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ISSN 2348-0416
USA CODEN: JASRHB

Journal of Applied Science And Research, 2016, 4 (2):33-43

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Haematological investigation in Black kites, *Milvus migrans govinda* in Bundelkhand region, India

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ABSTRACT

As a top consumer in food chains, raptors forage over large geographical areas and so might be expected to accumulate environmental toxicants. Total of 12 birds Milvus migrans govinda were collected from 2007-2011 for occurrence of blood parasites and effect of toxicants on haematology. Only 6 Kites were found suitable for preparation of blood smears. Haematozoan parasites were not found from any of 6 Milvus migrans govinda examined from Bundelkhand region. The blood was collected from wing veins in fresh dead eagles by using 2 ml syringe and needle. Feathers in the axillary region were plucked to isolate wing vein and the site was disinfected by 70% methylated ethanol. The blood smears were prepared, air dried, fixed with methanol, stained with Giemsa and examined for blood parasites. The haematological values obtained from the Black Kites, Milvus migrans govinda were within the range of healthy raptors. Absence of blood parasites in Milvus migrans govinda is also striking, considering that they have a strong preening behaviour against ectoparasites which are vectors of haematozoa. Present study on Milvus migrans govinda will be important for future comparisons of population health in Bundelkhand region.

Key-word : Raptors, Haematological, Bundelkhand region, Preening, Haematozoa.

INTRODUCTION

Raptors are excellent bioindicators of a healthy ecosystem. More emphasis is being placed on the blood as a health monitoring tool for wild birds (Dawson and Bortolotti, 1997; Newman *et al.*, 1997; Olsen *et al.*, 2001; Balbontin and Ferrer, 2002). Very little studies focused on the use of haematological parameters of birds for biomonitoring of environmental conditions. Heavy metals such as lead, cadmium has been identified as environmental contaminants toxic to eagles and posing serious threats to declining populations of other raptors worldwide. Physiological stress indicators such as haematological parameters could be useful to evaluate the effects of contaminants heavy metals on birds. Haematotoxicity happen when some of these different blood components are present or structural anomalies occurring in blood components interfere with normal functioning. Haematological parameters can be considered indicators of toxicity in bird's studies (Pushpendra Pathak and K. S. Rana, 2012). Use of pesticides, in modern agriculture has increased tremendously. Persistent organic pollutants such as organochlorine pesticides are distributed in food webs globally and, due to their bioaccumulative properties and persistence, they biomagnify to their greater concentrations in higher-level consumers (Senthil Kumar *et al.*, 2002).

Blood indices are considered physiological parameters of the whole body. Thus, present study has

been planned for haematological investigation of *Milvus migrans govinda* in Bundelkhand region. Establishing normal baseline values for blood of *Milvus migrans govinda* will be important for future comparisons of population health in Bundelkhand region.

Study Area

Bundelkhand region is located between 23° 20’ and 26° 20’ N latitude and 78° 20’ and 81° 40’ E longitude bounded by the Yamuna in the north, the Chambal in north west, erupted ranges of the Vindhya in the south east. The area is generally rocky. Granites of varying types are the predominant geological material found across in the region. These are followed by gneisses and sedimentary strata such as sandstone and limestone. In the southern extremity of the Bundelkhand, imposing escarpments of limestone and sandstone form the backbone of the Vindhyan range, which has effectively isolated the region from the south and is traditionally viewed as the division between northern and southern India. Bundelkhand region is an important agricultural region in Uttar Pradesh and 36.42% of the area is covered with forest. Bundelkhand region accounts for 10.8% of the total forest area and ranks first in terms of forest area of the state. Bundelkhand region including 13 districts of Uttar Pradesh and Madhya Pradesh. Bundelkhand region includes Jhansi, Lalitpur, Jalaun, Hamirpur, Banda and Mahoba in Uttar Pradesh and Sagar, Chattarpur, Tikamgarh, Panna and Damoh in Madhya Pradesh including parts of Gwalior, Datia, Shivpuri and Chanderi (Figure 1). The river network of the region comprises of various big and small rivers like, Yamuna, Chambal, Betwa, Dhasan, Son, Sindh and Kane. The main tributaries of the Yamuna are the Betwa, Ken, Baghain, Pahuj and Dhasan rivers, most of which are important sources of water.

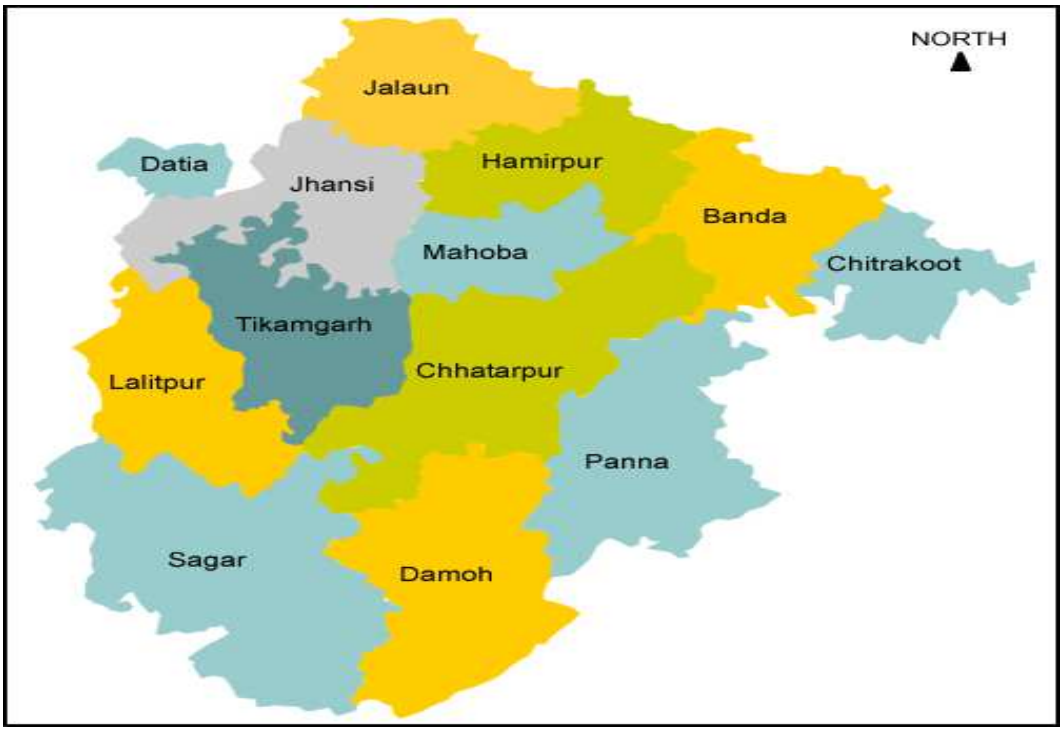

Figure 1: Map of study area “Bundelkhand region”
Source: http://www.apnabundelkhand.com/

MATERIALS AND METHODS

Materials

The blood was collected from wing veins in fresh dead eagles by using 2 ml syringe and needle.

Feathers in the axillary region were plucked to isolate wing vein and the site was disinfected by 70% methylated ethanol. The blood smears were prepared, air dried, fixed with methanol, stained with Giemsa and examined for blood parasites (Bennett, 1970).

RESULT AND DISCUSSION

Total of 12 birds *Milvus migrans govinda* were investigated for occurrence of blood parasites and effect of toxicants on haematology. Only 6 Kites were found suitable for preparation of blood smears. Haematozoan parasites were not found from any of 6 *Milvus migrans govinda* examined from Bundelkhand region. The blood parasites studies on birds in general have found low infestations rate. The absence of blood parasites in Black Kites, *Milvus migrans* is notworthy. The life style and foraging habitat of Black Kites, *Milvus migrans* probably reduces their susceptibility to some vectors of blood parasites.

Absence of blood parasites in *Milvus migrans govinda* is also striking, considering that they have a strong preening behaviour against ectoparasites which are vectors of haematozoa. Despite of the presence of suitable vectors and the right host-parasite relationship in the environment, raptors could be free of blood parasites if the competence of their immune system is extremely high. Absence of blood parasites can be an artefact caused by low parasite detectability or by intra specific variation in the susceptibility of the host to parasitisation depending on strong preening behaviour or habitat. Blood parasites often incur a fitness cost to the infected bird, sometimes resulting in death of the bird. Some raptors, however, are apparently free of blood parasites, presumably due to the lack of exposure to blood parasite vectors such as ectoparasites. Protective immunity in raptors may be also responsible for the absence of infections by blood parasites. According to some previous studies, the incidence and prevalence values of blood parasites are mainly dependent on the abundance and activity of ectoparasite vectors. Although the densities of vectors were not estimated, the data on the presence or absence of parasite vectors for all 12 *Milvus migrans govinda* was recorded. However, such extrinsic factors, immunological capacity of raptors and absence of the perfect host parasite relation might also be important.

The heterophils were lower in *Milvus migrans govinda* when compared to infected Philippine scops owls, *Otus megalotis*. The lymphocytes were higher. This is similar to the haematologic response of poultry to stress, which is characterized by the release of corticoids resulting to heterophilia and lymphopenia (Powers, 1994). Samour (1996) also described the increase in the heterophils and decrease in the lymphocytes during the periods of stress. The PCV values are higher than those obtained by Manigbas (2000) which were 29% for the Scops Owl, *Otus megalotis* and 32% for the Philippine eagle owl, *Bubo philippensis* (Table 1). These values can serve as benchmark to determine what could be the normal versus anaemic states. This finding is remarkably different from Butterworth (1996) who reported acute weight loss and anaemia due to *Haemoproteus* spp. in Snowy owls, *Nyctea scandiae*. This can be attributed to the pathologic response because of being unexposed, due to the absence of typical vectors. The mean estimated WBC count were 16158.33 (infected Scops owl), 17111 (non infected *Milvus migrans govinda*). Increase in leukocytes is seen in conditions of stress and disease in birds (Altman, 1997) as well as in cases of Chlamydiosis, Aspergillosis and Tuberculosis. There was no significant effect on the PCV and estimated WBC on the values obtained from the *Milvus migrans govinda* (Table 2) and the *Haemoproteus* spp. - infected owls. There was no increase in the heterophils and the lymphocytes of the *Otus megalotis* infected with *Haemoproteus* spp. The haematological values obtained from the Black Kites, *Milvus migrans govinda* were within the range of healthy raptors.

Table 1: The haematological parameters of Philippine scops owl, *Otus megalotis* and Philippine eagle owl, *Bubo philippensis*

	PCV (%)	**Total Protein (mg/dL)**	**WBC (x 10^3/L)**	**Heterophil (%)**	**Eosinophil (%)**	**Lymphocyte (%)**	**Monocyte (%)**	**Basophil (%)**
Haemoproteus spp infected scops owl, *Otus megalotis* (N=3)	40.33	6.76	16158.33	64.33*	6.33	27.67	1.33	0.33
Philippine eagle-owl, *Bubo philippensis*	38.66	4.33	26,044.00	50.00	5.00	40.00	4.66	0.33

Table 2: The haematological parameters of *Milvus migrans govinda*

S. No	**Sample no**	**PCV (%)**	**Total Protein (mg/dL)**	**WBC (x 10^3/L)**	**Heterophil (%)**	**Eosinophil (%)**	**Lymphocyte**	**Monocyte**	**Basophil**
1	MAS	42.00	5.03	17134	45.01	1.3	66.01	2.87	.011
2	MA3	41.03	4.98	17010	44.91	1.09	68.09	2.01	.032
3	MA4	41.09	5.56	17189	44.02	2.56	68.58	2.76	.081
4	MA5	43.13	6.04	17152	43.11	2.80	59.92	2.94	.037
5	MA6	40.96	5.85	17100	45.22	3.09	63.98	2.14	.021
6	MA7	43.06	6.21	17082	43.45	3.85	64.28	3.00	.021
Avg.		41.87	5.61	17111	44.28	2.44			

Discussion

Lead is one of the most toxic elements, because it binds and inactivates essential enzymes. Renal, gastrointestinal, nervous and haemapoietic systems are affected by the lead intoxication (Baykov *et al.,* 1996). There are several evidences indicate effects of metals in blood as Jitka Osickova *et al.*, (2012) reported that decreased haemoglobin and haematocrit levels are associated with lead-exposure and reflect the extent and efficiency of oxygen carrying capacity of blood and thus the bird´s ability for physical performance. Over-exposure to Pb is also known to cause reduced total-blood haemoglobin concentration, elevated levels of protoporphyrin and decreased packed cell volume(PCV) (Franson, 1986; Pain, 1995). In some cases, haematologic parameters may provide an indication of lead intoxication. A hypochromic, regenerative anaemia occurs in some affected birds (S. E. McDonald, 1988). However, death can be acute without premonitory signs (G. Dumonceaux, 1994). Mateo *et al.,* (1999) reported that the number of raptors affected by elevated blood Pb levels

0.2 ppm. P. Gomez-Ramirez *et al.,* (2011) suggested that blood lead levels above 15 µg/dl caused adverse effects. Franson (1996) suggested threshold ranges in Falconiformes for lead with subclinical effects levels (generally indicated by a biochemical perturbation) expressed as 0.2 ppm to 1.5 ppm in blood, 2 ppm to 4 ppm in liver and 2 ppm to 5ppm in kidney. Some evidences on cadmium toxicity are given as M. R. Spivey Fox (1971) reported growth depression, anaemia, elevated plasma transferrin levels caused by lethal concentration of cadmium in Japanese quail, *Coturnix japonica.* Scheuhammer (1987) suggested that a cadmium level over 3 ppm in liver and over 8 ppm in kidney might indicate an increased environmental exposure to that metal. Eisler (1985) suggested maximum liver cadmium levels via food items, which led to elevations in blood, liver and kidney tissues in the Smelter Hill associated Kestrel, *Falco* nestlings reached 0.378 µg/g or approximately 3% of the toxic threshold (13 µg/g). B. W. Cain *et al*., (1983) studied on Mallard ducklings, *Anas platyrhynchos* were fed cadmium in the diet at 0, 5, 10, or 20 ppm from 1 day of age until 12 weeks of age. At 4-week intervals six males and six females from each dietary group were randomly selected, bled by jugular venipuncture, and necropsied. Significant decreases in packed cell volume (PCV) and hemoglobin (Hb) concentration and a significant increase in serum glutamic pyruvic transaminase (GPT) were found at 8 weeks of age in ducklings fed 20 ppm cadmium. Mild to severe kidney lesions were evident in ducklings fed 20 ppm cadmium. Guillermo Blanco *et al*., (2004) observed levels of heavy metals in blood have detrimental effects on the health of prefledgling Black kites, *Milvus migrans* exposed to emissions from a solid waste incinerator near Madrid, central Spain. They concluded negative effects of Cd on the immune system. Hypothesis on copper is given as F. McGhee *et al.,* (1965) concluded that trace elements such as iron, zinc, cadmium and lead; interactions may be harmful to the organism. The lethal dose of copper in birds has considered at 80 ppm-160 ppm. E. van Wyk *et al.,* (2001) reported Cu concentrations below 500 µg/g, which is considered to be the level at which toxic effects in blood and tissues of vultures species African whitebacked, *Pseudogyps africanus*, Cape griffon, *Gyps coprotheres* and Lappetfaced *Torgos tracheliotos* in different regions of South Africa.

Many hypothesis are given on effects of zinc on haematology of birds as M. M. Christopher *et al.,* (2004) reported that toxic effects of zinc leading to haemolytic anaemia have recently been investigated in mallard ducks, *Anas platyrhynchos*. Zinc poisoning in birds, observed abnormalities include a greater number of immature red blood cells, hypochromasia, poikilocytosis and nuclear abnormalities, such as fusiform, elongated, and irregular nuclei (M. M. Christopher *et al.,* 2004). J. L. Stahl *et al.,* (1989) observed excess zinc result in a functional iron deficiency leading to reduced haeme synthesis and erythropoiesis. The lethal level of zinc in birds has considered at 312.4-2418 ppm (www.ivis.org). The interaction between zinc and iron may therefore play a major role in the development of anaemia in birds overexposed to zinc. In addition, zinc limits copper availability and decreases tissue copper and ceruloplasmin concentrations.

Some hypotheses have been suggested on high concentration of Fe as Rattner *et al.,* 2008) concluded that high concentration of Fe determined in the blood of the birds, fluctuation levels of metals in the blood may reflect the diet and mobilization quantities stored in the tissue or last dietary, dermal or respiratory. E. S. Dierenfeld *et al.,* (1994) concluded that all birds need some iron, to create blood and other tissues. They suggested that diets should be formulated to contain between 50 ppm and 100 ppm iron. Diet contribute to the development of haemosiderosis and iron storage disease in birds. Sensitive species have more efficient mechanisms of iron absorption. Factors such as iron level, iron source and interactions with other compounds all affect the availability of dietary iron (http://www.nashvillezoo.org). Mining activities generally represent a risk to wildlife inhabiting the surrounding areas (W. N. Beyer, 2004). Organisms living in these areas are chronically exposed to high levels of metals and related products that may cause detrimental health effects on individuals and populations (T. Eeva *et al.,* 1996). In Bundelkhand

region, a number of rock crushing unit (RCU), geo-granite mining, drilling and blasting operations are going on in large scale which generated huge amount of silica and other types of mixing dust. The other fine particles originated from Parichha thermal power plant, Hiedel diamond cement industry and also brick manufacturing process units emits in the form of flyash, lime particulate matters and soil born dust respectively (Abhimanyu Singh *et al.,* 2013). Metals like Cd, Pb, Cu and Fe concentrations were found to the above permissible limit at some places in different seasons and may cause health hazards in existing environment in Bundelkhand region (Gayatri Singh *et al.,* 2010). The nonessential heavy metals lead and cadmium are emitted and globally distributed mainly through industry, road traffic, and consumption of fossil fuels (Kenntner *et al.,* 2003). Thus, these metal lead, cadmium, copper, zinc and iron have been included in present study. Present study has been detected concentration of lead, cadmium, copper, zinc and iron below lethal concentration in *Milvus migrans govinda* in Bundelkhand region. Since the haemoglobin, PCV and haematocrit levels in the samples studied were within the normal range and showed no decrease therefore it is evident that the birds were not exposed to lead, cadmium, copper, zinc and iron toxicity. Based on previous studies, there is no reason to believe that levels of metals in *Milvus migrans govinda* represent any hazard, as it has been found no values approaching levels known to be toxic for any of the metals analysed.

Pesticides affect the human, environment and wildlife including birds. Because of persistent nature, organochlorines are no longer in use in several countries. But some of them like aldrin, dieldrin, lindane and endosulfan are still in use in developing countries (Anindita Mitra *et al.,* 2011). Most organochlorine inhibits gamma-amino butyric acid (GABA) receptor in brain and affects the central nervous system. They cause widespread population decline of raptorial birds like the Peregrine falcon, *Falco peregrinus,* Sparrow hawk, *Accipiter nisus* and Bald eagle, *Haliaeetus leucocephalus* (Anindita Mitra *et al.,* 2011). The chronic use and excessive doses of pesticides become part of food chain leading to a series of haematological, biochemical, reproductive and pathological changes in the body (Ahrar Khan *et al.,* 2012). Long term exposure to these products causes countless abnormalities and reduces the life span of organisms (Hussain *et al*., 2011; Naz *et al.,* 2011). Blood findings are important for the assessment of various systemic functions and health of animals under various environmental conditions and most importantly, for diagnosis of drug or chemical induced haemolysis (Atamanalp and Yanik, 2003). There are many evidences of effects of pesticides in haematology of raptors as Vassilis Goutner *et al.,* (2009) investigated the levels of organochlorines in blood of Cinereous vultures, *Aegypius monachus* and Griffon vultures, *Gyps fulvus* at Dadia national park, Greece. All 16 organochlorine pesticides analysed were detected from both vulture species blood samples. Nevertheless, endrin, γ-chlordane and *p, p* − DDT in Cinereous vulture, *Aegypius monachus* and dieldrin, endrin, γ-chlordane, endosulfan II and *p, p* − DDT in Griffon vulture, *Gyps fulvus*. β-HCH, lindane and endosulfan sulphate followed in levels, the last being more important in Griffon vulture, *Gyps fulvus*. They concluded that residue levels detected in vultures sampled in Dadia national park were too low to affect them. Martinez-Lopez *et al.,* (2009) reported degree of exposure to lindane and endosulfan in the blood of booted eagle nestlings, *Hieraaetus pennatus* (1999–2003). The highest blood lindane concentrations were obtained 1 year prior to its prohibition by the European Union. They concluded that concentrations of organochlorine pesticides in the blood of booted eagle nestlings may be used to monitor the use of those pesticides over a particular agricultural region and alert the authorities of possible environmental or health risks. Newton and Wyllie (1992) examined 1029 dead Sparrow hawk, *Accipiter nisus* and 1055 dead Kestrels, *Falco tinnunculus* from various parts of Britain and Organochlorine poisoning was also reported a cause of death. The main chemical which caused poisoning was HEOD derived from the insecticides aldrin and dieldrin. Sparrow hawk, whose deaths were attributed to HEOD poisoning contained 5-85 μg/g in their liver. Other deaths were

attributed to poisoning by heptachlor epoxicide. Mandal *et al.,* (1986) reported haematological changes produced by lindane (γ- HCH) in six species of birds were investigated as to indicate whether it can be used as an early warning system for pesticide toxicity in India. Anaemia and decreased haemoglobin concentration have been documented after birds were exposed to lindane. The effects of various chlorinated hydrocarbons on avian species was studied by S. J. Ithuri (1984) and concluded that continuous administration of DDT in feed reduced haemoglobin concentration in blood and reduced haematocrit value. He also observed increase in the haematocrit and haemoglobin value during chronic aldrin toxicity. In field trials, Barn owls, *Tyto alba* in an oil palm plantation in Malaysia were tolerant of secondary ingestion of warfarin for rat control but the population declined from 20 breeding pairs to two individuals after use of second-generation anticoagulants, firstly coumachlor, then brodifacoum; carcasses showed signs of haemorrhaging (Duckett, 1984). These hypotheses are not supported by present study. Haematology constituents were determined in blood collected from 6 *Milvus migrans govinda* from Bundelkhand region. The heterophils were lower in *Milvus migrans govinda* when compared to infected Philippine scops owls, *Otus megalotis*. The lymphocytes were higher. This is similar to the haematologic response of poultry to stress, which is characterized by the release of corticoids resulting to heterophilia and lymphopenia (Powers, 1994). Samour (1996) also described the increase in the heterophils and decrease in the lymphocytes during the periods of stress. The PCV values are higher than those obtained by Manigbas (2000) which were 29% for the Scops owl, *Otus megalotis* and 32 % for the Philippine eagle owl, *Bubo philippensis*. These values can serve as benchmark to determine what could be the normal versus anaemic states. This finding is remarkably different from Butterworth (1996) who reported acute weight loss and anemia due to *Haemoproteus* spp. in Snowy owls, *Nyctea scandiae*. This can be attributed to the pathologic response because of being unexposed, due to the absence of typical vectors. The mean estimated WBC count were 16158.33 (infected Scops owl), 17111 (non-infected *Milvus migrans govinda*). Increase in leukocytes is seen in conditions of stress and disease in birds (Altman, 1997) as well as in cases of chlamydiosis, aspergillosis and tuberculosis. There was no significant effect on the PCV and estimated WBC on the values obtained from the *Milvus migrans govinda* and the *Haemoproteus* spp. infected owls. There was no increase in the heterophils and the lymphocytes of the *Otus megalotis* infected with *Haemoproteus* spp. The haematological values obtained from the Black Kites, *Milvus migrans govinda* were within the range of healthy raptors. Present study result indicates that the haematological status of *Milvus migrans govinda* is not affected by pesticides.

CONCLUSION

This study is the first account of presence of blood parasites and effect of toxicants on haematology in Bundelkhand region. Collected haematological data provide baseline parameters of *Milvus migrans govinda* in Bundelkhand region. Raptors are at the top of the food chain, so they have no natural enemies. But their population is declining day by day that means humans have done something to harm the Raptor's wild habitat. Rapid industrialization and urbanization has resulted in water bodies becoming highly toxic which is reflected by the accumulation of heavy metals in food chain and raptor are at the top of the food chain. As further research is conducted, the information of present study will aid in the haematology as a tool for the management of raptor populations.

ACKNOWLEDGEMENTS

Authors are thankful to Prof. Madhu Tripathi, Head, Department of Zoology, University of Lucknow, Lucknow. We are thankful to Forest department of Uttar Pradesh for providing the permission to study. Our sincere acknowledgements to all the local people of Bundelkhand region

for their cooperation and help.

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