronization **(Pavelic et al., 2001)** of natural zeolite from Zeolith Bentonit Versand, Germany .

Antiviral assays of natural zeolite, micronized zeolite

Zeolite treatment

Due to sedimentation of zeolite in its water suspension, it is not possible to treat a cell culture with zeolite and further follow up morphological changes of cells upon viral infection. For this reason, different viral titers (10^{5} : 10^{1}) and MEM supplemented with 2% Fetal calf serum (negative control) were treated with zeolite at concentrations ranging from(10 ,20,30,40,50)µg /ml. After incubation (15 h, 4°C, constant rotation), the suspension (media and zeolite) was centrifuged (10 min, 4°C, 3000 rpm) to separate the liquid from the solid phase .

Cytotoxic assay of Natural Zeolite on BHK-21 and Vero cells

BHK-21 and Vero cells were seeded in 96–well micro-titre plates (Greiner-Bio one, Germany), for 24 h at 37°C in 5% CO_2. When the cell monolayers were confluent, the medium was removed from each well and

replaced an equal volume of (100 ul) growth media alone (cell controls) and with 100 µl of two-fold serial dilutions of zeolite in fresh medium containing 2% fetal calf serum was added to the confluent cell monolayer ,for cell controls 100 µl of media without samples was added .The plates were incubated at 37 °C and examined 2 days. After incubation, cytotoxicity was determined by examining cellular morphology by inverted light microscope. Morphological changes were scored **(Simoes et al., 1999)** after cell staining with crystal violet stain **(Doyle, et al., 1995).**

Antiviral assays using BHK and Vero cells cultures

Non-toxic dilution of zeolite (100 µl) was mixed with 100 µl of each type of FMDV solution (A, O, SAT2) and BEFV. The mixture was incubated for 30 min at 37°C, then 10-fold dilutions from each mixture were done (100 µl from the mixture add to 900 µl media…i.e.) 50 µl from each dilution inoculated in tissue culture plates BHK cells in 96 multi well-plates. The cultures were then incubated at 37°C. After 24-48 hours , the plates were examined for viral cytopathic effect (CPE) under an inverted light microscope . The drop in the virus titer was calculated regarding to the titer of the positive virus control. This method was done according to ***(Dragana, et al. 2008).***

Determination of the effect of different concentrations of ZEOLITE against different types of FMDV

Guinea pigs were inoculated with 0.5 ml intradermoplante from the adapted viruses (10^7 $\log_{10}$ $TCID_{50}$ / ml) **(Korani et al., 2013**) and different concentrations of zeolite (Six Guinea pigs for each concentration). Besides that, a group of Guinea pigs was inoculated with virus only as a control. After 7 days post inoculation, all the Guinea pigs were examined to evaluate the antiviral action of zeolite.

RESULTS AND DISCUSSION

Two types of viruses were chosen on the basis of their morphology. The ephemeral fever virus capsid is surrounded by a lipoprotein envelope while Foot and mouth disease viruses are not enveloped. Both test viruses are single stranded RNA viruses while ephemeral fever virus has negative sense genome. Both test viruses have specific CPE appears in (BHK) and Vero cell culture , within 24 h - 48 h .

Table (1): Cytotoxicity of zeolite

Concentration of ZEOLITE (µg / ml)	Cytotoxicity %
100	100%
80	75 %
70	50%
60	25%
50	0%
40	0%
30	0%
20	0%
10	0%

The optimal dilution of zeolite is 50 µg / ml could be used safely on the cells

These results showed that there was a direct relationship between increasing concentration of zeolite and the cytotoxicity of the treated cells. At concentration of 100 µg/ml, the cytotoxicity was about 100% and when the concentration decreased till reaching 50µg/ml there was no cytotoxicity found in the treated cells.There was a clear reduction in the FMD virus titer of different strains was achieved by incubating the virus with nontoxic dose of zeolite at concentration of (50 µg/ml) compared with the non-treated virus (virus control).The result also showed the inhibitory concentration 50% (IC_{50}) of zeolite which give 50% mortality of infected baby mice with FMDV.The observed percentages of antiviral effect also depended on the type of virus as shown in Table (2).

Table (2) showed that the effect of concentration (10, 20, 30, 40, 50 µg /ml) of zeolite on FMD virus O/panAsia, with different titer . 50 µg /ml of zeolite is more effective concentration on the virus (5.7 % , 14.2 % , 33.1 % , 78.8 % and 94.6 % (with virus titer 10^{-5}) inhibition of CPE, respectively While FMD virus A, with the 10, 20, 30, 40, 50 µg /ml of zeolite induced a maximum of 6 % , 17 % , 36.2 % , 73 % , 96.3 % (with virus titer 10^{-5}) inhibition of CPE, respectively .Also FMD virus SAT 2, with the 10, 20, 30, 40, 50 µg /ml of zeolite induced a maximum of 5.8 % , 18 % , 38 % , 77 % and 92 % (with virus titer 10^{-5}) inhibition of CPE, respectively .And BEF virus, Concentrations of 10, 20, 30, 40, 50 µg/ml of ZEOLITE induced a

maximum of 17 % , 15.7 % , 42.4 % , 64.6 % and 98 % (with virus titer 10^{-5}) inhibition of CPE, respectively .

Table (2): Percentage of inhibition of the cytopathic effect using different dilution of FMDV and BEFV titer and different dilution of zeolite **.**

virus		ZEOLITE (µg/ml)	Virus titer				
			10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
			inhibition of the cytopathic effect %				
FMD	Type O	10	5.7	3.3	0	0	0
		20	14.2	12	10.3	6	2.8
		30	33.1	27	21	15	12.5
		40	78.8	52	45	41.2	38
		50	94.6	81	79	68.2	61
	Type A	10	6	4.8	0	0	0
		20	17	13.4	10	8.5	5
		30	36.2	25	20.8	15.7	12
		40	73	62	55.4	41	37
		50	96.3	86	77.2	69	65
	Type SA T2	10	5.8	3.1	0	0	0
		20	18	15.4	10.8	6.5	3
		30	38	25.4	21	15	12.8
		40	77	56	45.3	41	38.3
		50	92	84	76.1	65.3	58
BEF		10	17	15	11	9.5	9
		20	15.7	14	12.9	9	8
		30	42.4	38	34.4	29.3	22
		40	64.6	56	49.5	39.3	32.9
		50	98	90	84	77	70.8

The results in **table (2)** showed that zeolite at concentration of 50 µg/ml revealed 100 % reduction in the virus titre after treatment of the virus externally before infection of the cells compared to the non-treated virus and this result is agreed with that obtained by **(Khandelwal et al., 2014 and Galdiero et al., 2011)** who studied the mechanism of antiviral action against different viruses. The results investigated that concentration of 50 µg/ml has not prophylactic effect for the cells before infection with the virus but when this concentration was used after adsorption of the virus to the cells it exhibited a (**94.6** , **96.3**,92 & 98% reduction in the virus

titer (FMD O,A,SAT2 & BEFV) respectively . These results meaning that this concentration had an antiviral effect against infection of FMDV and BEFV. From table (3) the results revealed that the inhibitory concentrations 100% (IC%) was 50 µg / ml , 40 µg / ml and 30 µg / ml which give 100% inhibition of infection in inoculated Guinea pigs with FMDV three types .

Table (3): The effect of different concentrations of zeolite against different types of FMDV in guinea pigs.

Concentration of zeolite	Number of Guinea piga	FMD V					
		Type O		Type A		Type SAT2	
		Infected Guinea pigs	*IC %	Infected Guinea pigs	IC%	nfected Guinea pigs	IC%
50 µg / ml	6	0	100	0	100	0	100
40 µg / ml	6	0	100	0	100	0	100
30 µg / ml	6	0	100	0	100	0	100
20 µg / ml	6	4	33	5	17	5	17
10 µg / ml	6	6	0	6	0	6	0
Virus control	6	6	0	6	0	6	0

*IC = inhibitory concentration

The inhibition of viral proliferation must probably be unspecific and independent of virion size, structure and genome type. As zeolite consists of a mixture of particles of approximately 1 um in diameter and an internal pore size of 0.35nm, virions ranging from 20 to 200 nm in size were probably incorporated within the mesoporous zeolite aggregate and/or adsorbed on the surface of their crystalline microstructure during the 15h treatment of virally infected culture media. This would be the most plausible explanation because a similar phenomenon is used in the method of viral concentration by capture on borosilicate glass powder although the particle size is much larger (100–200 nm) **(Korani et al., 2013**). Furthermore, zeolite adsorb essential minerals and amino acids from culture media (**C ˇ ecˇuk, D. and Grce, 1992).** Inhibition of viral proliferation by capture and/or adsorption of versions onto zeolite

crystalline microstructure. Another possible mechanism of action of zeolite onto viral particles is its ion exchange capability that could destabilize morphology of viral particles, namely as lipoprotein structure (viral envelope) is less resistant to environment than protein (viral capsid), this could explain why BEF (enveloped) were more destabilized than FMD viruses (non-enveloped) by zeolite.

The exact mechanism of action of zeolite based on the ion exchange property of their interaction with viral particles in an aqueous solution (culture media), needs further investigation, extensive biochemical analysis of media and virion changes. the mechanisms of action of zeolite upon different types of viruses are probably non-specific which makes it more interesting than conventional antiviral drug **(Hunter 1985).** Such inactivation of viral particles by zeolite would be extremely interesting for viruses that infect the digestive tract such as enteroviruses and adenoviruses, and because zeolite can be orally administrated without toxicity **(Pavelic et al., 2001)** it could be used for therapeutic purposes. Besides that, zeolite could be used as traditional natural antidiarrheal therapy such as clay and activated charcoal **(Hunter 1985) and (Rodman, M.J. 1980) .** This is why new efficient and inexpensive potential drugs such as zeolite could be helpful to inhibit, if not eradicate, viral infections.

The control of FMD and BEF relies on slaughter of the exposed animals and vaccination with chemically inactivated vaccines. There had been several attempts to develop antiviral drug therapy that affect specific viral protein targets. In this study we studded the antiviral activity of Zeolite against Foot and Mouth disease virus Bovine ephemeral fever virus , the results found in **table (1)** it was showed clearly that the cytotoxicity of the cells was directly proportional to the concentration of Zeolite . Increased concentration Zeolite was accompanied by changes in cell morphology, at concentration 100 µg/ml, where cells became rounded and nuclei were more prominent and the cells were found to float in the medium. At concentration 50 µg/ml the cells appear normal and there was no cytotoxicity in the cells. Our study indicates an inhibitory effect of zeolite upon viral proliferation.The inhibitory effect was represented by the inhibition of specific viral CPE on cell culture compared to the same without treatment with zeolite.

CONCLUSION

Finally, it can be concluded that the zeolite has antiviral activity against FMDV and BEFV with concentration 30-50 µg /ml , but further studies on farm animals need to establish the effective dose , side effects and period of treatment.

REFERENCES

Alexandersen S, Zhang Z, Donaldson AI and Garland AJ.(2003): The pathogenesis and diagnosis of foot-andmouth disease. J Comp Pathol.129(1):1-36.

Arzt J, Juleff N, Zhang Z and Rodriguez LL.(2011): The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. Transbound Emerg.;58(4):291–304

Azab, A. M.; Khodeir, M.H.; Attyat, M. Kotb and El-Gallad, S.B.Kh. (2002): Susceptibility of different cell cultures to bovine ephemeral fever virus. 6thVet. Med. Zag. Conf. 41-55.

Breck D.W.J, (1964) : Chem. Educ. 41 : 678.

Brown, F. (2003) : The history of research in footand-mouth disease. Virus Res. 91 : 3-7.

C ˇ ecˇuk, D. and Grce, M. (1992) : Rev. Epide´m. Sante´ Publ. 40 : 182.

Doyle, A.; Griffiths, J.B. and Newel D.G. (1995): Cell and Tissue Cultures : Laboratory Procedures.John Wiley and Sons, England .

Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V and Galdiero M.(2011): Silver nanoparticles as potential antiviral agents.Molecules, 24;16(10):8894-918.

Grubman, M. J. (2005): Development of novel strategies to control foot-and-mouth disease: marker vaccines and antivirals. Biologicals **33:**227-234.

Grubman MJ and Baxt B.(2004): Foot-and-mouth disease. Clin Microbiol Rev. 17(2):465–93.

Hill JA and Cowen LE.(2015): Using combination therapy to thwart drug resistance. Future Microbiol. ;10:1719-26.

Hamoda, F.K.; Khalaf-Allah, S.S. and Khodeir, M.H. (2002): Some clinical, epidemioclogical and laboratory studies on bovine ephemeral fever (Three day sickness). Vet. Med. J. Giza, Vol.50, No.2: 203-220 .

Hunter,] J.M. , Science 228 (1985) 1040.

Khandelwal,N.,Kaur,G.,Kumara,N. and Tiwari,A (2014):Application of Silver nanoparticles in viral inhibition: A new hope for antivirals.Digest Journal of Nanomaterials and Biostructures Vol. 9, No. 1, 175 - 186.

Korani M, Rezayat SM and Arbabi Bidgoli S (2013): Sub-chronic Dermal Toxicity of Silver Nanoparticles in Guinea Pig: Special Emphasis to Heart, Bone and Kidney Toxicities. Iran J. Pharm Res.12(3):511-9.

Lefebvre DJ, Neyts J and De Clercq K.(2010): Development of a foot-and-mouth disease infection model in severe combined immunodeficient mice for the preliminary evaluation of antiviral drugs.Transbound Emerg Dis;57(6):430-3.

Lefebvre DJ, De Vleeschauwer AR, Goris N, Kollanur D, Billiet A, Murao L, Neyts J and De Clercq K. (2014): Proof of Concept for the Inhibition of Foot-and-Mouth Disease Virus Replication by the Anti-Viral Drug 2'-C-Methylcytidine in Severe Combined Immunodeficient Mice.Transbound Emerg Dis.;61(6):e89-91.

Maeda, T. Nose, Y. (1999) : Artif. Organs 23 : 129.

Macpherson, M.and Stocher, B., (1962) : Polyma transformation hamster cell clones, an investigation of genetic factors affecting cell competence. Virology, 16: 147-151.

Martin-Klein, I. , Flegar Mastric, Z., Zadro, R., Breljak, D. ,Stanovic Janda, S. , Stojkovic, R. , Marus ic´, M., Radacic´, M. and Boranic, M., (2001) : Food Chem. Toxicol. 39 :717.

Mumpton, F.A. (1999) : Proc. Natl. Acad. Sci. USA 96 :3463.

Nandi, S. and Negi, B. S. (1999): Bovine ephemeral fever: A review Comp. Immun. Microbiol. Infect. Dis. 22: 81-91

Nayak,B.K.,Chitra,N.,and AnimaNanda.(2015): Comparativeantibiogram analysis of AgNPs synthesized from two AlternariaSpp. With amoxicillin antibiotics. J.Chem.Pharm.Res. 7,727–731.

Pacheco JM, Smoliga GR, O'Donnell V, Brito BP, Stenfeldt C, Rodriguez LL and Arzt J. (2015): Persistent Foot-and-Mouth Disease Virus Infection in the Nasopharynx of Cattle; Tissue-Specific Distribution and Local Cytokine Expression. PLoS One.; 10(5):e0125698.

Pavelic´, K., Katic´, M., S˘verko, V., Marotti, T., Bos˘njak, B., Balog, T. , Stojkovic , R., Radac ic , M. C˘ olic, M. and Poljak-Blazi , M. (2002) : J. Cancer Res. Clin. Oncol. 128 :37.

Pavelic , K., Hadzija, M., Bedrica, L., Pavelic , J., Đikic, I., Katic, M.;Kralj, M., Herak Bosnar M.., Kapitanovic, S. , Poljak-Blazi M., Krizanac, S., Stojkovic, R., Jurin, M., Subotic´, B. and C olic´, M.(2001) : J. Mol. Med. 78 : 708.

Rodriguez, G.,Fuentes, M., Barrios, A. , Iraizoz, A., Perdomo, I. and,Cedre, B. (1997) : Zeolites 19 : 441.

Rodman, M.J. (1980) : R.N. 43 :58.

Simoes CMO, Amoros M and Girre L (1999) : Mechanism of antiviral activity of triterpenoid saponins. Phytoth Res 21: 317-325.

Stenfeldt C, Eschbaumer M, Pacheco JM, Rekant SI, Rodriguez LL and Arzt J.(2015): Pathogenesis of Primary Foot-and-Mouth Disease Virus Infection in the Nasopharynx of Vaccinated and Non-Vaccinated Cattle.PLoS One.;10(11):e0143666.

Upadhyayula S and Michaels MG. (2013): Ganciclovir, Foscarnet, and Cidofovir: Antiviral Drugs Not Just for Cytomegalovirus.J.Pediatric nfect. Dis. Soc.; 2(3):286-90

Upadhyayula S and Michaels MG. (2013): Ganciclovir, Foscarnet, and Cidofovir: Antiviral Drugs Not Just for Cytomegalovirus.J.Pediatric nfect. Dis. Soc.; 2(3):286-90

Van der Westhuizen, B. (1967) : Studies on bovine ephemeral fever: Isolation and preliminary characterization of a virus from natural and experimentally produced cases of bovine ephemeral fever. Onderstepoort J. Vet. Res., 34: 29-40.

Vagnozzi, A.; Stein, D.A.; Iversen, P.L. and Elizabeth, R. (2007) : Inhibition of foot-and-mouth disease virus infections in cell cultures with antisense morphino oligomers. Journal of Virology, 81 (21): 11669-11680.

Zhang,Z., Doel,C.and John B. Bashiruddin,(2015): Interleukin-10 production at the early stage of infection with foot-and-mouth disease virus related to the likelihood of persistent infection in cattle.Vet Res. 46:132