Golender N, et al., J Dairy Res Tech 2019, 2: 007
DOI: 10.24966/DRT-9315/100007

HSOA Journal of Dairy Research & Technology

Research Article

Epizootic Hemorrhagic Disease Virus Type 6: Disease, RNAemia and Abortions - The Israeli Experience

Natalia Golender[1], Yevgeny Khinich[1], Michael Bernstein[2], Nir Edery[3], Marisol Guini[1], Evgeny Eyngor[1] and Velizar Bumbarov[1]*

[1]*Division of Virology, Kimron Veterinary Institute, Beit Dagan 50250, Israel*

[2]*Division of Bacteriology, Kimron Veterinary Institute, Beit Dagan 50250, Israel*

[3]*Division of Pathology, Kimron Veterinary Institute, Beit Dagan 50250, Israel*

Abstract

Epizootic Hemorrhagic Disease (EHD) is non-contagious viral disease affecting mostly white-tailed deer and cattle. It can cause fever, hemorrhages, excessive salivation, agalactia, loss of body weight, abortion and occasionally death. A large outbreak of epizootic hemorrhagic disease virus serotype 6 (EHDV-6) was identified in several Israeli cattle farms towards the end of 2015.

In this study, we examined the persistence of EHD-6 viral RNA circulation in whole blood (RNAmia) from six naturally infected sentinel calves. RNAmia duration was estimated based on real-time reverse transcription polymerase chain reaction (RT-qPCR) of whole blood samples. Our results indicate that EHDV RNAmia lasted for approximately four months from the presumed date of the initial natural infection.

Additionally, based on the data of PCR-positive field samples from sick animals and aborted cattle fetuses, along with isolated viruses from samples taken at the same period of time as a routine diagnostic procedure, we conclude that the outbreak took place between late September and November 2015. Analysis of abortion cases throughout this period (all aborted fetuses and placentas tested for abortogenic pathogens), suggests that EHDV-6 was involved in the part of abortion cases in this cattle population.

To our knowledge this is the first study of field monitoring of EHDV RNAmia, and first EHDV RNA detection in aborted fetuses.

Keywords: Abortion; EHDV-6; Orbivirus; Reoviridae; RNAmia; Viremia

***Corresponding author:** Velizar Bumbarov, Division of Virology, Kimron Veterinary Institute, Beit Dagan 50250, Israel, E: Mail: velizarb@moag.gov.il

Citation: Golender N, Khinich Y, Bernstein M, Edery N, Guini M, et al. (2019) Epizootic Hemorrhagic Disease Virus Type 6: Disease, RNAemia and Abortions - The Israeli Experience. J Dairy Res Tech 2: 007.

Received: July 27, 2019; **Accepted:** August 14, 2019; **Published:** August 26, 2019

Introduction

Epizootic Hemorrhagic Disease (EHD) is an infectious, non-contagious viral disease of ruminants transmitted by insects of the genus *Culicoides*. The virus belongs to the genus *Orbivirus* within the family *Reoviridae*. At least seven serotypes are currently recognized (http://wahis2-devt.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.01.04b_EHD.pdf).

EHDV has been identified in Japan, Africa, North America, Australia and Mediterranean Basin. EHD in cattle was first described in Japan ("Ibaraki disease" or EHDV-2; Omori T.et al., 1969), but the 2006 outbreak of EHDV-7 in Israel [1], EHDV-6 in several countries of Mediterranean Basin [2] and the 2012 outbreak in the US (EHDV-2, EHDV-6 and EHDV-1) has increased the concerns of EHDV affecting cattle [3].

In 2015 EHDV-6 outbreaks in cattle were recorded in Tunisia and Israel (http://www.promedmail.org/post/3685159). Clinical signs observed in Israel, were characterized by a drop in milk production, weakness, excessive salivation, lameness, recumbency, pyrexia, milderythema of nasal and oral mucosae, weight loss, abortion and occasional death of animals [4].

Orbiviruses such as *Bluetongue* virus (BT) can circulate in the host for a prolonged time after the clinical signs have resolved. RNAemia can continue for a period of 25 months [5]. However, the data concerning EHDV circulation is scarce, and little is known regarding the duration of RNAemia or sub-clinical infection in cattle. Additionally, EHDV has never been reported as identified or isolated from fetuses. In this study, we monitored the duration of RNAmia in naturally infected calves. Additionally, we estimated duration of the EHDV-6 outbreak in Israel in 2015.

Materials and Methods

RNA extraction, RT-qPCR and conventional RT-PCRs

RNA extractions from field samples (EDTA-anticoagulated whole blood, lung, intestine, liver, heart, kidney, placenta and brain) and from ECE virus isolates followed by pan-EHDV RT-qPCR and conventional RT-PCR were performed as described previously [4,6].

In order to identify Simbu group viruses in bovine aborted fetuses, the in-house Simbu serogroup-specific Uni-S conventional RT-PCR was performed using a One-Step RT-PCR kit (Qiagen, Hilden, Germany).

Gel purification

PCR products resulting from positive samples were consequently purified using the MEGA quick-spin™ Total Fragment DNA

Purification Kit (iNtRON Biotechnology, Gyeonggi-do, South Korea) and analyzed on the ABI 3730xl DNA Analyzer, Hylabs, Rehovot, Israel.

Monitoring of RNAmia and antibody appearance in naturally infected calves

Whole blood and serum samples from six randomly-selected calves (approximately 8 months old at the beginning of monitoring) were sampled monthly from July 2015 to April 2016 and consequently tested by RT-qPCR for presence of EHDV RNA. The serum samples were tested for the presence of EHDV antibodies using LSIVet Ruminant EHDV – Serum ELISA Ab, Lissieu, France.

Virus isolation

EHDV was isolated on Embryonated Chicken Eggs (ECE) and Cell Cultures (CC) as described by [7-9].

BVD Ag ELISA tests

All internal organs from aborted fetuses (brain, lung, spleen, liver, intestine, heart and kidney) and placentas were homogenized in PBS (pH 7.2) to a final concentration of 1:10. The supernatant from these centrifuged samples was used for further BVD antigen ELISA testing by the IDEXX BVDV Ag/Serum Plus Test, Liebefeld-Bern, Switzerland, according to the manufacturer's protocol.

Bacteriological testing

Internal organs from aborted fetuses and placentas were transferred after necropsy for bacteriological testing. Tissue samples, including abomasal contents, were streaked onto MacConkey, blood, nutrient, Skirrow (*Campylobacter*) and *Brucella* (selective trypticase soya) agar plates. Testing for *Mycoplasma spp*. was performed by inoculation of abomasal contents into Fry's mycoplasma broth (Freundt, 1983) and platedonto mycoplasma agar. Placental, lung and abomasal smears were stained by Stamp's modified Zeihl Neelsen method and with an FITC monoclonal antibody for *Chlamydia spp.* (Cellabs, Australia). Abomasal contents were inoculated into tetrathionate broth for *Salmonella* enrichment which was streaked onto MacConkey and Brilliant Green agar after 24 hours incubation. All cultures excluding Salmonella were incubated in a 5% carbon dioxide atmosphere.

MAT for *Leptospira Spp*.

In order to detect exposure to *Leptospira spp.,* the Microscopic Agglutination Test (MAT), which is recommended by the OIE, was performed (http:// www.oie. int /doc/ged/D12009.PDF). Antigens representing serovars *Canicola, Gripotyphosa, Pomona, Icterohaemorrhagiae, Tarassovi, Ballum, Bratislava* and *Hardjobovis* were used. Initially, two-fold serum dilutions were used from 1:50 to 1:200. Positive samples were diluted until an end point was reached. The final titer of a sample was the last dilution of sera in which agglutination of at least 50% of antigen was observed.

IFAT for *Neospora caninum* (*N. caninum*)

The presence of antibodies to *N. caninum* was performed by Indirect Fluorescent Antibody Test (IFAT) as described by [10].

Gross pathology examination

Bovine fetuses with or without placenta were examined post mortem. Tissue samples of lung, heart, spleen, kidney, brain and placenta were fixed in normal buffered 10% formaldehyde for future histopathological examination (The results will be published in a separate report in future).

Results

Routine EHDV diagnosis (field samples)

A total of 152 (out of 321) field samples from cattle showing clinical signs were found positive for EHDV by RT-qPCR. One hundred and eighty three were sampled between October and November 2015, of which more than half (105 samples) were positive. Thereafter, the number of field samples and percentage of positive results decreased gradually until March 2016. From April 2016 onwards, no positive field samples were detected [4] (Table 1).

Date	Adult samples*	VI in ECE*	Aborted fetuses*
Jul-2015	0/32-0%	NT	NT
Aug-2015	0/7-0%	NT	NT
Sep-2015	5/7-71%	2/5-40%	0/1-0%
Oct-2015	57/95-60%	7/56-12.5%	2/9-22.2%
Nov-2015	58/88- 65.9%	3/57-5.3%	3/5-60%
Dec-2015	17/46-36.9%	0/17-0%	6/11-54.5%
Jan-2016	7/17-41.1%	0/2-0%	0/4-0%
Feb-2016	2/15-13.3%	NT	NT
Mar-2016	7/49-14.3%	0/2-0%	NT
Apr-2016	0/23-0%	NT	NT

Table 1: Total and percent positive clinical samples by PCR and virus isolation, July 2015 to April 2016.

*Presented as: positive / total samples-percent
NT - Not Tested
ECE - Embryonated Chicken Eggs
VI - Virus Isolation

Monitoring of EHDV-6 RNAmia and antibodies in naturally infected calves

The EHDV-6 RNA levels in whole blood samples from monitored calves were quantified by RT-qPCR. The highest quantity of EHDV-6 RNA (21.7 - 27.4 Ct) was present in all whole blood samples from all calves during the first two months from the presumed date of infection, after which a gradual decline till undetectable by five months from the presumable date of infection (Table 2).

Date of sampling	Ct qPCR values	ECE VI*	CC VI*
12-Oct-2015	21.7-27.3	0/5	0/5
17-Nov-2015	23.4-27.4	0/6	0/6
8-Dec-2015	23.4-30.8	0/6	0/6
11-Feb-2016	26.1-34.9	NT	NT
31-Mar-2016	NA	NT	NT

Table 2: EHDV RT-qPCR results and virus isolation from whole blood samples from six monitored naturallyinfected calves (sentinels).

NA - Not Amplified; NT - Not Tested; VI -Virus Isolation
* - number of samples used for VI
CC - Cell Culture
ECE - Embryonated Chicken Egg

Seroconversion was detected in all six calves on the 12th of October 2015, but no clinical signs of disease were apparent.

The monitored calves did not show any clinical signs of the disease, as opposed to several dairy cows from the same farm.

Gross pathological changes in aborted fetuses and placentas

Most of the fetuses received for necropsy were at different stages of autolysis, with additional microbial contamination. Only in one fetus, hemorrhages in the heart (right atrium) were seen. In placental samples that were positive for EHDV by RT-qPCR, no gross pathological changes were detected (Table 3).

Date	Lab Num	Lab diagnosis of aborted fetuses			
day/m/y		Virol	Bact	Parasit	Pathol
29/09/15	188/15*	-	-	-	-
08/10/15	192/15[b]	EHDV	-	-	myocarditis
08/10/15	193/15[p]	SHUV	-	*N. caninum*	Mild placentitis
13/10/15	195/15*	-	-	*N. caninum*	-
14/10/15	198/15*	-	-	-	Autolytic
21/10/15	201/15[b]	EHDV	mix.cul	-	Autolytic
25/10/15	203/15[b]	-	mix.cul	-	-
26/10/15	204/15[b]	-	-	*N. caninum*	Hemorrhage in the right atrium
27/10/15	205/15[b]	-	-	*N. caninum*	Multifocal hepatitis
27/10/15	206/15[b]	-	-	-	-
08/11/15	208/15[b]	EHDV	-	*N. caninum*	encephalitis
19/11/15	213/15[b]	-	-	-	Autolytic
19/11/15	214/15 [b]	EHDV/BVD	*L. hardjo* **	-	Large cysts in kidney
23/11/15	221/15*	EHDV	-	-	Mild placentitis
25/11/15	224/15*	SHUV	-	-	Autolytic
01/12/15	230/15[b/p]	EHDV	-	-	
03/12/15	231/15[b]	EHDV	-	*N. caninum*	encephalitis
07/12/15	233/15[p]	-	*T. pyogenes*	-	-
09/12/15	237/15[p]	EHDV	-	-	-
13/12/15	244/15[b/p]	EHDV/ AKAV/ SHUV	-	-	Epi/endo-carditis, periportal hepatitis
15/12/15	248/15[p]	EHDV	-	-	-
16/12/15	249/15[b]	-		*N. caninum*	-
17/12/15	250/15[b]	-	-	*N. caninum*	Epi/endo-carditis, periportal hepatitis
21/12/15	253/1/15[p]	SHUV	mix.cul	-	-
21/12/15	253/2/15[p]	EHDV	mix.cul	-	-
31/12/15	259/15[b]	-	mix.cul	*N. caninum*	-
04/01/16	101/16[p]	-	mix.cul	-	-
05/01/16	102/16[b]	BVD	-	-	Periportal hepatitis
26/01/16	117/16[p]	-	mix.cul	*N. caninum*	Autolitic
31/01/16	120/16[p]	-	-	N. caninum	-

Table 3: Laboratory findings from aborted fetuses, which were tested for EHDV genome presence by specific RT-qPCR.

[b] - Brain sample
[p] - Placenta sample
[b/p] - Both in placenta and brain samples viral genome was identified
* - Mixed sample from different organs
** - Antibodies were found in serum of the aborted cow
T. pyogenes- Trueperella pyogenes
mix.cul- mixed culture

Virus isolation

Infectious EHDV was successfully isolated in 12 out of 139 EHDV-RT-qPCR positive whole blood samples taken from clinically diseased animals. Two isolates were obtained from samples received in September 2015, seven in October 2015 and three in November 2015. No virus was isolated after November 2015 (Table 1).

Laboratory findings from aborted fetuses

From September through January 2016, 30 aborted bovine fetuses were tested for potential abortogenic pathogens including EHDV, Simbu group viruses, BVD, *Leptospirosis, Brucellosis* and *N. canimum*. Antibodies to *Leptospira borgpetersenii* serovars *Harjio* were detected in the serum of one of the aborting dams (Table 3), while all were negative for *Brucella spp*. Eleven aborted fetuses were EHDV positive by RT-qPCR, four - for Simbu group viruses by PCR (SHUV and AKAV), two -for BVD (Ag ELISA), eleven fetuses had antibodies against *N. Caninum* by IFAT and *Trueperella pyogenes* was isolated from one placenta.

Six of the thirty aborted bovine fetuses were negative (Table 3) for all tested abortion-related pathogens (routine diagnosis).

EHDV RNA was found in 7 out of 16 (43.75%) brain and 6 out of 13 (46.15%) placenta samples. All samples from fetal internal organs were negative for EHDV. The first EHDV positive sample from aborted fetus was found at the beginning of October, and the last one in mid-December. The majority of infected aborted cattle fetuses were collected during November and December of 2015 (Table 1 and 3). Generally, eleven of thirty aborted fetuses were PCR-positive for EHDV. EHDV as the sole abortive agent was detected in seven fetuses, while in the remaining four EHDV positive samples additional abortogenic pathogens were identified (Table 3).

Other potential abortigenic factors

All cases sampled in this study were from intensively managed zero-grazing dairy cattle. The balanced, whole-feed rations were monitored for the presence of nitrates and mycotoxins by the feed producers and were distributed to several other holdings with no apparent detrimental effects.

No aberrant use of pesticides was reported on these farms and no indications of the presence of toxic substances were noted.

Discussion

The naturally EHDV-6 infected sentinel calves observed in this study, did not exhibit clinical signs, suggesting probable subclinical infection. This is consistent with previous findings where calves experimentally infected with Turkish and Moroccan isolates of EHDV-6 also remained healthy and no clinical signs were observed [11]. EHDV RNAmia was seen in the blood in calves at least four months from the presumable date of natural infection based on seroconversion and qPCR. Despite high RNA levels detected in the blood samples of the monitored naturally infected calves, attempts to obtain virus isolates both using ECE and two different cell culture lines (Vero and BHK-21) failed (Table 2). EHDV-6 isolation in ECE was successful only in one of five RT-qPCR positive whole blood samples from affected cows with clinical signs within the same farm (data not shown), during the same time period.

Multifocal mononuclear infiltrates were seen in two brain samples, which were positive only by EHDV RT-qPCR leading to a diagnosis of encephalitis (data not shown in this report).

No gross changes were seen in any tested placentas. Additionally, in a few samples, antibodies against *N. caninum* were found (Table 3).

Bluetongue virus (BTV) is closely related to EHDV, is known to be capable to cross the placental barrier, as seen with the attenuated vaccine strains belonging to serotypes 2, 9, 10, 11, 13 and 17 [12,13], as well as with wild type BTV-8 and BTV-1 [14]. Congenital abnormalities (e.g., hydranencephaly, cavitary encephalopathy, cerebral hyperaemia) have been observed in the field as well as in experimental studies following transplacental BTV-8 infection of fetuses [15-18]. No placental lesions were observed in experimental infection of a pregnant goat with BTV-8 [15], as well as EHDV positive placentas, investigated during this outbreak.

Previously, only the EHDV-2 strain (Ibaraki virus) was considered to cause abortion, in addition to severe clinical disease in cattle [19,20]. In this study, the absence of commonly diagnosed abortifacient pathogens of cattle in Israel implicates the involvement of EHDV-6 in abortions seen in some of the cattle population from September 2015 until January 2016. EHDV was the only pathogen identified in 7 out of 30 aborted fetuses. Consequently, EHDV-6 may be considered implicated in the abortions in other cows during this period, where no other agents were detected.

It should, however, be noted that the cases of abortion occurred several weeks after the presumed onset of the EHDV-6 outbreak, emphasizing the delay from the appearance of viremia to fetal loss. The presence of the virus in the brains of aborted fetuses suggests that fetal infection took place several weeks prior to fetal death *in utero*. The high percentage of EHDV-positive placenta samples (7 out of 13 for EHDV-6 -46.15%, Table 3) indicates that this could be a valuable diagnostic tool to confirm viral infection in cases of bovine abortion- as was found previously with Bovine Herpesvirus-1 and 4 infection [21,22] and SBV [23].

Considering all tested abortogenic pathogens, our finding of EHDV-6 viral genome in aborted cattle fetuses suggest that the virus was significant in inducing abortion in cattle during that period.

Conclusion

Based on the percentage of positive field samples, virus isolation from clinically ill milking cows and PCR results from aborted fetuses, we conclude that the outbreak lasted from late September to November 2015. Additionally, the RNAmia observed in monitored calves corresponds to the estimated duration of the outbreak; the last positive samples in RT-qPCR were obtained in March 2016, leading to the conclusion that possible exposure of cattle to EHDV-6 took place during November-December 2015 (Table 1). Considering the short EHDV-6 viremia, it is likely that the virus was isolated from newly infected animals, as successful virus isolation was achieved only from field samples received from late September until the beginning of November 2015.This confirms the data obtained by experimental infection of cattle with EHDV-7, where the viremic period was less than 2 weeks in most animals [24].

The presence of EHDV-6 RNA in brains from aborted fetuses and placentas during the period of observation may suggest that EHDV-6 is a significant abortogenic pathogen in the cases studied.

Based on the data from this study, the EHDV-6 outbreak in Israel lasted between late September and November 2015. Our findings indicate that the duration of RNAmia in naturally infected calves lasted approximately four months. However, the duration of viremia is still unclear and, possibly, significantly shorter.

Conflict of Interest

The author(s) declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgement

This work was partly supported by the Ministry of Agriculture Chief Scientist grant # 33-03-0004.

We are very grateful to the teams of the Field Government Veterinary Services for sampling the sentinel cattle, to all the veterinary practitioners in the field for collaboration, to Mrs. Veronika Peper, to Dr. Anita Koftunenko and to Mrs. Anna Gorohov for excellent technical assistance, to Dr. Avi Eldar, to Dr. Oran Erster, to Dr. Jacob Brenner and to Dr. Alexander Panshin for very useful scientific advices.

References

1. Yadin H, Brenner J, Bumbrov V, Oved Z, Stram Y, et al. (2007) Epizootic Haemorrhagic Disease Virus type 7 infection in cattle in Israel. Vet Rec 162: 53-56.
2. Savini G, Afonso A, Mellor P, Aradaib I, Yadin H, et al. (2011) Epizootic heamorragic disease. Res Vet Sci 91: 1-17.
3. Stevens G, McCluskey B, King A, O'Hearn E, Mayr G (2015) Review of the 2012 Epizootic Hemorrhagic Disease outbreak in domestic ruminants in the United States. PLoS One 10: 0133359.
4. Golender N, Khinich Y, Gorohov A, Abramovitz I, Bumbarov V (2017) Epizootic hemorrhagic disease virus serotype 6 outbreak in Israeli cattle in 2015. J Vet Diagn Invest 29: 885-888.
5. Vögtlin A, Hofmann MA, Nenniger C, Renzullo S, Steinrigl A, et al. (2013) Long-term infection of goats with bluetongue virus serotype 25. Vet Microbiol 166: 165-173.
6. Viarouge C, Breard E, Zientara S, Vitour D, Sailleau C (2015) Duplex Real-Time RT-PCR assays for the detection and typing of Epizootic Haemorrhagic Disease Virus. PLoS One 10: 0132540.
7. Freundt EA (1983) Culture media and classic mycoplasmas. In: Methods in Mycoplasmology, Vol.1 Mycoplasma Characterization.(Razin, S. and Tully, J.G.). Academic Press New York USA pp. 127-135.
8. Golender N, Wernike K, Bumbarov V, Aebischer A, Panshin A, et al. (2016) Characterization of Shuni viruses detected in Israel. Virus Genes 52: 806-813.
9. Komarov A, Goldsmit L (1951) A disease, similar to Blue Tongue in cattle and sheep in Israel. Ref Vet 8: 96-100.
10. Shkap V, Reske A, Pipano E, Fish L, Baszler T (2002) Immunologicalrelationship between Neosporacaninum and Besnoitiabesnoiti. Vet Parasitol 106: 35-43.
11. Batten CA, Edwards L, Bin-Tarif A, Henstock MR, Oura CA (2011) Infection kinetics of Epizootic Haemorrhagic Disease virus serotype 6 in Holstein-Friesian cattle. Vet Microbiol 154: 23-28.
12. Savini G, Lorusso A, Paladini C, Migliaccio P, Di Gennaro A, et al. (2014) Bluetongue serotype 2 and 9 modified live vaccine viruses as causative agents of abortion in livestock: a retrospective analysis in Italy. Transbound Emerg Dis 61: 69-74.

13. MacLachlan NJ, Conley AJ, Kennedy PC (2000) Bluetongue and equine viral arteritis viruses as models of virus-induced fetal injury and abortion. Anim Reprod Sci 61: 643-651.

14. Chauhan HC, Biswas SK, Chand K, Rehman W, Das B, et al. (2014) Isolation of bluetongue virus serotype 1 from aborted goat fetuses. Rev Sci Tech 33: 803-812.

15. Belbis G, Bréard E, Cordonnier N, Moulin V, Desprat A, et al. (2013) Evidence of transplacental transmission of bluetongue virus serotype 8 in goats. Vet Microbiol 166: 394-404.

16. Maclachlan NJ, Osburn BI (2008) Induced brain lesions in calves infected with bluetongue virus. Vet Rec 162: 490-491.

17. Vercauteren G, Miry C, Vandenbussche F, Ducatelle R, Van der Heyden S, et al. (2008) Bluetongue virus serotype 8-associated congenital hydranencephaly in calves. Transbound Emerg Dis 55: 293-298.

18. Wouda W, Roumen MP, Peperkamp NH, Vos JH, van Garderen E, et al. (2008) Hydranencephaly in calves following the bluetongue serotype 8 epidemic in the Netherlands. Vet Rec 162: 422-423.

19. Ohashi S, Yoshida K, Watanabe Y, Tsuda T (1999) Identification and PCR-restriction fragment length polymorphism analysis of a variant of the Ibaraki virus from naturally infected cattle and aborted fetuses in Japan. J Clin Microbiol 37: 3800-3803.

20. Omori T, Inaba Y, Morimoto T, Tanaka Y, Ishitani R (1969) Ibaraki virus, an agent of epizootic disease of cattle resembling bluetongue. I. Epidemiologic, clinical and pathologic observations and experimental transmission to calves. Jpn J Microbiol 13: 139-157.

21. Deim Z, Szeredi L, Egyed L (2007) Detection of bovine herpesvirus 4 DNA in aborted bovine fetuses. Can J Vet Res 71: 226-229.

22. Rodger SM, Murray J, Underwood C, Buxton D (2007) Microscopical lesions and antigen distribution in bovine fetal tissues and placentae following experimental infection with bovine herpesvirus-1 during pregnancy. J Comp Pathol 137: 94-101.

23. Martinelle L, Poskin A, Dal Pozzo F, De Regge N, Cay B, et al. (2015) Experimental Infection of Sheep at 45 and 60 Days of Gestation with Schmallenberg Virus Readily Led to Placental Colonization without Causing Congenital Malformations. PLoS One 10: 0139375.

24. Eschbaumer M, Wernike K, Batten CA, Savini G, Edwards L, et al. (2012) Epizootic hemorrhagic disease virus serotype 7 in European cattle and sheep: diagnostic considerations and effect of previous BTV exposure. Vet Microbiol 159: 298-306.

Journal of Anesthesia & Clinical Care

Journal of Addiction & Addictive Disorders

Advances in Microbiology Research

Advances in Industrial Biotechnology

Journal of Agronomy & Agricultural Science

Journal of AIDS Clinical Research & STDs

Journal of Alcoholism, Drug Abuse & Substance Dependence

Journal of Allergy Disorders & Therapy

Journal of Alternative, Complementary & Integrative Medicine

Journal of Alzheimer's & Neurodegenerative Diseases

Journal of Angiology & Vascular Surgery

Journal of Animal Research & Veterinary Science

Archives of Zoological Studies

Archives of Urology

Journal of Atmospheric & Earth-Sciences

Journal of Aquaculture & Fisheries

Journal of Biotech Research & Biochemistry

Journal of Brain & Neuroscience Research

Journal of Cancer Biology & Treatment

Journal of Cardiology: Study & Research

Journal of Cell Biology & Cell Metabolism

Journal of Clinical Dermatology & Therapy

Journal of Clinical Immunology & Immunotherapy

Journal of Clinical Studies & Medical Case Reports

Journal of Community Medicine & Public Health Care

Current Trends: Medical & Biological Engineering

Journal of Cytology & Tissue Biology

Journal of Dentistry: Oral Health & Cosmesis

Journal of Diabetes & Metabolic Disorders

Journal of Dairy Research & Technology

Journal of Emergency Medicine Trauma & Surgical Care

Journal of Environmental Science: Current Research

Journal of Food Science & Nutrition

Journal of Forensic, Legal & Investigative Sciences

Journal of Gastroenterology & Hepatology Research

Journal of Gerontology & Geriatric Medicine

Journal of Genetics & Genomic Sciences

Journal of Hematology, Blood Transfusion & Disorders

Journal of Human Endocrinology

Journal of Hospice & Palliative Medical Care

Journal of Internal Medicine & Primary Healthcare

Journal of Infectious & Non Infectious Diseases

Journal of Light & Laser: Current Trends

Journal of Modern Chemical Sciences

Journal of Medicine: Study & Research

Journal of Nanotechnology: Nanomedicine & Nanobiotechnology

Journal of Neonatology & Clinical Pediatrics

Journal of Nephrology & Renal Therapy

Journal of Non Invasive Vascular Investigation

Journal of Nuclear Medicine, Radiology & Radiation Therapy

Journal of Obesity & Weight Loss

Journal of Orthopedic Research & Physiotherapy

Journal of Otolaryngology, Head & Neck Surgery

Journal of Protein Research & Bioinformatics

Journal of Pathology Clinical & Medical Research

Journal of Pharmacology, Pharmaceutics & Pharmacovigilance

Journal of Physical Medicine, Rehabilitation & Disabilities

Journal of Plant Science: Current Research

Journal of Psychiatry, Depression & Anxiety

Journal of Pulmonary Medicine & Respiratory Research

Journal of Practical & Professional Nursing

Journal of Reproductive Medicine, Gynaecology & Obstetrics

Journal of Stem Cells Research, Development & Therapy

Journal of Surgery: Current Trends & Innovations

Journal of Toxicology: Current Research

Journal of Translational Science and Research

Trends in Anatomy & Physiology

Journal of Vaccines Research & Vaccination

Journal of Virology & Antivirals

Archives of Surgery and Surgical Education

Sports Medicine and Injury Care Journal

International Journal of Case Reports and Therapeutic Studies

Journal of Ecology Research and Conservation Biology

Submit Your Manuscript: http://www.heraldopenaccess.us/Online-Submission.php