Advances in Bioresearch
Adv. Biores., Vol9 (1) January 2018: 225-231

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.1.225231

Advances in Bioresearch

ORIGINAL ARTICLE

Histopathologic study of Aborted embryos with Neosporosis among Dairy farms of Alborz province

Dariush Khajeh, Hossein Khaj
M.Sc of Veterinary Parasitology, Islamic Azad University, Science and Research Branch, Bushehr, Iran
Animal Science, Agricultural and Natural Resources Research Center, Bushehr Province, Bushehr, Iran

ABSTRACT
Neospora caninum is one of the microscopic protozoa that was still not known as a cause of abortion in cows until 1989. This protozoa is more common in dogs and cows and rarely in the goat and sheep and other domestic and wild animals. Crossing the placenta, the parasite causes the infection of the fetus, which can lead to abortion in the 3rd to 8th gestation or birth of calves with congenital infections. Since this is a major cause of abortion among cattle herds and has caused huge economic losses to the livestock industry in Iran, so the determination of histopathologic lesions in different organs of the aborted fetuses can lead us to identify and fight this parasite as much as possible.For this purpose, 164 aborted fetuses are collected from dairy farms of Alborz province during 1 year from 2012-13 and transferred to the laboratory along with ice. After splitting the skull, a piece of the brain was removed from the brain and stored at -70 ° C and then prepared for PCR testing. The samples were prepared from the heart, brain, liver, kidney, thymus, spleen and lungs of 21 abortion fetuses that were positive for PCR and stained for histopathologic examination.The results show that out of 164 embryos, 67 cases were positive after the PCR. In other words, 40.85% are infected with Neospora parasite.
***Keywords**: fetus, cow, abortion, neosporosis, Alborz province*

Received 30.07.2017 Revised 23.09.2017 Accepted 20.12.2017

How to cite this article:
Dariush Khajeh, Hossein Khaj. Histopathologic study of Aborted embryos with Neosporosis among Dairy farms of Alborz province. Adv. Biores., Vol 9 [1] January 2018.225-231.

INTRODUCTION

Neospora caninum is one of the microscopic protozoa that was still not known as a cause of abortion in cows until 1989. This protozoa is an intracellular parasite that is an integral part of Apicomplex, which is so similar to toxoplasma [4]. It more often causes disease in dogs and cows, and rarely in the goat and sheep and other domestic and wild animals [6].

Crossing the placenta, the parasite causes the infection of the fetus, which can lead to abortion in the 3rd to 8th gestation or birth of calves with congenital infections [8]. It also causes paralysis and death in dogs [4]. It has a worldwide expansion and in some countries 30% of abortions in dairy and meat cattle are due to infection with this parasite. Neospora infection in Australia causes an annual loss of $ 85 million in the milk industry and $ 25 million in the meat industry.

Since this parasite is a major cause of abortion among cattle herds and has caused huge economic losses to the livestock industry in Iran, so the present study aimed to determine the percentage of aborted fetus by *Neospora* in Alborz province by detection of PCR and the histopathologic lesions that have been created in various aborted fetal organs.

MATERIALS AND METHODS

In order to carry out this project, 164 aborted fetuses were collected from dairy farms of Alborz Province during 1 year from 2012-13 and transferred to the laboratory along with ice. First, a piece of brain was removed from the brain of all embryos and numbered and kept at a temperature of -70 ° C and then prepared for PCR testing. In this way, 25 milligrams of brain of each embryo were separated and the N*eospora* DNA was isolated using DNA fragmentation kit of Dr. Shayan according to the brochure. With the help of primers designed from the Genbank PCR site, the PCR test was carry out. Primers used in this study were the NP21 primer with 328 bp.

NP21 (5'-GTG CGT CCA ATC CTG TAA C-3')
NP6 (5'-CAG TCA ACC TAC GTC TTC T-3')
Further, the amount of 5 µl of the DNA sample was extracted and .5 µm molar oligonucleotide primer, 1mM MgCl2, 200 mM of Taq, dNTPpolymerase and some dispersed distilled water were combined to reach 25µl. The mixture was then transferred to a Thermo Cycler to replicate 40 times at 96°C for denaturation, 50° for binding, and 74° for repetition of the cycle, and propagate the protozoal DNA. After the proliferation of DNA, 10 µl of the sample and 2µl of the sample buffer were transferred to 1% agarose gel containing 0.5 µl of titanium bromide in TEB buffer containing TEB (40 mMTris acetate, 2 mM EDTA) and placed in horizontal electrophoresis device set at 100 volts and 350 mA for 110 minutes in order to confirm it. Bands formed as a result of PCR were observed under ultraviolet light.
The samples were prepared from the heart, brain, liver, kidney, thymus, spleen and lungs of 21 abortion fetuses that were positive for PCR and fixed in formalin 10%. The samples were then transferred to tunghistokinette 2000leica, and they were formed as paraffin blocks. The paraffin blocks were then cut to .5 micron sheets using a leica device and stained with Hematoxylin-Eosin staining method.
Finally, the data were analyzed with Anova non-parametric equivalence (Kruskal-wallis), which had a significant difference with the significance level less than P <.01.

RESULTS

After referring 164 embryos to the laboratory during the years 2012 and 2013, PCR was performed on the brain suspension prepared from these embryos to detect neosporous parasites. Out of these 164 samples, 67 cases (40.85% of the embryos) were positive and formed a band of 328 bp. The results of examining organs such as the heart, kidneys, brain, spleen, six, thymus and liver of 21 samples that have been randomly selected from positive cases are presented in Tables 1 through 3 and Figures 1-8.

Table 1. Detailed table of pathological lesions of various organs of the collected embryos

Organ / Type of lesion / No. of fetus	Heart	Brain	Kidney	Lung	Spleen	Thymus	Liver
1	Epicardium and myocardium lymphocytic	-	Distributed lymphocytosis and autolysis	Distributed lymphocytosis		-	-
2	Epicardium Lymphocytosis	Focal Gliosis + PVC	Autolysis	Lumbar lymphocytosis and mild edema	Megakaryocytosis + Normal tissue	Lymphocytosis around the lobules	Venous lymphocytosis
3	Focal lymphocytosis	+ Hyperemia	-	Normal	-	Mild Lymphocytosis	Focal lymphocytosis
4	Focal lymphocytes under the Epicardium	Focal gliosis	Focal and scattered Lymphocytosis in modular	Mild lymphocytosis between tissue	Mild lymphocytosis	Hyperemia and depletion of lymphocytes around lobules	Scattered lymphocytosis
5	Myocardial lymphocytic Lymphocytes - under the epicardium	Focal gliosis	Focal lymphocytic	Mild lymphocytic	Mild lymphocytosis	-	Mild venous lymphocytosis
6	The presence of mononuclear cells	Focal gliosis	Presence of lymphocytes	Mild lymphocytosis	-	-	Mild lymphocytosis
7	Lymphocytosis under epicardium and the presence of mononuclear cell	Focal gliosis	Lymphocytosis	Mild lymphocytosis	-	-	Mild lymphocytosis

Table 2. Detailed table of pathological lesions of various organs of the collected embryos

Organ Type of lesion No. of fetus	Heart	Brain	Kidney	Lung	Spleen	Thymus	Liver
8	-	Mild edema around vessel	Acute tubular necrosis	Hyperemia with mild edema	depletion of lymphocytes from tissue	Hyperemia of lymphocyte depletion	Hyperemia
9	Lymphocytosis + Autolysis	Mild edema and focal Gliosis	Autolysis	Presence of lymphocytes in tissue-pneumonia	Normal	Hyperemia	Autolysis and the presence of dispersed mononuclear cells
10	Focal lymphocytosis	-	Hyperemia	Mild lymphocytosis between tissues	Hematopoiesis	Inter-follicular depletion of lymphocytes	Scattered portalLymphocytosis
11	-	-	-	Acute lymphocytosis of pneumonia	-		Scattered lymphocytosis
12	Normal tissue		Focal-Approximate Lymphocytosis	Mild scattered lymphocytic	depletion of lymphocytes	-	The presence of mononuclear cell
13	Myocarditis and Endocarditis multifocal Lymphocytosis		Multi-focal lymphocytosis in modular	Mild lymphocytosis	-	-	Multi-focal portal lymphocytosis
14	Lymphocytosis under epicardium		Mild hyaline cast + lymphocytosis		-	-	Degenerate and desiccated with the presence of fat in the tissue

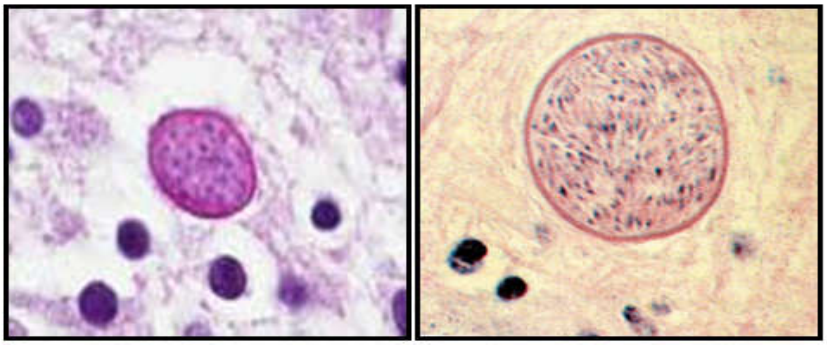

Figure 1:Neospora cysts in the brain tissue

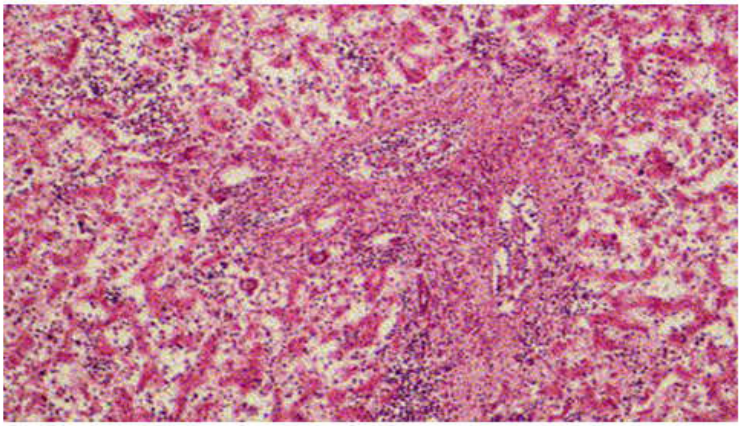

Figure 2: Portal hepatitis of inflammatory cells between accumulated hepatocyte cells and hepatocytes necrosis (H & E - x400)

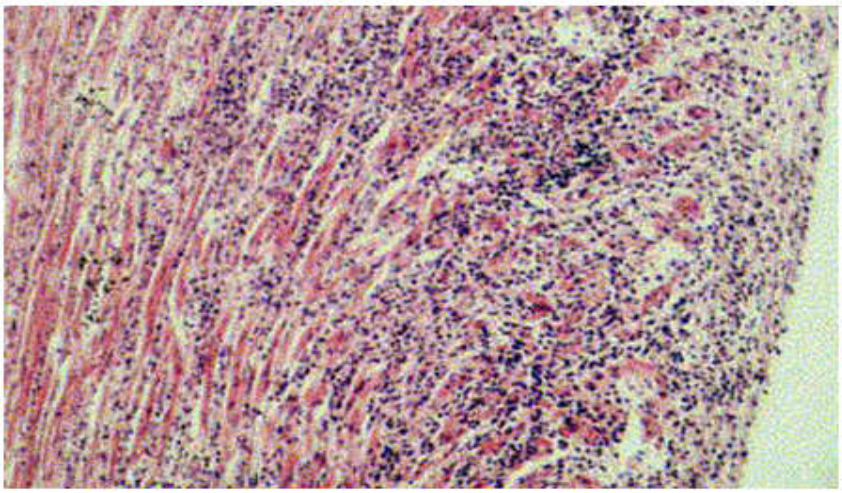

Figure 3: Embryo heart with cumulative myocarditis and infiltration of inflammatory cells in the heart cells (H & E - x400)

Table 3. Detailed table of pathological lesions of various organs of the collected embryos

Organ / Type of lesion / No. of fetus	Heart	Brain	Kidney	Lung	Spleen	Thymus	Liver
15	Focal lymphocytosis	Multifocal gliosis	-	Normal tissue	Focal lymphocytosis	Focal lymphocytosis	Focal lymphocytosis
16	Focal presence of lymphocytic cells		Focal presence of lymphocytic autolysis cells	The presence of lymphocytes in the lobules	Mild depletion of lymphatic tissue	depletion of lymphocytes from inside the follicles	The presence of diffuse lymphocytes
17	Multi-focal presence of lymphocytes		Mild Hyperemia with mild lymphocytosis	Mild presence of lymphocytic cells	Hematopoiesis of hyperemia	Intercostal edema and hyperemia along with the depletion of lymphatic cells from the inside of the follicles	
18	The presence of lymphocytic cells under epicardium and myocardium	Focal gliosis	Mild autolysis	Mild edema	Autolysis with hyperemia		Mild hyperemia + autolysis
19	Mild presence of lymphocyte	Normal	Autolysis	Inter-lobule and under-cavity edema	Autolysis		Mild lymphocyte presence + Multi-focal necrosis
20	Focal presence of lymphocytes	-	Focal presence of lymphocytes	The inter-lobule presence of lymphocytes	Mild depletion of lymphocytes	depletion of lymphocytes from follicles	Extensive and diffuse presence of lymphocytes
21	Focal presence of lymphocytes	-	Focal and intra-tissue presence of lymphocytes	Focal and intra-tissue presence of lymphocytes	-	-	Diffuse lymphocytosis with multi-focal necrosis with portal hepatitis

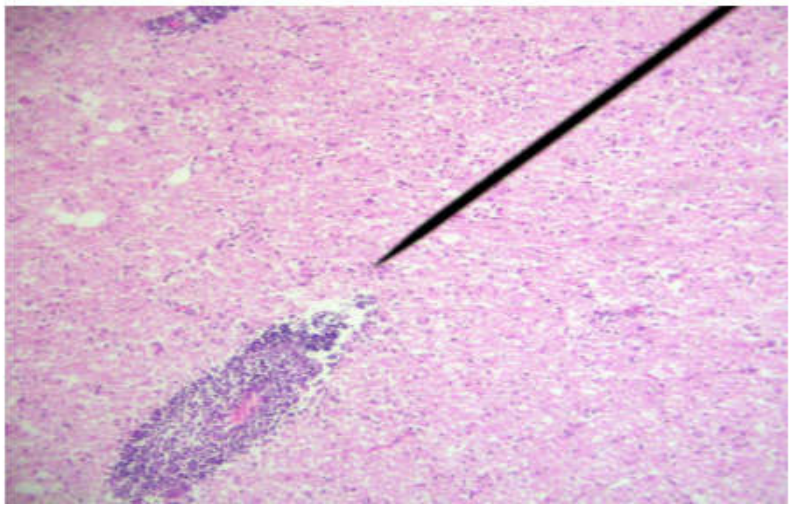

Figure 4: Non-purulent encephalitis and sleeve accumulation of mononuclear inflammatory cells in the microscope and robin around the abducted embryo's cerebrospinal fluid (PVC) (H & E - x400)

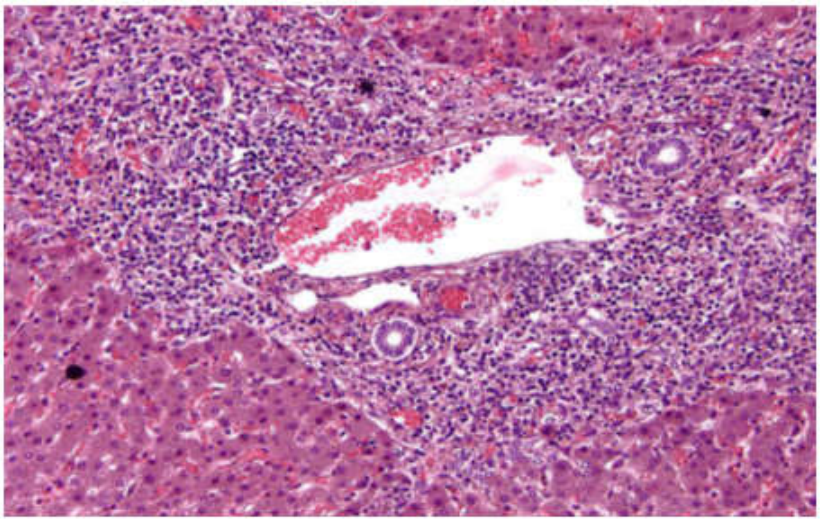

Figure 5: The aborted fetus' liver, in which infiltration of inflammatory cells in the liver port space representsthe parietal portal hepatitis. (H & E - x400)

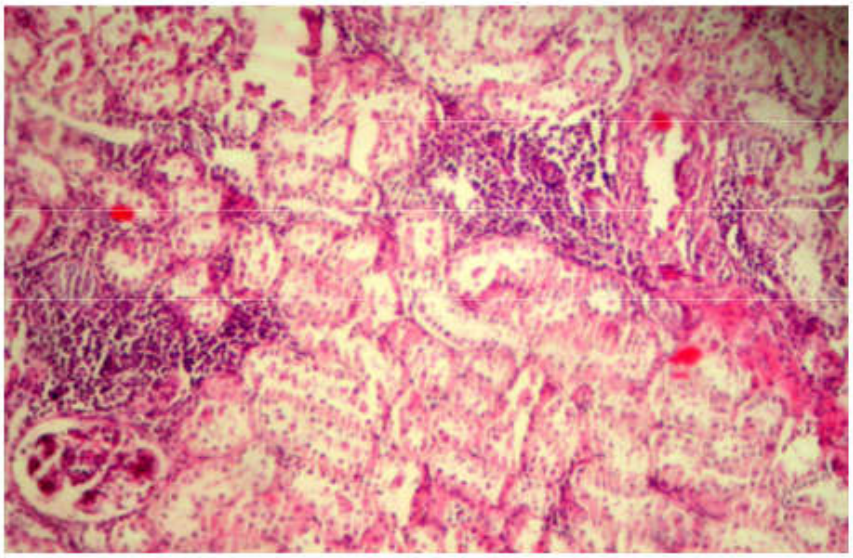

Figure 6: The kidney afflicted with the interstitial nephritis of the aborted fetus, which shows the penetration of mononuclear inflammatory cells in its interstitial space. (H & E - x400)

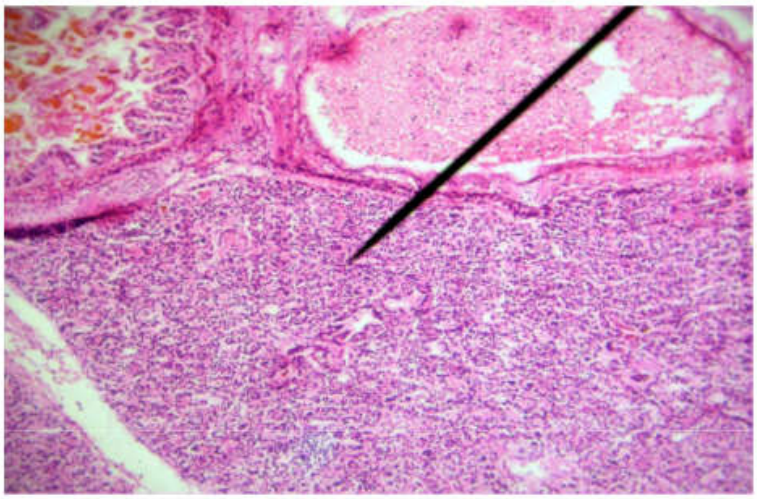

Figure 7: The lung with interstitial pneumonia of the aborted fetus, where infiltration of mononuclear inflammatory cells in the interstitial space of the lung and the wall of the lung alveoli is seen. (H & E - x400)

DISCUSSION AND CONCLUSION

Reproduction is one of the main factors in the dairy industry, and the survival of this industry depends on its prosperity. Pregnancy and abortion are one of the most important inhibitors of reproductive performance in cattle herds. Neosporosis is caused by *Neospora caninum* protozoa and is an important disease in cows and dogs around the world. *Neospora caninum* causes abortion in dairy cows. Most abortions occur in 5-6 months of pregnancy, which may be in the form of the death of the embryo in the womb, mummification, autolysis, stillbirth, birth of the newborn with clinical symptoms, and the appearance of a seemingly healthy but infected baby in a chronic form. According to the results, neosporosis accounts for a high percentage of abortion in dairy farms in Alborz province. The results of PCR on 164 embryos collected from 2012 to 2013 showed 67 infected cases (equivalent to 40.8% of the total embryos) that showed a band of about 328bp and positive PCR. Statistical analysis of data on pathological lesions in different organs of the embryos (heart, lung, brain, liver, spleen, and thymus) has been shown in Tables 1 through 3, and interpretation of these results are as follows:

There is a significant difference between heart lesions, so that focal lymphocytosis is the most frequent among other lesions, and myocardial lymphocytosis and epicardium lymphocytosis are in second and third place, respectively.

As shown in Tables 1 through 3, the highest percentage of lesions in the brain is multifocal gliosis (52.4%), followed by edema with gliosis (42.9%).

According to these tables, the highest rate of lesions in the kidney, mild lymphocytic infiltration with a frequency percentage of 23.8%, followed by focal lymphocytosis with a percentage of 19%.

In the study of the spleen, lymphoid tissue depletion with the frequency percentage of 19% is the most common lesion.

According to these tables, in the thymus, the mild lymphocytic was the most prevalent lesions (52.5%).

Also, the highest rate of lesion in the liver was hyperemia with a high prevalence of 19%, and severe lymphoid infiltration with a prevalence of 14%.

According to the results, the greatest lesion seen in all organs is the lymphocytic and mononuclear cells infiltration.

In a study by Alexander *et al*. on a herd of 300 cows vaccinated against rabies, brucellosis, leptospirosis and calves diarrhea, animals that had positive antibodies against *Neospora caninum* were identified by indirect immunofluorescence (IFA) and samples were taken from heart, brain, spinal cord, tongue, liver and kidney of aborted fetuses for pathology, histochemistry and PCR. In the PCR test, the protozoan DNA was found in all organs of the fetus including the brain, tongue, spinal cord, heart and kidneys. The rate of positive serological tests in animals with a history of abortion was 38.46% and in animals without abortion history was 20.51%. In pathology tests, encephalomyelitis included gliosis and the presence of mononuclear cells [2].

In another study by Salehi *et al*., [8] examined brains of embryos aborted from seropositive neosporous cattle and 7 newly-born breeding cattle that were serum positive were transferred to the laboratory for

N-PCR and pathology. Mild infection, focal hemorrhage, degeneration of Cotyledons of placenta cavity, and hyperemia were seen in 6 cases and edema was in the brain of all embryos. In 3 of these 12 brains, spot hemorrhage of neuron necrosis and gliosis as well as encephalitis in brain cavity and PVC, and lymphocytic were seen. Also, the infectious contamination with Eliza was 38.8% in four herds of dairy cows around Tehran and NP 21, NP6 primers were measured at 328 bp (8). The results of the above studies are consistent with the results obtained in this study. According to Dubey, Neosupora is one of the major factors in abortion in cows, which is because the protozoa first enters the placenta or the heart and destroys or causes the release of maternal prostaglandins causing abortion. The destruction of embryo tissues is due to the lack of oxygen and enough food from the yellow body. In addition, the reduction of congenital immunity in the fetus causes inflammation of the placenta and release of inflammatory substances in the placenta, which causes abortion [2].

In a study by Angkana *et al.* in a zoo in Thailand on a rhino after a sudden death, the Neospora parasite was isolated from the heart and brain of the rhino. Also, in the study of pathology, the liver was enlarged and necrotic yellow points were seen at its edges. The lungs had edema, and there were also bloodshed in the lungs and diaphragm and spleen. The left and the mesenteric kidneys had necrotic points, the mesentery and adrenal lymph glands were enlarged and had ecchymosis bleeding. In the liver, in addition to bleeding, the presence of giant cells and macrophages was observed [3].

In a study conducted by Macaldowie *et al*., pregnant cows were experimentally infected with *Neospora caninum* in three ways: intravenous injection, subcutaneous and oral infusion, and after 14, 28, 42, and 56 days, livestock were slaughtered and studied. . On the 28th day of pregnancy, immunohistochemistry findings showed that neospora antigen was present in the embryo's body, and infection was observed with neutrophilic and eosinophilic infections in the placenta [5].

In a study conducted by Khordadmehr *et al.* on the effects of neospora on the chick embryo by injection into the egg, it was observed that after the death of the embryos, mild bleeding was at the internal surface of the egg and on the body of the embryos [7].

In the study of Salehi [8] on the embryo's brain, neuronal necrosis and gliosis, PVC and lymphocytic were also observed, which were also seen in this studies.

The presence of mononuclear and lymphocytic cells and bleeding in the organs is one of the most important complications of the disease, which was also evident in various studies by researchers.

In addition, the statistical analysis of the present study showed a significant relationship between infection with neosporosis and the number of abortions in the cows. For this reason, it seems that this protozoan can be considered as one of the important factors in the occurrence of abortion in cattle in the region of Alborz and other parts of the country. It is recommended that in case of dealing with suspicious cases of repeated abortions in dairy cattle herds, examination of the status of infection with the *Neospora caninum* should be considered.

REFERENCES

1. Heidari, H. (2007). Newly identified Neospora protozoa. Daneshjoo Publications, First edition.
2. Alexandre. D., Munhoz, P.P&Rosangela, M. (2011). Bovine abortion associated with *Neospora* canium: diagnosis and epidemiological aspects of a dairy cattle herd in the northeast region of soapanlo state, Brazil. Braz J Vet Pathol, 2011, 4(2), 112-116.
3. Angkana, S., Montakan. V.,Tanit. K.,Sedsada, T., Boripantsiriaroonrat, M., Nopadon. P., (2010). Systemieneosporosis in a white khinoceros journal of 200 and wildlife medicine.J Vet Med Educ, 41 (1): 164-167.
4. Belinda, W. (2004), Neosporacanimuminfeetion in cattle, NSW agriculture. 1-3.
5. Macaldowie, C., Maley, S.W., Wright, S., Bartely, P., Redondo, E., Buxton, D., Innes, EA.(2004). placental pathology associated with fetal death in cattelinculated with *Neospora caninum* by two different routes in early pregnancy. J. comp. pathol. 131. (2-3): 142-56.
6. JDubey, .P.,Buxton, D., Wouda, W. (2006), pathogenesis of Bouineneosporosis. J. comp. path. Vol, 134, 267-289.
7. Khordadmehr, M.,Namavaril, M.,Khodakaramazizolla, M., Mansourian, A., (2013), Comparison of use of vero cell line and suspension culfure of murine macrophage to attenuation of virulence of *Neosproa canimum*, Research in veterinary science, 515-521.
8. Salehi, N., Haddadzadeh, H., Ashrafihelan, J., Shayan, P., Saderbzzaz, A. (2009), Molecular and pahthological study of bovine aborted fetuses and placenta from *Neospora caninum* infected dairy cattle, Iranian.