Parasitological and molecular investigation of *Babesia microti* in rodents of Sarab district of East Azerbaijan

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The cause of Babesiosis, piroplasmosis or red water fever is a small protozoon called babesia. Vast majority of animals including; aquatics, amphibians, rodents, reptiles, birds, mammals and human beings are infected with this parasite. Thus, it is considered one of the most important diseases of zoonotic parasitic infections and is introduced as a health problem in some countries of tropical areas, which at the same time, is of economical importance. The cause of Babesiosis in Iran is *Babesia microti* and rodents are one of the reservoirs' of the disease parasite. This investigation is the first molecular study for determining rodents as reservoir hosts of *Babesia microti* in Sarab city and surrounding villages. First of all 100 rodents of 4 different species consisting *Mus musculus*, *Meriones persicus*, *Cricetulus migratorius* and *Mesocricetus auratus* from different villages of Sarab city were hunted using live traps. Then blood samples of all hunted rodents were tested by parasitological studies (preparing Impression smear of spleen tissue and thin peripheral blood smear). By dissection and parasitological studies of all the rodents of 4 different species, in 3 *Mus musculus* which were mice, no schizont of Babesia was seen in impression smear of spleen tissue but pear shaped intra erythrocytic babesia parasite was present in thin blood smear study. Infection Rate was 3% and in the end, parasitological test results were confirmed using molecular method of PCR. The isolated parasite genus was determined to be *Babesia microti*. It was specified in this study that rodents, especially *Mus musculus* could be as reservoir hosts for babesiosis in Sarab city of East Azerbaijan province of Iran.

Key words: *Babesia Microti, Rodents, PCR, Sarab, Iran.*

Histopathological evaluation of effect's nano- hydroxyapatite / zirconia stabilized with yttria in tibia Bone healing of rabbit

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The aim of this study was an evaluation of the biological effects of zirconia -stabilized yttria on bone, using an in vivo model: nano–hydroxyapatite powder with zirconia-stabilized yttria and non-treated controls were inserted in rabbit tibia and then histopathologically analyzed. Twenty New Zealand white male rabbits randomly divided into two groups of 10 rabbits in each group. Anesthesia was induced using intramuscular ketamine hydrochloride (20 mg/kg), acepromazine (0.1 mg/kg) and atropine sulfate (0.02 mg/kg), then rabbits connected to anesthetic machine using isoflorane 1%. A cortical hole of 3mm diameter and 8mm depth in each tibia was drilled. In group I, the defect was left empty, where as in group II, the bone defect was packed with nano–hydroxyapatite/zirconia stabilized with yttria. Histological evaluations were performed at four weeks after the implantation. After four weeks the woven bone was formatted in group I. Mixed lamellar and woven bone formation was seen in group II and there was a little inflammation in this group. Foreign body reaction was negative. Bone growth was observed as well as the bone outside the implant and bone inside the implant. Bone lacunas contained osteocyte that demonstrated bone tissue was active and vital. Histopathological changes on two groups were scored and statistical analysis showed that the total average scores in group II were significantly higher than the group I ($p<0.05$). Histopathological analysis of the bone healing was shown to be significantly improved by the nano–hydroxyapatite/zirconia stabilized with yttria compared with the control group, suggesting that this biomaterial promote the healing of cortical bone, presumably by acting as an osteoconductive.

***Key words**: Nano Hydroxiapatite Zirconia, Yttria, Tibia, Rabbits.*

Survey on GRA6 gene in differentiating *Toxoplasma gondii* genotypes, using PCR-RFLP method, in sheep aborted fetuses in Ardabil area, Iran

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Toxoplasma gondii, is an isosporean intracellular protozoa in warm blooded animals. Its importance relies on public health hazards and significant economical losses in man and some domestic and wild animals. Its life cycle develops in two hosts: Cat and other felids are definitive hosts which produce oocyts and man and many domestic and wild animals are the accidental intermediate hosts which harbor tissue cysts in their bodies. This parasite causes abortion in man and some herbivorous animals, too. Because the infection rate of toxoplasmosis in sheep aborted fetuses of Ardabil was over 75 %, by PCR method, we decided to use GRA6 gene of *Toxoplasma* in order to reveal the strains and their pathogenicity in this area. All 75 fetuses were tested by PCR-RFLP method. However, this gene was not amplified in none of the fetuses. Meanwhile, results of bioassay, following intraperitoneal injection of fetal brains to albino mice, revealed that no tissue cyst developed in mice brains. In conclusion, it is probable that the amount of *Toxoplasma* organisms was not sufficient for the gene amplification. In addition, it seems that the low number of the parasite as well as the presence of non-virulent murine strain of *Toxoplasma,* may not be the cause of sheep abortion in Ardabil area.

Key words: *Toxoplasma Gondii, GRA6, Sheep, Ardebil, Iran.*

Changes in the blood biochemical indices of sheep in experimental hypermagnesemia

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Hypermagnesemia is rarely encountered, but when it is it usually suggests either compromised renal function or is an iatrogenic phenomenon resulting from the zealous administration of oral magnesium salts. The aim of this study was to investigate the physiological effects of experimentally induced hypermagnesemia on serum biochemical changes. Magnesium sulfate solution (40%) was intravenously infused (three times, at 30-min intervals) to 5 healthy sheep (experimental group). Meanwhile, 5 healthy sheep received 0.9% saline solution (IV) and kept at the similar condition as the control group. In both groups serum was collected before and after administration at different time points (blood samples were taken 5 minutes before and after administration). Aspartate transaminase (AST), alanine transminase (ALT), glucose, blood urea nitrogen (BUN), creatinine, calcium, magnesium, K+ and Na+ levels were measured. Biochemical analysis of the studied parameters showed a significant increase (P<0.05) in serum magnesium levels following magnesium sulfate infusion compared to the control group. Glucose levels showed significant increase at T4 and T5 compared to T0-T3 in the experimental group. Potassium levels showed significant decrease in T3 compared to the baseline. No significant difference was observed in the activities of AST and ALT between groups. Changes in BUN, creatinine, Calcium and Na+, AST and ALT were not significant before and after magnesium sulfate infusion. While, experimental hypermagnesemia can be accompanied with a decrease in serum K+, increase in serum Mg level and an increase in glucose levels.

Key words: *Experimental Hypermagnesemia, Biochemical Indices, Sheep.*

Correlation of necrosis with angiogenesis and proliferation in heterotopic xenograft model of breast cancer

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Breast cancer is considered as one of the most important malignancies in women. Each year, a considerable population of women dies from this disease. To date, basic and clinical cancer research has been developed. The most portions of these researches have been devoted to the breast cancer. All new anticancer drugs must successfully pass preclinical *in vitro* and *in vivo* assays to enter clinical trials. To assay the validity of heterotopic xenograft models of breast cancer from the viewpoint of correlation of necrosis with angiogenesis and proliferation index. BT-474 cell line of breast cancer was inoculated heterotopically and bilaterally to 10, 6-8 week old athymic nude mice. Severity of necrosis was determined by H&E staining. Angiogenesis and proliferation index were calculated from immunohistochemistry slides stained by CD34 and Ki-67, respectively. Then, the correlation of necrosis with angiogenesis and proliferation was assessed. There was seen a positive correlation between necrosis and proliferation ($p < 0.001$, $r = 0.90$). Such a correlation was also observed between necrosis and proliferation index ($p < 0.001$, $r = 0.93$). Our results highlight the validity of heterotopic xenograft model of breast cancer. Theses results demonstrate that this model can give researchers a useful tool to assess pathologic complete response (pCR) at the preclinical level with a good translational potential.

Key words: *Breast Cancer, Xenograft Models, Necrosis, Angiogenesis, Proliferation.*

Investigating growth indices and feeding habits of Pink ear emperor *Lethrinus lentjan* in coastal waters of Hormozgan province

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Pink ear emperor *Lethrinus lentjan* from family Lethrinidae, a protogynous hermaphrodite, is an important species for fisheries in the Persian Gulf, with the highest amount of catch and food acceptability in Hormozgan province. Ninety samples were collected in summer 2012 from Bandar Abbas coastal waters by gillnet and gargoor and were investigated for growth and feeding indices based on standard protocols. The results indicated that the relationship between standard length and weight can be expressed as: $y= 0.044x^{2.874}$, which means that pink ear emperor, has an isometric growth in coastal waters of Hormozgan province. Moreover, condition factor was estimated 3.01 for this species. Regarding feeding indices, CV index was calculated 66.66 which rank pink ear emperor as a relatively low eating fish. Food preference index revealed that small fishes (66.3%), crabs (34.4%), Squid (6.9%) and bivalves (3.4%) constitute the prey items of *Lethrinus lentjan* in coastal waters of Hormozgan province.

Key words: *Growth Indices, Food Habits, Persian Gulf, Lethrinus Lentjan.*

Comparison of infection rate of *Mycoplasma agalactiae* in affected sheep with goats to contagious agalactia in Kerman province

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Mycoplasma agalactiae is considered to be the classic agent of contagious agalactiae in sheep and goats. This disease is particularly widespread around the word. Iran is one of the countries that contagious agalactia is present. The aim of this study was to compare the infection rate of *M. agalactiae* in affected sheep with goats to contagious agalactia by Polymerase Chain Reaction (PCR) method in Kerman province. This comparative study was performed on all sheep and goats received suspected samples within the periods of 6 months in Kerman province. A total of 142 sheep and 85 goat samples were collected from conjunctival excretion, milk secretion and joint exudates. Mycoplasma genus was detected from samples, using specific primers of 16S rRNA gene with specific band of 163 bp, by PCR method. All mycoplasma positive samples were investigated, using specific primers of surface lipoprotein with specific band of 375 bp, for *M. agalactiae* infection. Out *of 142 samples*, 59 (41%) sheep samples and 46 (54%) goats' samples were positive with mycoplasma genus. Also 17 (29%) sheep samples and 28 (61%) goats' samples were infected with *Mycoplasma agalactiae*. The results were shown that the infection with *Mycoplasma agalactiae* in goats was more than sheep. In conclusion, *Mycoplasma agalactiae* was the main agent of contagious agalactiae in sheep and goats in Kerman province.

Key words: *Contagious Agalactia, Mycoplasma Agalactiae, Polymerase Chain Reaction, Sheep, Goat, Kerman Province.*

The effect of genetic variation in hemagglutinin gene of H9N2 influenza viruses in diagnosis using RT-PCR

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Genetic variations in the hemagglutinin gene of influenza viruses are an important characteristic. These variations may cause limitation of RT-PCR assay for detection of influenza subtypes. In this study, a total of 6 samples, suspected to type A influenza virus, were isolated from industrial birds' trachea in North Khorasan Province and RT-PCR analysis were performed. Results showed that all of the 6 suspected samples were detected positive as type A influenza viruses. These isolates were then determined their subtypes using RT-PCR H9N2 specific primers. The result showed that 4 out of 6 isolates were positive as H9N2 and other two isolates did not have any bond on gel electrophoresis and considered negative. For further evaluation, all of the hemagglutinin genes of viruses were sequenced and confirmed as H9N2 viruses using BLAST software in Gene Bank. It was also found, that primers binding sites with hemagglutinin genes in two last viruses were mismatched in three nucleotide acids with 3' end of forward and reverse primers. These factors may reduce the sensitivity of RT-PCR tests and cause false negative results. Therefore, it would recommend updating RT-PCR primers and protocols in a timely manner.

Key words: *Influenza Virus, H9N2, Hemagglutinin Gene, Primer, RT-PCR.*

Histopathological study of ammonia on status gill kidney and liver of Persian Sturgeon

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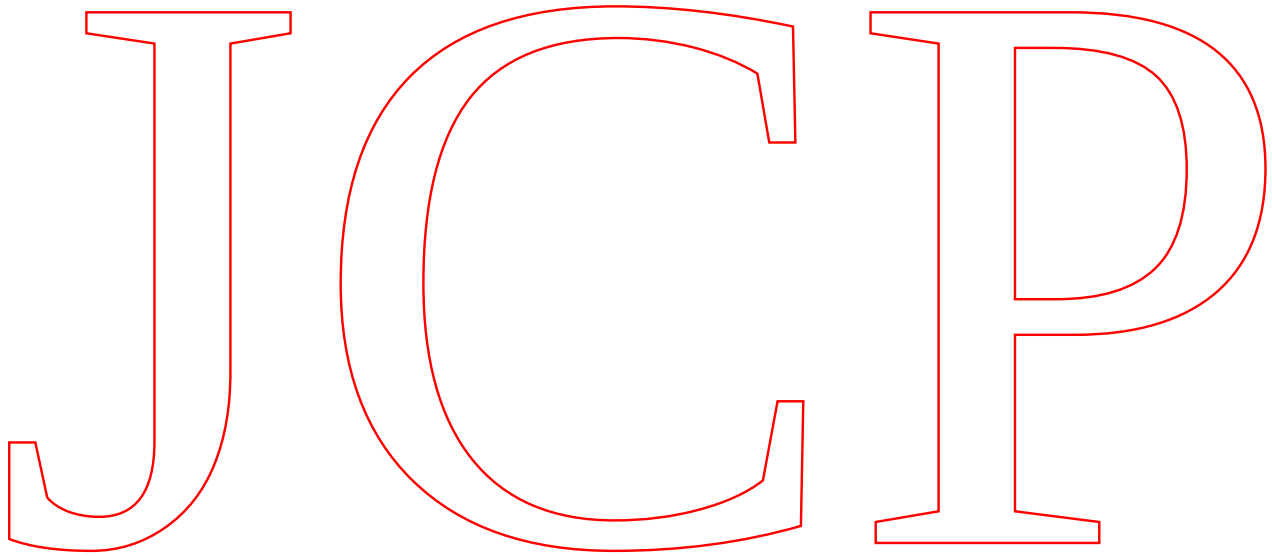
Nitrogenous compounds is one of the most important environmental pollution and Ammonia is the most important and dangerous one. Generally in water pollution classification the presence of Nitrite, Nitrate and Ammonia implies that there are inorganic chemical contaminants in the water. In this research the effect of Ammonia acute toxicity on baby *Acipenser persicus* weighted average of 3 grams in lab situation was studied. The tests were done on 7 cases and 3 repetitions and a whole number of 10 fish by stagnant water method for 96 hours. Fish were exposed to different whole Ammonia concentrations which were 0, 5, 7, 9/5, 13/2, 18 & 25 mg in liter for baby *Acipenser persicus*. First, the amount of Ammonia LC50 in 24, 48, 72 & 96 hours was gained, this was calculated for baby *Acipenser persicus* respectively 12.6033, 9.8218, 8.0038, 6.3708 mg in liter whole ammonia. After finding LC50 amounts, we chose one low level of LC50 which is 25% concentration and one high level of LC50 which is 75% concentration and the test was repeated by 4 cases and 3 repetitions. Concentration range for this material was 1/592, 3/185 & 4/778 mg in liter whole Ammonia. Then possible microscopic and Histopathologic lesions of this type of fish's gill, liver and kidney tissue were studied. The results showed that after exposing to Ammonia, some phenomena such as hyperemia, hyperplasia, adhesions secondary lamella, primary lamella swelling, hemorrhage and cell necrosis is observed in the gill, and in the liver some other phenomena such as hyperemia, stagnation of bile, cell necrosis and cell atrophy is observed and finally in the kidney complications like hyperemia, interstitial tissue degeneration, cell necrosis, dilatation of Bowman's space, homosydryn is observed. Generally, the most injuries are observed in these fish's gill.

Key words: *Acipenser Persicus, Ammonia, Histopathology, Lethal Concentration(LC_{50}).*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