Iranian Journal of Veterinary Medicine

Heavy metal bioaccumulation and its potential relation with incidence of canine parvovirus infection in golden jackals, North Iran

Namroodi, S.[*]**, Rezaie, H., Milanlou, D.**

Department of Environmental Sciences, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran

Key words:
bioaccumulation, heavy metal, jackals, parvovirus, road-killed

Correspondence
Namroodi, S.
Department of Environmental Sciences, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran
Tel: +98(17) 32524493
Fax: +98(17) 32427040
Email: snamroodi2000@yahoo.com

Received: 28 November 2016
Accepted: 11 March 2017

Abstract:

BACKGROUND: Heavy metal toxicity has been confirmed to be a critical threat to animals' health. It has been proved that heavy metals can cause immunosuppression. Although, it is said that damage of immune function plays a contributing role in the increasing incidence of infectious diseases. The increasing use of rural habitats by jackals makes them suitable to monitor the anthropogenic activities impact on the health status of the animals. **OBJECTIVES:** We examined whether exposure to immunosuppressive heavy metals is associated with infectious disease in golden jackals (as representative of wild canids). So mercury and lead concentrations, frequency of CPV-2 infection and the relation between heavy metal concentrations and CPV-2 infection incidence were analyzed in golden jackals. **METHODS:** 30 Road-killed golden jackals were necropsied. Concentrations of Pb and Hg were measured by AAS in kidney and liver samples. VP2 gene of the CPV genomic DNA was applied to detect CPV-2 infection in fecal samples by PCR. **RESULTS:** Mean concentrations (mg/kg wet weight) of Hg and Pb were 0.15 ± 0.11 and 0.25 ± 0.18 in kidneys, and 2.8 ± 0.91 and 4.7 ± 1.03 in livers. CPV-2 was detected in 8 (24%) samples. Mean concentrations of Hg and Pb were meaningfully higher in the jackals that were CPV-2 infected compared to non- CPV-2 infected jackals. **CONCLUSIONS:** This pilot study has linked heavy metals bioaccumulation to viral infection. Further work is required to estimate the exact role of heavy metals in susceptibility of jackals to CPV-2 infection.

Introduction

Environmental contamination by heavy metals is one of the most dangerous anthropogenic influences on living organisms worldwide. This is due to both the occurrence of high numbers of metals' sources inside the earth's crust and human activities (Langner et al., 2011; Markert et al., 2011). Some of them (Hg and Pb) are very toxic for organisms even in low concentration and have a wide range of toxic effects on humans and animals (Lehmann, et al., 2011). Toxic effects of heavy metals frequently linked to chronic exposure include mutagenicity, carcinogenicity, teratogenicity, immunosuppression, poor body condition and impaired reproduction (Beyioersmann and Hartwig, 2008; Garcia-Leston et al., 2012; Lehmann et al., 2011).

The data related to adverse effects of heavy metals contaminations in the wild carnivores is rare. However, Hg and Pb are known to bioaccumulate and to magnify in some carnivores such as marine mammals, and have been introduced as a cause of great concern in terms of their general health (Larbi, et al., 2014). Moreover, adverse effects of non-essential heavy metals on the immune system of animals have often been stated (Jaishankar et al., 2014).

Moreover, it has been accepted that impairment of immune function plays a contributing role in the increasing incidence of infectious diseases in animals. Accordingly, nowadays pollution and pathogens represent a serious threat to the health of animals (Morley, 2010).

Golestan Province located in the north Iran is a very important region for conservation of biodiversity in Iran. More than 50% of the total Iranian mammals' species is found in Golestan Province (Majnoonian et al., 1999). Golestan's geographical situation and climate make it suitable for agricultural and industrial activities (Sharbati, 2012). Hence, it suffers from a large extent, large scale human activities and pollution.

Despite the fact that many kinds of environmental changes, such as heavy metal contamination threaten the survival of wild species in Golestan Province, heavy metals contamination and their effects on occurrence of infectious diseases in wild animals have not been surveyed by researchers in Iran.

Canine parvovirus (CPV-2) is widely circulating in the global canine population and one of the important causes of morbidity and mortality in wild and domestic canids (Goddard and Leisewitz, 2010). For example, it has been documented that CPV-2 infection was one of the major factors in decline of the Isle Royale wolf population (Peterson and Krumenaker, 1989).

Golden jackal (*Canis aureus*) is the biggest and the only jackal species outside Africa. It is the most extensively dispersed canid species, occurring in Northern and Eastern Africa, Asia Minor, the Middle East, Central and Southern Asia, and South-eastern Europe (Jhala and Moehlman, 2004). Golden jackal is supposed to be adaptable species due to its omnivorous diet which mainly consists of small mammals, livestock remains and carcasses, and human refuse (Lanszki et al. 2010; Ćirović et al. 2014; Markov and Lanszki 2012; Jaeger et al. 2007). Due to theirs opportunistic scavenging behavior, jackals are able to live in different ecosystems, with high local densities (Jhala and Moehlman, 2004; Šálek et al. 2014).

Jackals' population, being in close contact with human made pollutant, can act as a suitable reservoir for pathogens (Ćirovi et al., 2015). In addition, they may play a role in the risk of infection disease spillover from unvaccinated rural dogs to wild canids.

Golden Jackal has been introduced as one of the most abundant species of wild canids in Golestan Province (Ziae, 2008). Consequently, it can be used as a sentinel of its ecosystem for scientific studies in North Iran.

Animals respond in a different way to environmental pollution, and the presence of toxic materials in the environment and their increase in animal tissues need to be studied for a better understanding of the effects of contaminants on each of the organisms of the ecosystem.

The main objective of this study was to survey the impact of anthropogenic activities on golden jackals through heavy metal contamination and frequency of CPV-2 infection in golden jackals. So, Hg and Pb concentrations and CPV infection incidence were analyzed in road killed golden jackals to survey the relation between heavy metals concentrations and the susceptibility of golden jackals to CPV-2 infection incidence.

Materials and Methods

Sampling: Fecal samples and liver & kidney tissues were collected from 30 road-killed jackals from the main traffic road of Golestan Province which is located near numerous villages. Samples were used only from carcasses for which the close time of death could be evaluated as less than 12-24 h.

CPV-2 detection: Fecal samples were obtained in the form of a rectal swab, using sterilized swabs, and directly transferred to labeled sterile vials containing PBS. The samples were centrifuged at 10,000 rpm for 5 minutes and supernatant was used in the PCR. Total DNA were extracted from the supernatant by use of Bioneer extraction kit. The custom manufactured forward (5' GAAGAGTGGTTGTAAATAATA 3') and reverse (5' CCTATATCACCAAAGTTAGTAG 3') primers specific for the VP2 gene of the CPV-2 genomic DNA were applied to amplify the DNA of 681 bp in length having the following cyclic conditions. Initial denaturing temperature of 94 °C for 5 min for one cycle, 30 cycles of denaturation at 94 °C for 30 sec, primer annealing at 55 °C for 2 min, 72 °C for 2 min, 30 cycles and final extension of 5 min at 72 °C (Pereira et al., 2000). The amplified products were electrophoresed on 1% agarose gel, stained with ethidium bromide and then visualized under the UV transilluminator against the 100 bp DNA ladder.CPV-2 Vaccine strain and ultra-water were used as positive and negative control, respectively.

Heavy metal analysis: To measure Hg and Pb concentrations in kidney and liver tissue samples, wet tissue weight was recorded. Then samples were digested with concentrated nitric and perchloric acid (1:1). After heating on a hot plate for 1 h, the digested samples were examined two times for measuring Hg and Pb concentrations with an atomic absorption spectrophotometer using an air/acetylene flame (GBc I, Australia) (FAO, 1993).

Statistical analysis: SPSS 18 and Excel 2010 software were used for statistical analyses. Mean concentrations of Pb and Hg were represented in mean WW ± standard deviation. Differences in trace elements concentrations of different tissues and incidence of CPV-2 infection were calculated by T-test.

Results

Frequency of CPV-2 infection: There was no apparent clinical sign of CPV-2 infection in road-killed jackals. VP2 partial gene was detected in 8 of 30 (24%) fecal samples (Fig. 1). There was no difference between female and male jackals in frequency of CPV-2 infection.

Tissues Hg and Pb concentrations: Mean Hg concentrations in livers and kidneys of sampled golden jackals were 0.25

Table 1. Group Statistics. 1= Kidney, 2= Liver.

	Salembimar	N	Mean	Std. Deviation	Std. Error Mean
Hg K[1]	1.00	22	.1101	.08563	.01826
	2.00	8	.2913	.05718	.02022
Hg L[2]	1.00	22	.1809	.09851	.02100
	2.00	8	.4463	.21804	.07709
Pb K	1.00	22	2.4436	.66175	.14109
	2.00	8	3.9637	.46325	.16378
Pb L	1.00	22	4.3155	.83583	.17820
	2.00	8	5.8238	.68930	.24371

Table 2. Results of Independent Samples T-test between heavy metals concentration and CPV2 incidence. 1= Kidney, 2= Liver

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Hg K1	Equal variances assumed	.683	.415	-5.519	28	.000	-.18111	.03281	-.24833	-.11390
	Equal variances not assumed			-6.649	18.886	.000	-.18111	.02724	-.23815	-.12408
Hg L2	Equal variances assumed	5.810	.023	-4.643	28	.000	-.26534	.05715	-.38241	-.14827
	Equal variances not assumed			-3.321	8.063	.010	-.26534	.07990	-.44934	-.08135
Pb K	Equal variances assumed	.292	.593	-5.956	28	.000	-1.52011	.25520	-2.04287	-.99735
	Equal variances not assumed			-7.032	17.949	.000	-1.52011	.21617	-1.97437	-1.06586
Pb L	Equal variances assumed	.002	.964	-4.557	28	.000	-1.50830	.33100	-2.18631	-.83028
	Equal variances not assumed			-4.996	15.052	.000	-1.50830	.30191	-2.15160	-.86499

± 0.18 and 0.15 ± 0.11 mg/kg, respectively. Although Pb concentrations in livers and kidneys of sampled golden jackals were 4.7 ± 1.03 and 2.8 ± 0.91 mg/kg, respectively, no significant difference was observed between levels of Hg and Pb concentration in male and female jackals. Livers Hg and Pb concentrations were higher than kidneys.

Correlation between levels of heavy metals concentrations and incidence of CPV-2 infection: There was a positive correlation between CPV-2 infection incidence and levels of Hg & Pb concentrations in sampled golden jackals. Results of T-test revealed that mean concentration of the Hg and Pb was higher in CPV-2 infected jackals than CPV-2 negative jackals (Table 1 and 2).

Discussion

There is limited data regarding heavy metals contamination, frequency of CPV-2 infection and potential relationship between

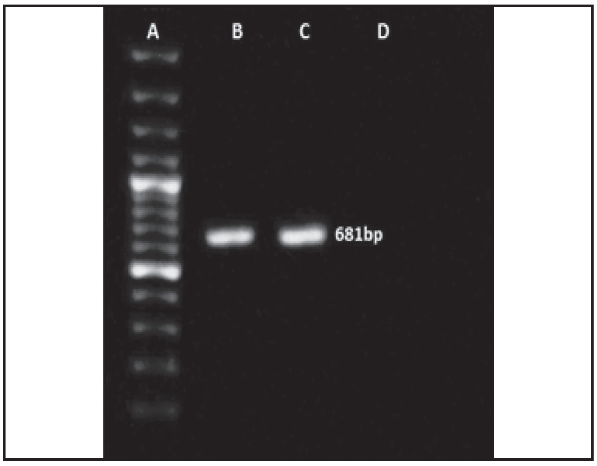

Figure 1. PCR results: A= DNA Ladder, B= Positive control, C= Positive sample, D= Negative control.

heavy metals contamination and incidence of viral diseases in golden jackals. For this study, as for other surveys explained in the scientific papers, the obtained results were step by step compared to linked results reported in the peer-reviewed scientific literature.

Frequency of Canine parvovirus infection incidence: Infectious diseases have been documented as important threats to wildlife population throughout the world and have been introduced in some areas where populations of some species such as Africa wild dogs have been declining (Prager et al., 2012).

CPV-2 was detected in 8 out of 30 (24%) fecal samples by PCR which seems a high value. CPV-2 can persist in the environment for a long period of time, and can also be spread not only by direct contact but also through vectors such as flies (Greene and Apple 2011). Therefore, this information together with the suitable climate situation of Golestan Province for CPV persisting in environment can also be part of the explanation for high CPV-2 infection of sampled golden jackals.

Measurement of serum antibody titer can be a useful method to reveal the history of animals CPV-2 exposures. But it is not valid for determination of CPV-2 infection incidence in canids (Greene and Apple 2011). Hence, in this study CPV-2 infection was surveyed in golden jackals to measure the actual CPV-2 contamination risk that golden jackals are facing in Golestan Province.

With regard to CPV, detection of antibody titer to CPV-2 has been frequently used to analyze canids' population.

There are very limited epidemiological surveys on CPV-2 infection in wild canids. One of the first reports of CPV-2 infection of wild canids was documented by Mech and Fritts (1987). They reported CPV-2 infection led to the death of 11 wolf pups in a captive wolf colony in Minnesota (USA). Mech and Goyal (1993) explained that the small population of wolves has been significantly affected by CPV-2 mortality.

Results of serological studies which are not comparable with our study have shown high frequency of wild canids and free-living dogs' exposures to CPV-2 in some countries.

13 of 19 wolves' serum from Montana (USA) were recorded as positive for CPV-2 antibody (Johnson, 1984).

Based on data achieved in this study, it cannot be concluded exactly whether CPV-2 spill over from golden jackals to other wild canids species in Golestan Province. However, the presence of CPV-2 in sampled golden jackals suggests the possible danger of CPV-2 infection in other wild canids and the need for designing continues via monitoring plans for actively restricting this lethal disease through vaccination.

So, it seems that the government should be encouraged to vaccine rural dogs and also the golden jackals' population in north Iran, to protect endangered species from

CPV-2 infection.

Therefore, this study must be extended to include the testing of higher numbers of golden jackals in North Iran.

Pb and Hg contamination: Assessment of environmental contamination can be indirectly surveyed by analysis of toxic elements concentration in living organisms.

To the best of our knowledge, this is the first study that surveyed the concentrations of Pb and Hg in golden jackals, which are at the top of the food web and may be very sensitive to any levels of non-essential heavy metals, in Iran.

Pb is a very toxic heavy metal that disrupts numerous physiological processes and it does not have any biological function in animals (Kalisinska et al., 2012, Najeeb et al., 2014). Lead toxicity can cause anemia, impaired nervous system and kidney function, delayed healing of fractures and immunosuppression (Liu, 2003). There are many natural and anthropogenic sources of Pb in the environment. The chief causes of lead contamination are industrial activities, coal combustion, automotive industry, agricultural activities (Najeeb et al., 2014).

Pb concentrations in livers and kidneys of sampled golden jackals were 4.7 ± 1.03 and 2.8 ± 0.91 mg/kg, respectively. Toxic threshold of Pb concentration in golden jackals has not been surveyed. However, based on laboratory tests on rodents from the order Rodentia, it was found that Pb bone concentration near the level of 50 mg/kg dw, could lead to adverse effects of this metal on skeletal formations (Andrews, et al., 1989).

As there was no data about the health status of sampled road killed jackals, the toxic effect of Pb on sampled jackals cannot be concluded. Nevertheless this metal can be toxic for animals even at very low levels.

There are just two similar studies on golden jackals in the world and most of the related surveys have been done on foxes.

Ćirović1 and his colleagues (2014) surveyed concentrations of seven trace elements (Pb, Cd, Zn, Cu, Fe, Mn, and Ni) in livers of 129 golden jackals from Serbia. The highest and lowest Pb concentration were recorded 23.00 mg/kg ww and 3.46 mg/kg ww, respectively, with average range of 9.59 mg/kg.

Markov and his colleagues (2016) detected Pb concentration of 6.88 ± 1.67 mg/kg dw and 4.03 ± 1.32 mg/kg dw in livers and kidneys of golden jackals from Bulgaria, respectively, which seems lower than results of the current study.

Pb skin concentration in red foxes from Spain, Croatia and Poland has been documented 0.053 mg/kg, 0.043 mg/kg and 1.445 mg/kg, respectively (Milla´n et al., 2008, Bilandzic et al., 2010).

Hg is a well-known environmental toxicant and pollutant which causes severe changes in the body tissues and causes a wide range of adverse health effects such Gastro-intestinal disorders, respiratory tract irritation, renal failure, neurotoxicity and immunosuppression (Scheuhammer et al. 2008).

The chief sources of Hg contamination include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, metallurgy, burning coal and discharges of industrial wastewater (Liao et al., 2006).

Mean Hg concentrations in livers and kidneys of sampled golden jackals were 0.25 ± 0.18 and 0.15 ± 0.11 mg/kg, respectively. To the best of our knowledge, there is not any report on golden jackals Hg contamination. So, we compared our results with other canids' species such as foxes.

Hg poisoning in wild canids has been rarely documented. One known case was documented in a Swedish wild fox with Hg poisoning signs: running around, staggering, apparently blind without olfactory sense. A mixed sample from the liver and kidney from that individual contained 30 mg Hg/kg ww (Borg et al., 1969).

Although there were a few previous reports on Hg contamination in foxes as representative of wild canids, the Hg threshold value for wild canids (above which adverse effects happen) was not reported. Such data is accessible for the domestic dogs (*Canis lupus*) which also belong to the family Canidae. The laboratory tests performed on the dogs revealed that the typical concentration of Hg in the liver and kidney is <0.1 mg/kg wet weight, ww (~0.3 mg/kg dw), while 2.8 and 3.3 mg/kg ww are introduced as lethal (9.3 and 11 mg/kg dw) (Farrar et al., 1994).

Generally, the effects of Hg contamination on sampled golden jackals in this study are not clear. However, as it can be toxic to cells in low concentration, the toxic effect of Hg on sampled jackals seems possible (Borg et al., 1969).

From the results of similar studies, starting from the 1980s, red foxes and Arctic foxes were found to have kidney and liver Hg levels not higher than 10 mg/kg dw, which is equal to ~2.5 mg/kg ww (Kalisinska et al., 2012).

In recent years, the highest Hg concentrations in the liver and kidney of the red fox ranged from about 4.2 to 5.4 mgHg/kg dw and were observed only in single individuals in Poland and Spain. In both cases, the considerable Hg concentrations were due to unusual environmental conditions and diet (Cybulski et al., 2009, Millan et al., 2008).

Kalisinska and her colleagues (2012) determined the concentrations of total Hg in samples of liver, kidney and skeletal muscle of 27 red foxes from northwestern Poland.

The median concentrations of Hg in the liver, kidney and skeletal muscle were 0.22, 0.11 and 0.05 mg/kg dw, respectively. As it can be concluded, the results of this study are lower than the results of current study. Although when compared with the kidney and liver Hg levels reported by Cybulski et al. (2009) for the farmed silver fox (0.056 and 0.082 mg/kg dw, respectively), golden jackals in this study had higher levels of Hg in the kidneys and livers.

Observed differences in noted results originate from many factors, including difference in diet of each species, habitat condition, type of biological samples, and biological characteristics of each species of animals (Millan, et al., 2008).

Sources of Hg and Pb have not been diagnosed in this study. However, that consumption of contaminated foods has been introduced as one of the major means of heavy metal contamination (Millan, et al., 2008). Golden jackals usually feed on small mammals which can absorb trace elements from soil, water, plant, air and have been introduced as a potential source of contamination for predatory animals inhabiting natural and agricultural ecosystems (Fritsch, et al., 2010).

So, Hg and Pb contaminations in sampled road -killed jackals are strongly correlated with rural and wild regions condition of Golestan Province and indirectly show heavy metal contamination of rural and wild ecosystems in this province.

In this study like most of the studies which have been done on tissues heavy metals contaminations of animals, higher concentration of heavy metals was observed in livers than kidneys (Kalisinska et al., 2012, Markov et al., 2016).

One main part of the explanation is liver tissue activities in detoxification of blood in the body of the animals. Conversely, Lopez et.al, detected a higher concentration of Hg in kidneys (0.053 mg/kg ww) than livers (0.032 mg/kg ww) in free living dogs.

There are conflicting results regarding the effects of gender on heavy metals contamination in animals. Gender effect on heavy metals accumulation is assumed to be related to the biology of the species. The extent of golden jackal`s home range can be influenced by food resource distribution, extent of human pressure, type of habitat, and is estimated to be in the range from 1.1 to 20 km2 (Jhala and Moehlman, 2004).

Generally, male jackals have wider home ranges than females (Giannatos, 2004). Furthermore, the removal rate of foreign compounds per unit body weight in mammals decreases as body weight increases (Prater, 1980).

Thus, males being heavier and more mobile are more prone to collecting contaminants, including heavy metals although the difference in level of metals between genders occurs, no significant sex-related differences in heavy metals accumulation were found in this study. Similar results were reported by Cirovicl et al. (2014) in golden jackals and Kalisinska et al., (2012) in red foxes.

Conversely, in the survey of Millán et al., (2008) on levels of heavy metals and metalloids contamination in critically endangered Iberian lynx and other wild carnivores from southern Spain, liver concentrations of Se, Cd, Pb and Hg were higher in females than in males.

Totally, results of the current study revealed potential risk of heavy metal contamination for wild carnivores and rural habitats in Golestan Province regions and also showed that golden jackals can be a good indicator for environmental pollution.

Relation between Hg and Pb contamination levels and CPV-2 incidence: Presence of heavy metals in biological tissues reflects the chronic intake of heavy metals. Since there is no useful procedure for the removal of trace elements accumulation in various tissues, low levels of chronic intake can lead to harmful effects on long living organisms (Bilandzic et al., 2010).

The presence of Pb and Hg has been detected in most tissues of mammals, and it has been proved that even minimum concentrations of them can cause metabolic disturbances, reducing physical efficiency, weakening immune and enzymatic processes, and leading to many diseases and sometimes death (Rittschof et al., 2005). For example, the interactions between infectious diseases and pollutants in aquatic communities have become an increasing area of concern (Morley, 2010). In combination, these two types of stressors may potentially be synergistically harmful to affected population. Interestingly, a noteworthy positive connection between CPV-2 infection incidence and levels of Hg & Pb contaminations was detected in our study. Jackals with high levels of Hg and Pb tissues concentration were also positive for CPV-2 infection.

To the best of our knowledge, this survey is one of the principal epidemiological studies that provides evidence for a relationship between heavy metals contamination and incidence of viral disease in wild canids. The consequent immunomodulation by Pb and Hg contamination may increase sampled golden jackals susceptibility to CPV-2.

There are some studies with similar results which showed interactions between heavy metals contaminations and infectious and non- infectious diseases in humans and animals.

Nguyen and his colleagues (2016) reported a positive relationship between aerial heavy metals and itchy eyes and coughing in chronic cough patients.

Heavy traffic exposure, one source of heavy metals, has been associated with growth in the incidence and prevalence of childhood asthma and wheeze as well as allergic sensitization, bronchial hyper responsiveness and respiratory symptoms in children (Janssen, et al., 2003).

Bennett and his colleagues showed that long-term exposure to heavy metals, including immunosuppressive metals, like mercury (Hg), is associated with infectious disease in wild cetacean.

Like the results of this study, they found that mean liver concentration of Hg was significantly higher in the porpoises that died of infectious disease compared to healthy porpoises that died from physical trauma.

It has been documented that pretreatment of mice with Hg compounds enhances susceptibility to experimental infections with murine leishmaniasis or sporozoites.

Chou et al.(1998) used host-virus system to examine the effects of heavy metals (cadmium, copper, zinc, mercury) on clam susceptibility to viral infection. Introduction to virus followed by metals resulted in increased clam mortalities compared to controls of up to 52% after 5 weeks.

Liao et al. (2006) and Liao and Yeh (2007) showed that the immunomodulating effects of metal pollution is a significant factor influencing population dynamics of disease transmission, increasing susceptibility of molluscs to infection. However, size of host population, life stage or density and the way of stress exposure (virus +metal or metal + virus) are critical factors in disease dynamics.

Nevertheless, a number of mammalian studies have demonstrated that chemical exposure can cause reactivation of latent viral infections or that viruses may modify the detoxification of other pollutants (Sattar et al., 2007).

It has been supposed that survival and transmission of free-living viral stages in polluted conditions are influenced by the specific contaminants present (Sattar et al., 2007).

These studies, in line with the present study, suggest that heavy metal contamination can affect susceptibility rate of animals to diseases. However, the correlation found between CPV-2 infection and levels of heavy metals concentrations in the present study does not necessarily reveal a causal relationship between these two factors. Indeed, other factors associated to habitat geographical conditions could have been involved in occurrence of such a correlation. It is very likely that this correlation might also be enhanced by the habitat (sanitation, temperature, diet) of the golden jackals and further studies are needed to monitor long-term concentrations of heavy metals with long-term effect on immune system of golden jackals and also frequency of CPV-2 infection.

Moreover, the survey points to the necessity of extra investigation using golden jackals as bioindicators of environmental capacity, and not only with respect to heavy metals. It shows the importance and necessity of assessment of the potential risk associated with environmental pollution.

Acknowledgements

The authors thank all the local minibus drivers that helped us access the road-killed jackals Mr. poorghaz, Dr. kheirabad (employee of Golestan Province Environmental Department) and also students who helped us in collection of the samples.

References

Andrews, S.M., Johnson, M.S., Cooke, J.A. (1989) Distribution of trace elements pollutants in a contaminated grassland ecosystem established on metalliferous fluorspar tailings. Lead. Environ Pollut. 58: 73-85.

Bennett, P.M., Jepson, P.D., Law, R.J., Jones, B.R., Kuiken, T., Baker, J.R. (2001) Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environ Pollut. 112: 33-40.

Bilandzic, N., Dezdek, D., Sedak, M., Dokic, M., Solomun, B., Varenina, I., Knezevic, Z., Slavica, A. (2010) Concentrations of trace elements in tissues of red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) from suburban and rural areas in Croatia. Bull Environ Contam Toxicol. 85: 486-491.

Borg, K., Wanntorp, H., Erne, K., Hanko, E. (1969) Alkyl mercury poisoning in terrestrial Swedish wildlife. Viltrevy. 6: 301-379.

Ćirović, D.A., Penezić, M., Milenković, M.P. (2014) Winter diet composition of the golden jackal (*Canis aureus*) in Serbia. Mamma Biol. 79: 132-137.

Cybulski, W., Chalabis-Mazurek, A., Jakubczak, A., Jarosz, L., Kostro, K., Kursa, K. (2009) Content of lead, cadmium, and mercury in the liver and kidneys of silver foxes (*Vulpes vulpes*) in relation to age and reproduction disorders. Bull Vet Inst Pulawy. 53: 65-69.

FAO/ SIDA (1993) Manual of Methods in Aquatic Environmental Research, part9, Analysis of metals and organochlorines in fish. FAO Fisheries Technical Paper No.212.

Farrar, W.P., Edwards, J.F., Willard, M.D. (1994) Pathology in a dog associated with elevated tissue mercury concentrations. J Vet Diagn Invest. 6: 511-514.

Fritsch, C.R.P., Cosson, M., Coeurdassier, F., Raoul, P., Giraudoux, N., Crini, A. (2010) de Vaufl eury and R. Scheifl er. Responses of wild small mammals to a pollution gradient: Host factors influence metal and metallothionein levels. Environ Pollut. 158: 827-840.

García-Lestón, J., Roma-Torres, J., Vilares, M., Pinto, R., Prista, J., Teixeira, J.P., Mayan, O., Conde, J., Pingarilho, M., Gaspar, J.F., Pásaro. E., Méndez. J., Laffon. B. (2012) Genotoxic effects of occupational exposure to lead and influence of polymorphisms in genes involved in lead toxicokinetics and in DNA repair. Environ Int. 43: 29-36.

Giannatos, G. (2004) Conservation Action Plan for the golden jackal Canis aureus in Greece. WWF Greece.

Greene, C.E., Apple, M.J.G. (2011) Canine viral enteric pathogens. In Infectious Diseases of the Dog and Cat. W.B Saunders Elsevier. St.Louis. Mo., USA. 8: 75-76.

Goddard, A., Leisewitz, A.L. (2010) Canine Parvovirus. Vet Clin Small Anim Pract. 40: 1041-1053.

Janssen, N.A.H., Brunekreef, B., Van Vliet, P., Aarts, F., Meliefste, K., Harssema, H., Fischer, P. (2003) The relationship between air pollution from heavy traffic and allergic sensitization, bronchial hyperresponsiveness, and respiratory symptoms in Dutch schoolchildren. Environ Health Perspect. 111: 1512-1518.

Jaeger, M.M.E., Haque, P., Sultana, R.L., Bruggers. (2007) Daytime cover, diet and space-use of golden jackals (*Canis aureus*) in agro-ecosystems of Bangladesh. Mammalia. 71: 1-10.

Jaishankar, M., Tenzin, T., Anbalagan, N., Blessy, B., Krishnamurthy, N.B. (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol. 7: 60-72.

Jhala, Y.U., Moehlman, P.D., Jackal, G., Sillero-Zubiri, C.M., Hoffmann, D.W. (2004) Canids, Foxes, Wolves, Jackals and dogs. International Union for Conservation of Nature and Natural Resources. p. 156-161.

Johnson, P.T. (1984) Viral diseases of marine invertebrates. Helgolander Meer. 37: 65-98.

Kalisinska, E., Lisowski, P., Izabela, D. (2012) Kosik-Bogacka. Red fox *Vulpes vulpes* (L., 1758) as a bioindicator of mercury contamination in terrestrial ecosystems of North-Western Poland. Biol Trace Elem Res. 145: 172-180.

Langner, A.N., Manu, A., Tabatabai, M.A. (2011). Heavy metals distribution in an Iowa suburban landscape. J Environ Qual. 40: 83-89.

Lanszki, J.G., Giannatos, A., Dolev, G., Bino, M.H. (2010) Late autumn trophic flexibility of golden jackal *Canis aureu*s. Acta Theriologica. 55: 361-370.

Larbi, D.K, Bouslah, Y., Bouderbala, M., Boutiba, Z. (2014) Heavy metals in soft tissues of short-beaked common dolphins (*Delphinus delphis*) stranded along the Algerian West coast. Sci Res. 4 (2).

Lehmann, I., Sack, U., Lehmann, J. (2011) Metal ions affecting the immune system. Met Ions Life Sci. 8: 157-185.

Liao, C.M., Chang, C.F., Yeh, C.H., Chen, S.C., Chiang, K.C., Chio, C.P., Chou, B.Y.H., Jou, L.J., Lien, G.W., Lin, C.M., Shen, H.H., Wu, G.D. (2006) Metal stresses affect the population dynamics of disease transmission in aquaculture species. Aquaculture. 257: 321-332.

Liao, C.M., Yeh, C.H. (2007) Hard clam Meretrix lusoria to Hg-stressed birnavirus susceptibility revealed through stage-structured disease transmission dynamics. Aquaculture. 264: 101-118.

Liu, Z.P. (2003) Lead poisoning combined with cadmium in sheep and horses in the vicinity of non-ferrous metal smelters. Sci Total Environ. 309: 117-126.

Lopez-Alonso, M., Miranda, M., García-Partida, P., Cantero, F., Hernandez, J., Benedito, J.L. (2007) Use of dogs as indicators of metal exposure in rural and urban habitats in NW Spain. Sci Total Environ. 372: 668-675.

Majnoonian, H., Zehzad, B., Dareshouri, F.B., Meigouni, H. (1999) Golestan National Park. Department of Environment of Islamic Republic of Iran, Tehran, Iran. p. 450- 463.

Markert, B., Wuenschmann, S., Fraenzle, S., Graciana Figueiredo, A.M., Ribeiro, A.P., Wang, M. (2011) Bioindication of atmospheric trace metals with special references to megacities. Environ Pollut. 159: 1991-1995.

Markov, G., Kocheva, M., Gospodinova, M. (2016) Assessment of Heavy Metal Accumulation in the Golden Jackal (*Canis aureus*) as a Possible Bioindicator in an Agricultural Environment in Bulgaria. Bull Environ Contam Toxicol. 96: 458-64.

Mechand, S.H., Frui, I.S. (1987) Parvovirus and heartworm found in Minnesota wolves. Endangered Species Technical Bulletin Number 12, U.S. Fish and Wildlife Service, Washington D.C. p. 5-6.

Mechand, S. M., Goyal. (1993) Canine parvovirus effect on wolf population change and pup survival. J Wildlife Dis. 29: 330-333.

Millan, J., Mateo, R., Taggart, M.A., Lopez-Bao, J.V., Viota, M., Monsalve, L., Camarero, P.R., Blazquez, E., Jimenez, B. (2008) Levels of heavy metals and metalloids critically endangered Iberfian lynx and other wild carnivores from Southern Spain. Sci Total Environ. 399: 193- 201.

Morley, N.J. (2010) Interactive effects of infectious diseases and pollution in aquatic molluscs. Aquat Toxicol. 96: 27-36.

Najeeb, U., Ahmad, W., Zia, M.H., Malik, Z., Zhou, W. (2014) Enhancing the lead phytostabilization in wetland plant Juncus eff usus L. through somaclonal manipulation and EDTA enrichment. Arab J Chem. 5: 27-33.

Nguyen, T.T.T., Higashi, T., Kambayashi, Y., Anyenda, E.O., Michigami, Y., Hara, Y. (2016) A longitudinal study of association between heavy metals and itchy eyes, coughing in chronic cough patients: related with non-immunoglobulin E mediated mechanism. Int Environ Res Public Health. 13: 110-21.

Pereira, C.A., Monezi, T.A., Mehnert, D.U.D 'Angelo, M., Durigon, E.L. (2000) Molecular characterization of canine parvovirus in Brazil by PCR. Vet Microbiol. 75: 127-133.

Peterson, E., Krumenaker, H. (1989) Wolf approach extinction on Isle Royale: Abiologucal and policy conundrum. George Wright Forum. 6: 10-15.

Prager, K.C., Mazet, J.A.K., Munson, L., Cleaveland, S., Donnelly, C.A., Dubovi, E.J. (2012) 'The effect of protected areas on pathogen exposure in endangered African wild dog (*Lycaon pictus*) populations',. Biol Conserv. 150: 1-15.

Prater, S.H. (1980). The Book of Indian Animals. Bombay Natural History Society, Bombay, India.

Rittschof, D., and McClellan-Green, P. (2005) Molluscs as multidisciplinary models in environmental toxicology. Mar Pollut Bull. 5: 369-373.

Šálek, M.J., Červinka, B.C., Ovidiu, M., Krofel, D., Ćirović, I., Selenec, A., Penezić, S., Grill, J., Reigert. (2014) Population densities and habitat use of the golden jackal (*Canis aureus*) in farmlands across the Balkan Peninsula. Europ J Wildlife Res. 60: 193-200.

Scheuhammer, A.M., Basu, N., Burgess, N.M., Elliott, J.E., Campbell, G.D., Wayland, M., Champoux, L., Rodrigue, J. (2008) Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). Ecotoxicology. 17: 93-101.

Sharbati, A. (2012) The Ecotourism Potentials of Golestan Province. J Basic Appl Sci Res. 2: 564-570.

Ziaee, H. (2008) Field handbook of Iran's mammals. Introduction wildlife center institute publication, Tehran, Iran.

مجله طب دامی ایران، ۱۳۹۶، دوره ۱۱، شماره ۲، ۱۲۴-۱۱۳

تجمع زیستی فلزات سنگین و ارتباط بالقوه آن با بروز بیماری پاروویروس سگ‌سانان در شغال‌های طلائی، شمال ایران

سمیه نمرودی* حسن رضایی داوود میلانلو

گروه محیط زیست، دانشکده شیلات و محیط زیست دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران

(دریافت مقاله: ۸ آذر ماه ۱۳۹۵، پذیرش نهایی: ۲۱ اسفند ماه ۱۳۹۵)

چکیده

زمینه مطالعه: ثابت شده است که فلزات سنگین تهدیدی حیاتی برای سلامت حیوانات بوده و می‌توانند باعث کاهش قدرت سیستم ایمنی شوند. همچنین گفته می‌شود که تخریب سیستم ایمنی، نقش کمکی مهمی در بروز بیماری‌های عفونی ایفا می کند. افزایش استفاده از زیستگاه‌های روستایی توسط شغال‌ها، آنها را برای بررسی اثرات فعالیت‌های انسانی بر سلامت حیوانات، مناسب می‌سازد. **هدف:** در این مطالعه ما به بررسی این موضوع که آیا تماس با فلزات سنگینی که باعث کاهش قدرت سیستم ایمنی می‌شوند، با بروز بیماری عفونی در شغال طلایی، به عنوان نماینده سگ‌سانان، ارتباط دارند پرداختیم. **روش کار:** ۳۰ شغال تلف شده بر اثر تصادف جاده‌ای کالبدگشایی شدند. غلظت فلزات سنگین سرب و جیوه در نمونه‌های کبد و کلیه با استفاده از دستگاه جذب اتمی اندازه‌گیری شد. ژن VP۲ از ژنوم دی‌ان‌ای پاروویروس سگ‌سانان جهت شناسایی CPV-۲ در نمونه‌های مدفوع با استفاده از PCR، استفاده گردید. **نتایج:** متوسط غلظت (وزن مرطوب) فلزات جیوه و سرب بترتیب ۰/۱۵±۰/۱۱ mg/kg و ۰/۲۵±۰/۱۸ mg/kg در کلیه و ۲/۸±۰/۹۱ mg/kg و ۴/۷±۱/۰۳ mg/kg در کبد بود. CPV-۲ در ۸ (۲۴٪) نمونه شناسایی گردید. متوسط غلظت سرب و جیوه در شغال‌هایی که آلوده به CPV-۲ بودند، نسبت به شغال‌هایی که آلوده نبودند بالاتر بود. **نتیجه‌گیری نهایی:** این مطالعه مقطعی، تجمع بافتی فلزات سنگین را با عفونت ویروسی مرتبط ساخت. کارهای بیشتری جهت برآورد دقیق نقش فلزات سنگین در مستعد ساختن شغال‌های طلائی به آلودگی به CPV-۲ لازم است.

واژه های کلیدی: تجمع زیستی، فلز سنگین، شغال، پاروویروس، تلفات جاده ای

---

*) نویسنده مسؤول: تلفن: ۳۲۵۲۴۴۹۳ (۱۷)۹۸+ نمابر: ۳۲۴۲۷۰۴۰ (۱۷)۹۸+ Email: snamroodi 2000@yahoo.com