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RESEARCH ARTICLE

Determination of lipid peroxidation biomarkers in Vero cell line inoculated with Bovine Ephemeral Fever Virus

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Özet

Avcı O, Yavru S, Dik I. Bovine Ephemeral Fever Virus inokule edilen Vero hücre kültürlerinde lipid peroksidasyon biomarkırlarının belirlenmesi.

Abstract

Avci O, Yavru S, Dik I. Determination of lipid peroxidation biomarkers in Vero cell line inoculated with Bovine Ephemeral Fever Virus.

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Amaç: Bu çalışma Bovine Ephemeral Fever Virus (BEFV, Genbank No: GQ229452.1) inokule edilen Vero hücre kültürlerinde lipid peroksidasyon biomarkırlarını belirlemek amacı ile yapıldı.

Gereç ve Yöntem: BEFV inoküle edildikten sonra 4'er saat ara ile 5 gün boyunca hücre süpernatantları toplandı. Hücre süpernatantlarındaki süperoksid dismütaz (SOD), katalaz (CAT), glutasyon peroksidaz (GPX) enzimleri, glutasyon (GSH) ve malondialdehit (MDA) miktarı ticari olarak temin edilen ELISA kitleri ile ölçüldü. Bunun yanında ayrıca BEFV'nin meydana getirdiği sitopatojenik etki (CPE) invert mikroskop yardımı ile periyodik olarak değerlendirildi.

Bulgular: Araştırmada BEFV'nin CPE'si inokulasyonu takiben 72. saatte elde edildi. Maksimum SOD miktarı 56. saatte tespit edilirken, CAT ve GPX minimum seviyeleri ise sırasıyla 8. ve 104. saat olarak belirlendi. GSH miktarı sırasıyla 30., 60., 84. ve 120. saatlerde maksimum; 44., 92. ve 112. saatlerde minimum seviyede ölçüldü. MDA miktarında ilk 8 saatte ani düşüş meydana geldiği belirlendi. CAT, MDA ve SOD düzeylerinin BEFV'in neden olduğu CPE oluşumundan önce azaldığı tespit edildi.

Öneri: Lipid peroksidasyon biyomarkırlarının BEFV'nin patogenezinde yararlı olabileceği öngörülmektedir. BEFV enfeksiyonunda oluşacak oksidatif hasarın azaltılması ile ilgili çalışmaların planlanmasında yardımcı olabilir.

Anahtar kelimeler: BEFV, SOD, CAT, GPX, MDA

Aim: The aim of the present study was to determine of lipid peroxidation biomarkers in Vero cell line inoculated with Bovine Ephemeral Fever Virus (BEFV, Genbank No: GQ229452.1).

Materials and Methods: Cell supernatants were collected 4 h/day for 5 days after BEFV inoculation. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) enzymes, glutathione (GSH) and malondialdehyde (MDA) values were analyzed from the test media by commercially available ELISA kits. In addition to this, cytopathogenic effects (CPE) of BEFV in cell culture were evaluated periodically by invert microscope.

Results: In this research, CPE of BEFV was observed at 72 h post-inoculation. Maximum level of SOD was determined at 56 h, while minimum levels of CAT and GPX were determined at 8 and 104 hours, respectively. Maximum GSH levels were determined at 30, 60, 84 and 120 hours while minimum GSH concentrations were measured at 44, 92 and 112 hours. A sudden decrease of MDA level was observed in the first 8 hours occurred. In addition, CAT, MDA and SOD levels decreased before developing BEFV-caused CPE.

Conclusion: It is concluded that lipid peroxidation biomarkers can be useful in the pathogenesis of BEFV. It may prove helpful in the design of future protect from decreasing of oxidative damage associated with BEFV infection.

Keywords: BEFV, SOD, CAT, GPX, MDA

Introduction

Bovine Ephemeral Fever (BEF), caused by Bovine Ephemeral Fever Virus (BEFV, order Mononegavirales, family Rhabdoviridae, genus Ephemerovirus), is a viral vector borne disease (Murray 1997), and virus causes acute infection, suddenly fever, anorexia, depression (Wang et al 2001), arthritis and immobility joint swelling (Mellor 1996), noncontagious, that affects both cattle and buffalo (Uren et al 1992). BEFV has a negative sense RNA, five structural proteins (G, N, P, M, L), and propagated in one-day old baby mice after intracerebral inoculation. Although bovine kidney, hamster lung, Aedes albopictus (St. George 1985), Baby Hamster Kidney-21 (Nandi and Negi 1999) and Vero (Yeruham et al 2010) cell cultures can be preferred for virus propagation in vitro.

Oxidative stress can be determinate in all cells during physiologic events for vitality or harbinger of physiopathology damage. Unpaired electron in atomic or molecular structure is called free radical. Free oxygen radicals on biological systems firstly reported by Gershman et al. in 1954 (Turrens 1991). Oxidant structures are produced during various metabolic events in organism. Autoxidation of polyunsaturated fatty acids (PUFAs) has been realizable with oxygen. Malondialdehyde (MDA) is formed as a result of lipid peroxidation. MDA, is a dialdehyde, contains 3-carbon and carbonyl group at C1 and C3. It can be learned the severity of lipid peroxidation by measurement of MDA levels (Fernandez et al 1997, Aydin et al 2009, Aydin et al 2010). Infectious diseases are reported to be a source of free oxygen radicals (Maksimenko and Vavaev 2012). Oxidative stress in viral infections was reported in the studies (Raju et al 2000, Crist et al 2013, Yahya et al 2013, Fraisier et al 2014).

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) enzymes, and glutathione (GSH) play as antioxidants in cells (Guemouri et al 1991, Nguyen et al 2004). SOD, firstly defined by McCord and Fridovich in 1969 (Guemori et al 1991), provides conversion of superoxide to hydrogen peroxide and oxygen (Jung et al 2003). The CAT is a common enzyme in all living cells that catalyzes the decomposition of hydrogen peroxide to water and oxygen (Peng et al 2014). GPX is an important cytoplasmic enzyme, containing four selenium atoms in structure (Zhou et al 2006). GSH is a tripeptide made of cysteine, glycine and glutamate. It is an important antioxidant that prevents damage to important cellular components caused by free radicals. It plays a critical role in cellular antioxidant defense systems and protects the liver against reactive oxygen species (Mitchel and Russo 1987).

It has been reported that free oxygen radicals can be increased by aging (Giacomoni et al 2000, Halliwell and Whiteman 2004), and oxidative stress can occur in cancer, diabetes (Zherebitskaya et al 2009), neurodegenerative disorder (Halliwell 2001, Andersen 2004, Lin and Beal 2006), and viral infections (Schwarz 1996, Israel and Gougerot-Pocidalo 1997, Peterhans 1997). Infectious diseases can be sources of free oxygen radicals (Maksimenko and Vavaev 2012). It is likely that antioxidant enzyme levels has shown significant change in viral infections (Lutsenko 2013, Stukelj et al 2013), and oxidative stress can occurs (Raju et al 2000, Crist et al 2013, Yahya et al 2013, Fraisier et al 2014).

It was hypothesized that oxidative stress and free radicals can be formed in permanent Vero cell which inoculated with BEFV. The purpose of the present study was to determine and compare the lipid peroxidation markers in Vero cell inoculated with BEFV.

Material and Methods

Cell culture and virus

Vero cell line and BEFV (Genbank No: GQ229452.1) were supplied from Virology Department, Faculty of Veterinary, University of Selcuk. Cells were cultivated in 25 cm^2 flasks (Corning, USA) with Dulbecco's Modified Eagle Medium (DMEM, Biological Industries, Israel) supplemented with 5% heat-inactivated fetal calf serum (FCS, Biological Industries, Israel), and 1% antibiotics (10000 IU/mL penicillin G, 10 mg/

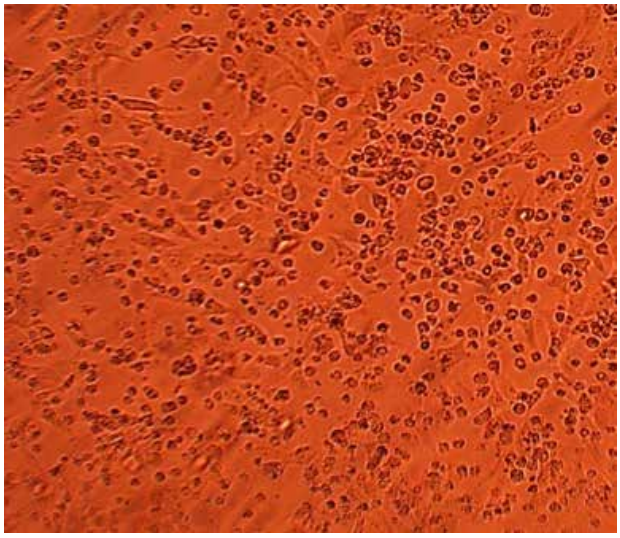


Figure 1. CPE after 72.h post-inoculation (X40).

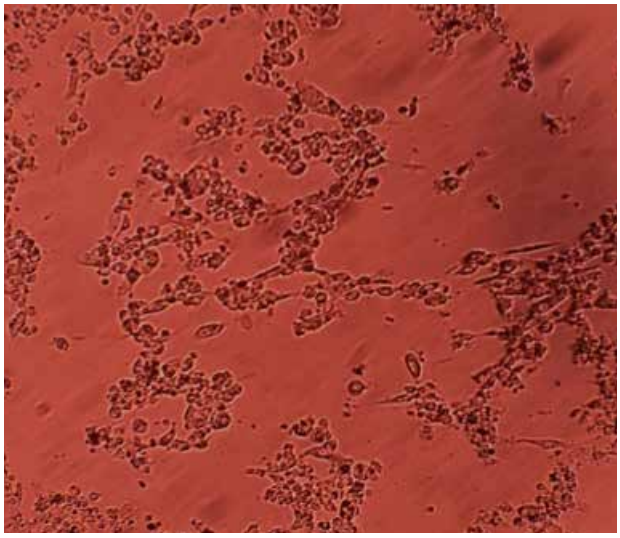
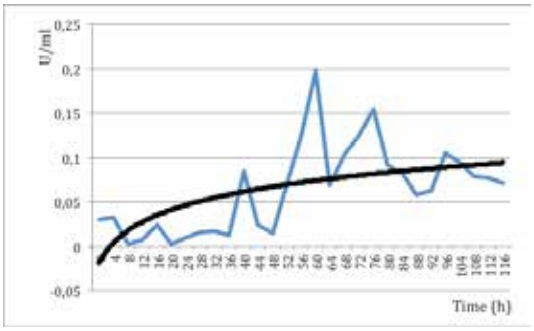
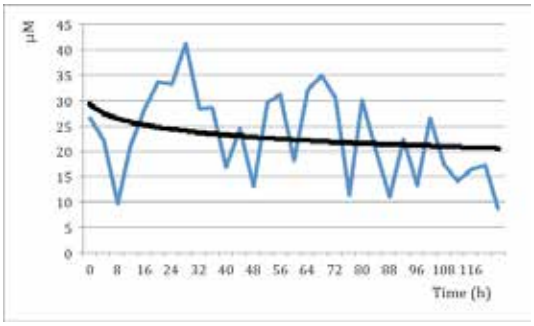


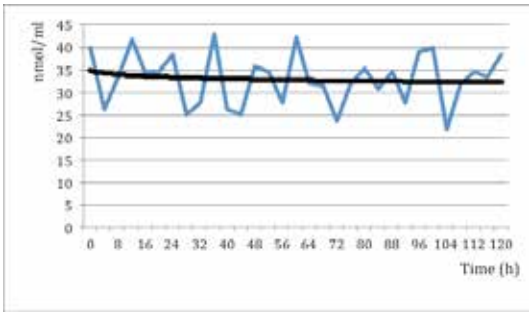
Figure 2. CPE after 120.h post-inoculation (X40).



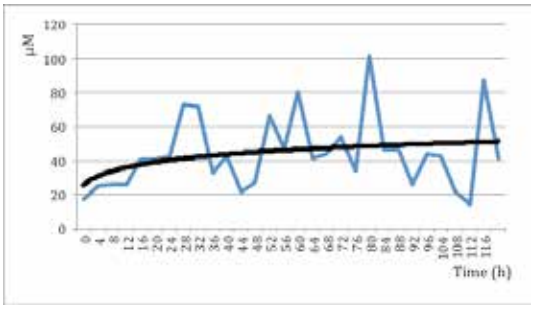
Graphic 1. Periodic SOD values after BEFV inoculation.



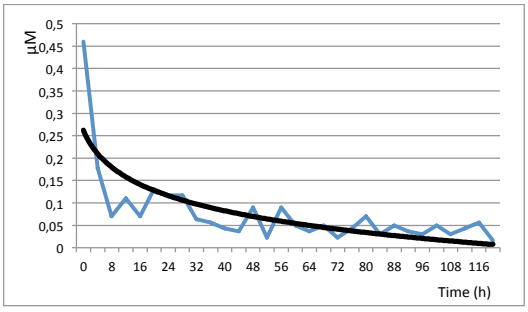
Graphic 2. Periodic CAT values after BEFV inoculation.



Graphic 3. Periodic GPX values after BEFV inoculation.



Graphic 4. Periodic GSH values after BEFV inoculation.



Graphic 5. Periodic MDA values after BEFV inoculation.

mL streptomycin, and 25µ/mL amphotericin B; Biological Industries, Israel) at 37°C and 5% CO_2 incubator. A total of 30 flasks were used during experiment. After 100 $TCID_{50}$ (0.5 mL) of the BEFV was inoculated by non-adsorption method medium were collected at 4 h intervals for 5 days. The supernatants were centrifuged for 10 min at 5000 g (+4°C) and stored at -80°C until use.

ELISA Analyses

Levels of SOD (Cayman, USA), CAT (Cayman, USA), GPX (Cayman, USA), GSH (OxisResearch, USA) and MDA (OxisResearch, USA) in the supernatants of the Vero cell cultures were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The tests were performed as per the manufacturer's instructions. The plates were then read on an automatic micro plate reader (Rayto RT 2100C, China). This study protocol has been approved by ethics committee of Veterinary Medicine of Selcuk University (Report No: 2014/02).

Results

Cytopathogenic effects of BEFV on Vero cell line after 72 and 120 h post-inoculation were shown in Figure 1 and 2, respectively. SOD, CAT, GPX, GSH and MDA concentrations of BEFV were shown in Graphics 1, 2, 3, 4 and 5, respectively.

Discussion

It has been recognized that oxidative stress plays an important role in diverse viral infections (Valyi-Nagy and Dermody 2005, Hahn et al 2008, Williams et al 2010).

In the current research, BEFV changed oxidative status of Vero cell line. BEFV caused fluctuation in the enzymatic antioxidants including SOD (Graphic 1), CAT (Graphic 2) and GPX (Graphic 3) activities and non-enzymatic antioxidant GSH (Graphic 4) level. In the experimentally induced infectious studies, fluctuations of antioxidant enzymes were reported when these enzymes were measured from tissues (Yazar and Tras 2001). In this study, maximum level of SOD was measured at 56 h, whereas minimum levels of CAT and GPX were determined at 8 and 104 h, respectively. Maximum GSH concentrations were measured at 30, 60, 84 and 120 h, while minimum concentrations were determined at 44, 92 and 112 h, respectively. In addition, CAT and SOD activities decreased before developing CPE. These results may be reflect that BEFV change oxidative status in Vero cell line in vitro, and BEFV firstly effect CAT and SOD activities. It has been shown that oxidative damage is related with acute encephalitis in mice experimental infected with herpes simplex virus type 1 (Schachtele et al 2010). Also it has also been reported that oxidative injury can be occur in rabies by Jackson et al. (2011). It is known that different viral proteins may target cell mitochondria and impair mitochondrial morphology (Lichty et al 2006). Oxidative stress is strongly induced by stimulation of the mitochondrial Ca2+ in hepatitis C infection (Li et al 2007). The significantly reduced ($P<0.01$) level of Ca, inorganic phosphor, and total protein in blood sera of cattle infected with BEFV reported by Sahin et al (2004).

Interestingly, MDA levels, which is accepted oxidative stress biomarker, dramatically decreased at 8 hour after BEFV inoculation (Graphic 5). It has been reported that decreased MDA level may be measured when samples contain insufficient lipid substrate (Shukla et al 1997). It has been speculated that Vero cell line may has lower lipid substrate in the current research.

Conclusion

It has been concluded that SOD, CAT, GPX and MDA can be used as a novel early lipid peroxidation biomarkers of oxidative stress in Vero cell inoculated with BEFV. Antioxidant supplementations may be useful for decreasing of oxidative damage caused by BEFV in vitro studies.

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References

Andersen JK, 2004. Oxidative stress in neurodegeneration: Cause or consequence? Nat Med, 10, 18-25.

Aydin I, Bulbul A, Avci GE, Celik HA, 2009. Serum oxidative status and adenosine deaminase activity in dogs with transmissible venereal tumour. Bull Vet Inst Pulawy, 53, 771-774.

Aydin I, Bulbul T, Polat ES, Yazar E, 2010. Serum antioxidant status and adenosine deaminase activity during the gestational period of sheep. Revue Med Vet, 161, 479-484.

Crist MB, Melekhin VV, Bian A, Shintani A, Milne GL, Kalliampur AR, Dageforde LA, Haas DW, Hulgan T, 2013. Higher serum iron is associated with increased oxidant stress in HIV-infected men. J Acquir Immune Defic Syndr, 64, 367-373.

Fernandez J, Alvarez JA, Lopez JA, 1997. Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chem, 59, 345-353.

Fraisier C, Rodrigues R, Vu Hai V, Belghazi M, Bourdon S, Paranhos-Baccala G, Camoin L, Almeras L, Peyrefitte CN, 2014. Hepatocyte pathway alterations in response to in vitro Crimean Congo hemorrhagic fever virus infection. Virus Res, 179, 187-203.

Giacomoni PU, Declercq L, Hellemans L, Maes D, 2000. Aging of human skin: Review of a mechanistic model and first experimental data. IUBMB Life, 49, 1-5.

Guemouri L, Artur Y, Herbeth B, Jeandel C, Cuny G, Siest G, 1991. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clin Chem, 37, 1932-1937.

Hahn K, Robinson B, Anderson C, Li W, Pardo CA, Morgello S, Simpson D, Nath A, 2008. Differential effects of HIV infected macrophages on dorsal root ganglia neurons and axons. Exp Neurol, 210, 30-40.

Halliwell B, 2001. Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. Drugs Aging, 18, 685-716.

Halliwell B, Whiteman M, 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol, 142, 231-255.

Israel N, Gougerot-Pocidalo MA, 1997. Oxidative stress in human immunodeficiency virus infection. Cell Mol Life Sci, 53, 864-870.

Jackson AC, Kammouni W, Fernyhough P, 2011. Role of oxidative stress in Rabies virus infection. Adv Virus Res, 79, 127-136.

Jung O, Marklund SL, Geiger H, Pedrazzini T, Busse R, Brandes RP, 2003. Extracellular superoxide dismutase is a major determinant of nitric oxide bioavailability: In vivo and ex vivo evidence from EC-SOD deficient mice. Circ Res, 93, 622-629.

Li Y, Boehning DF, Qian T, Popov VL, Weinman SA, 2007. Hepatitis C virus core protein increases mitochondrial ROS production by stimulation of Ca+2 uniporter activity. FASEB J, 21, 2474-2485.

Lichty BD, McBride H, Hanson S, Bell JC, 2006. Matrix protein of Vesicular stomatitis virus harbours a cryptic mitochondrial-targeting motif. J Gen Virol, 87, 3379-3384.

Lin MT, Beal MF, 2006. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature, 443, 787-795.

Lutsenko MT, 2013. Enzymatic activity of peripheral blood erythrocytes in pregnant women with exacerbation of herpesvirus infection. Bull Exp Biol Med, 154, 505-507.

Maksimenko AV, Vavaev AV, 2012. Antioxidant enzymes as potential targets in cardioprotection and treatment of cardiovascular diseases. Enzyme antioxidants: The next stage of pharmacological counterwork to the oxidative stress. Heart Int, 7, doi: 10.4081/hi.2012.e3.

Mellor PS, 1996. Culicoides: Vectors, climate change and disease risk. Vet Bull, 66, 301-306.

Mitchel JB, Russo A, 1987. The role of glutathione in radiation and drug induced cytotoxicity. BJ Cancer, 55, 96-104.

Murray MD, 1997. Possible vectors of bovine ephemeral fever in the 1967/68 epizootic in northern Victoria. Aust Vet J, 75, 220.

Nandi S, Negi BS, 1999. Bovine ephemeral fever: A review. Comp Immunol Microbiol Infect Dis, 22, 81-91.

Nguyen AD, Itoh S, Jeney V, Yanagisawa H, Fujimoto M, Ushio-Fukai M, Fukai T, 2004. Fibulin-5 is a novel binding protein for extracellular superoxide dismutase. Circ Res, 95, 1067-1074.

Peng C, Wang X, Chen J, Jiao R, et al., 2014. Biology of ageing and role of dietary antioxidants. Biomed Res Int, Article ID 831841.

Peterhans E, 1997. Reactive oxygen species and nitric oxide in viral diseases. Biol Trace Elem Res, 56, 107-116.

Raju TA, Lakshmi AN, Anand T, Rao LV, Sharma G, 2000. Protective effects of quercetin during influenza virus-induced oxidative stress. Asia Pac J Clin Nutr, 9, 314-317.

Sahin T, Camkerten I, Yaralioglu Gurgoze S, 2004. Some biochemical parameters with Bovine Ephemeral Fever in cows. YYU Vet Fak Derg, 15, 71-73.

Schachtele SJ, Hu S, Little MR, Lokensgard JR, 2010. Herpes simplex virus induces neural oxidative damage via microglial cell Toll-like receptor-2. J Neuroinflamm, 7, 35.

Schwarz KB, 1996. Oxidative stress during viral infection: a review. Free Radic Biol Med, 21, 641-649.

Shukla A, Rasik AM, Patnaik GK, 1997. Depletion of reduced glutathione, ascorbic acid, vitamin E and antioxidant defence enzymes in a healing cutaneous wound. Free Radic Res, 26, 93-101.

St. George TD, 1985. Studies on the pathogenesis of bovine ephemeral fever in Sentinel cattle. I. Virology and serology. Vet Microbiol, 10, 493-504.

Stukelj M, Toplak I, Svete AN, 2013. Blood antioxidant enzymes (SOD, GPX), biochemical and haematological parameters in pigs naturally infected with porcine reproductive and respiratory syndrome virus. Pol J Vet Sci, 16, 369-376.

Turrens JF, 1991. The potential of antioxidant enzymes as pharmacological agents in vivo. Xenobiotica, 21, 1033-1040.

Uren MF, St. George TD, Murphy GM, 1992. Studies on the pathogenesis of bovine ephemeral fever in experimental cattle III. Virological and biochemical data. Vet Microbiol, 30, 297-307.

Valyi-Nagy T, Dermody TS, 2005. Role of oxidative damage in the pathogenesis of viral infections of the nervous system. Histol Histopathol, 20, 957-967.

Wang FI, Hsu AM, Huang KJ, 2001. Bovine ephemeral fever in Taiwan. J Vet Diagn Invest, 13, 462-467.

Williams R, Yao H, Peng F, Yang Y, Bethel-Brown C, Buch S, 2010. Cooperative induction of CXCL10 involves NADPH oxidase: Implications for HIV dementia. Glia, 58, 611-621.

Yahya RS, Ghanem OH, Foyouh AA, Atwa M, Enany SA, 2013. Role of interleukin-8 and oxidative stress in patients with hepatocellular carcinoma. Clin Lab, 59, 969-976.

Yazar E, Tras B, 2001. Effects of fluoroquinolone antibiotics on hepatic superoxide dismutase and glutathione peroxidase activities in healthy and experimentally induced peritonitis mice. Revue Med Vet, 152, 235-238.

Yeruham I, Ham MV, Stram Y, Friedgut O, Yadin H, Mumcuoglu KY, Braverman Y, 2010. Epidemiological investigation of bovine ephemeral fever outbreaks in Israel. Vet Med Int, Article ID 290541.

Zherebitskaya E, Akude E, Smith DR, Fernyhough P, 2009. Development of selective axonopathy in adult sensory neurons isolated from diabetic rats: Role of glucose-induced oxidative stress. Diabetes, 58, 1356-1364.

Zhou L, Xiang W, Potts J, Floyd M, Sharan C, Yang H, Ross J, Nyanda AM, Guo Z, 2006. Reduction in extracellular superoxide dismutase activity in African-American patients with hypertension. Free Radic Biol Med, 41, 1384-1391.