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Cross Infection with Gastro-intestinal Tract Parasites between Red panda (*Ailurus fulgens* Cuvier, 1825) and Livestocks in Community Forest of Ilam, Nepal

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Abstract: *From the Ilam community forest total 55 faecal samples of Red Panda (n=14) and livestocks (n= 41) were collected to analyses the cross- transmission of GI parasites between Red Panda and livestocks and vice-versa. For the examination, direct fecal smear, concentration techniques were utilized to identify the ocyst, cyst, eggs and larvae of parasites in feces. All the samples were found positive either for protozoan or helminth parasites. The recorded protozoan parasites were Eimeria sp., Entamoeba sp, and Balantidium sp. with their prevalence 64.28%, 57.14% and 14.28% respectively in Red Panda and 60.97%, 21.95% and 7.31% respectively in livestocks. In case of helmith parasites, seven nematode were recorded in Red Panda with different prevalence rate, Oxyuris (100%), Ascaris (57.14%), Trichostrongylus (50%), Strongyloides (50%), Trichuris (42.8%), Crenosoma (42.85%) and Hook Worm (35.7%) but trematode and cestode were absent while in livestock six nematode Oxyuris (87.8%), Ascaris (60.97%), Strongyloids (53.65%), Trichostrongylus (41.465), Hook Worm (39.02%) and Trichuris (31.70%) and one each of trematode and cestode: Paramphiostomum sp. 2.43% and Moniezia sp. 14.63% respectively. There was no statistical significant difference in prevalence of gastrointestinal parasites between Red Panda and livestocks (P > 0.05) which could be attributed to sharing of same pastures land. So, grazing system of livestock in the habitat of Red Panda should be stop.*

Keywords: *Red panda; Distribution; Status; Conservation; Habitat; Oxyuris*

1. Introduction

Red Panda is an endemic and a flagship species in the Himalayan region (Roberts and Gittleman, 1984) which threatened with extinction worldwide (Wang *et al.*, 2008). This species is an arboreal, shy, nocturnal and solitary in nature however sometime seen in a pair or group and travel a linear distance of 1.57 Km during breeding season and active only in the late in the afternoon and/or early evening hours (Yonzon, 1989). The IUCN classifies the Red Panda as vulnerable status, suggesting a likely extinction globally if conservation measures are not initiated soon (Wang *et al.*, 2008). On the basis of habitat suitability index,

Nepal is home to approximately 1.9% of total global population of Red Panda and 38% potential habitat of Red panda covered by Protected Areas where as remaining 62% by community managed and national forest (DNPWC/MoFSC/GoN, 2010). Currently, Red Pandas are believed to occur at low densities with a patchy distribution due to habitat fragmentation, loss of foraging habitat, human and livestock disturbances, poaching, and disease (Patterson-Kane *et al.*, 2009, Dorji *et al.*, 2012, Sharma *et al.*, 2014)

Parasitic infection is the one of disease in Panda (Zhang *et al.*, 2006). Parasitic infection in animals as well as human negatively impact on body weight gain, quality of reproduction due to loss of appetite, nutrient uptake and utilization (Gross *et al.*, 1999) even death in wild animals (Rao and Acharjyo, 1984, Hossain and Perry 1994). By using same grazing site by domestic and wild animals, there are chances of cross- transmission of parasites between wild to domestic or vice-versa (Hoberg and Brooks 2008, Agosta *et al.*, 2010). A wide range of parasites have been identified that can infect both wild and domestic animals (Kock *et al.*, 2002). The better understanding of cross- transmission help to makes management policy for both wild and domestic animals in different geographical area (Morgan *et al.*, 2005). Historically, study of parasites in wild animals have been descriptive account (Thapa 2013, Chaudhary 2014 and Shrestha 2015) rather than clinical effect that the parasites cause i.e. morbidity and mortality.

2. MATERIALS AND METHODS

The study was carried out in Maimajhuwa, Mabu, Jamuna and Jogmai VDCs of Ilam, it was designed to include all the Red Panda and livestock which shared same land for grazing. From the GIS system study area was selected, for this elevation was 2200-4000m and size of grids were 1.7 × 1.7 (2.89) Km^2 while in each grid two transects were made. Fresh faecal Samples of both domestic animals as well as red Panda were collected from transects and opportunistically from the study area. Altogether 55 faecal samples were collected preserved in 2.5% Potassium dicromate. The entire faecal samples were examined in the laboratory of CDZ by both direct smear and concentration method for detection of intestinal parasites and intensity of parasites of Red Panda and livestock.

3. RESULTS

All the samples (Red Panda and livestocks) collected from study area were found positive for parasitic infection. Cyst, oocyst, eggs and larvae of different parasites were observed in both Red Panda and livestocks. During coprological examination three and seven genera of protozoan and nematode parasites were recorded in Red Panda while trematode and cestode were absent. Incase of livestock, three and six genera of protozoan and nematode parasites and one each genera of cestode and trematode were recorded. *Eimeria sp.* occyst showed the highly prevalent with 64.28% in Red Panda and 82.92% in Livestocks among protozoan paarasites while *Oxyuris sp.* egg showed the highest prevalent among nematode parasites with 100% and 87.8% in Red Panda and livestocks respectively. There was no statistical significant difference in prevalence of gastrointestinal parasites between Red Panda and livestocks ($p > 0.05$). However, *Entamoeba sp*. showed the significant difference between them ($p < 0.05$) (Table 1).

Table 1. *Comparative analysis of GI parasites in between Red Panda and livestock*

S.N	Class	Parasites	Prevalence in Red Panda	Prevalence in livestock	X^2-Value	P-Value
1.	**Sarcodina**	*Entamoeba sp.*	8(57.14%)	9(21.95%)	6.052	0.014
2.	**Sporozoa**	*Eimeria* with micropyle	9(64.28%)	20(48.28%)	1.007	0.316
		Eimeria without micropyle	8(57.14%)	25(60.97%)	0.064	0.800
3.	**Litostomatea**	*Balantidium sp.*	2(14.28%)	3(7.31%)	0.613	0.592*
4.	**Nematoda**	*Oxyuris sp.*	14(100%)	36(87.8%)	1.878	0.314*
5.		*Strongyloid sp.*	7(50%)	22(53.65%)	0.056	0.813
6.		Hook worm	5(35.7%)	16(39.02%)	0.048	0.826
7.		*Trichostrongylus sp.*	7(50%)	17(41.46%)	0.309	0.578
8.		*Crenosoma sp.*	6(42.85%)	0	19.723	0.000*
9.		*Ascaris sp.*	8(57.14%)	25(60.975)	0.064	0.800
10.		*Trichuris sp.*	6(42.85%)	7(31.70%)	0.574	0.449
11.	**Cestode**	*Moniezia sp.*	0(0%)	6(14.63%)	_	-
12.	**Trematode**	*Paramphiostomum sp.*	0(0%)	1(2.43%)	-	-

*= Fisher exact test accepted due to less than five expected value

From the analysis it showed that the transmission of parasites between Red Panda and livestock were possible. To know the parasites sharing groups with Red Panda livestocks were categories into cattle and goat/sheep. Morphologically similar parasitic egg/ cyst and larvae were compared.

There was no statistical significant difference in prevalence of gastrointestinal parasites in between Red Panda and Cattle and Red Panda and goat/ sheep ($p > 0.05$) (Table: 2 and Table: 3).

Table 2. *Prevalence of gastro-intestinal parasites in Red Panda and cattle*

S.N	Class	Parasites	Prevalence in Red Panda	Prevalence in cattle	X^2-Value	P-value
1.	**Sarcodina**	*Entamoeba sp.*	8(57.14%)	7(53.84%)	1.944	0.163
2.	**Sporozoa**	*Eimeria* with micropyle	9(64.28%)	10(47.61%)	0.940	0.332
		Eimeria without micropyle	8(57.14%)	13(61.53%)	0.079	0.778
3	**Litostimatidea**	*Balantidium sp.*	2(14.28%)	2(9.52%)	0.188	1.00*
4	**Nematoda**	*Oxyuris sp.*	14(100%)	19(90.47%)	1.414	0.506*
5		*Strongyloid sp.*	7(50%)	10(47.61%)	0.019	0.890
6.		Hook worm	5(35.7%)	9(42.85%)	0.179	0.673
7		*Trichostrongylus sp.*	7(50%)	11(52.38%)	0.19	0.778
8.		*Ascaris sp.*	8(57.14%)	12(57.14%)	0.00	1.00
9.		*Trichuris sp.*	7(42.85%)	8(38.09%)	0.079	0.778
10.		*Crenosoma sp.*	6(42.85%)	0	1.414	0.506*

*= Fisher exact test accepted due to less than five expected value

Table 3. *Prevalence of gastro-intestinal parasites in Red Panda and goat/sheep*

S.N	Class	Parasites	Prevalence in Red Panda	Prevalence in Goat and Sheep	X^2-Value	P-value
1.	**Sporozoa**	*Eimeria* with micropyle	8(57.14%)	7(53.84%)	0.304	0.581
		Eimeria without micropyle	9(64.28%)	8(61.53%	0.054	0.581
2	**Sarcodina**	*Entamoeba sp.*	8(57.14%)	0	10.556	0.002*
3	**Litostomatea**	*Balantidium sp.*	2(14.28%)	0	2.006	0.481*
4	**Nematoda**	*Oxyuris sp.*	14(100%)	11(84.61%)	2.326	0.222*
5.		*Strongyloid sp.*	7(50%)	8(61.53%)	0.363	0.547
6.		Hook worm	5(35.71%)	5(38.46%)	0.22	0.883
7.		*Trichostronggylus sp.*	7(50%)	6(46.15%)	0.40	0.842
8.		*Ascaris sp.*	8(57.14%)	7(53.84%)	0.30	0.863
9.		*Trichuris sp.*	6(42.85%)	5(38.46%)	0.054	0.816
10.		*Crenosoma sp.*	6(42.85%)	0	7.163	0.016*

*= Fisher exact test accepted due to less than five expected value

4. Discussion and Conclusion

A total of 14 faecal samples of Red Panda and 41 samples of livestock were collected from the community forest of Ilam and examined by concentration methods. All the samples of both Red Panda and livestock were found to be positive either for protozoan or helminths. This prevalence rate of Red Panda was almost similar as compared to 93.02% reported in Red panda from Rara National Park (RNP) (Shrestha *et al.*, 2015) and 100% in Kothi Bhir community area (KBCA), Rolpa (Lama *et al.*,2015). But higher than the reports of Bertelsen *et al.*, (2010) and Pradhan *et al.*, (2011) which showed 35% and 46.66% parasitic infection from European zoo and Darjeeling, India respectively. The prevalence of gastrointestinal parasites of livestock in this study was similar with report of Bandyopadhyay *et al.*, (2010) who reveal 92.4% prevalence rate in India and the prevalence was higher as compared to 81.82%, 28.25%, 66.29% observed by Byanju *et al.*, (2011), Laha *et al.*, (2012) and shirale *et al.*, (2009) respectively. High prevalence of parasites in Red panda and livestock clearly indicates that the parasites shared between them probably due to sharing the same pasture area.

From the economic and sanitary point of view, coccidian parasites are the most prevalent among protozoa. Coccidian parasite infects large number of wild animals including Red Panda and Raccoons. *Eimeria* is the most common Coccidian parasites among wildlife and livestock. The prevalence of *Eimeria* with micropyle and without micropyle in Ilam almost similar with *Eimeria* reported from RNP (Shrestha *et al.*, 2015). High prevalence of *Eimeria* infection has been also reported from Raccoons of America (Dubey *et al.*, 2000, Wright and Gompper 2005, Dubey 1982, Foster *et al.*, 2004, and Adams *et al.,* 1981). Large number of livestock were also showen to be infected (Ntonifor *et al.*, 2013, Kanyari *et al.*, 2009, Swai *et al.*, 2006, Biu *et al.*, 2009). In the

present study, *Eimeria* was found to be 64.28% which was higher than 35% and 48% observed by Kanyari *et al.*,(2009) in sheep and goat respectively, 17.8% by Laha *et al.*, (2012) and 20.9% by Ntonifor *et al.*, (2013) and lower than 85.7% (Apio *et al.*, 2013) and 76.5% (Matsubayashi *et al.*, 2009).

Almost similar prevalence of *Eimeria* in Red Panda and livestock may be due to the herbivorous food habitat of Red Panda sharing the same grazing area with livestock. Other Coccidian parasites such as *Isospora sp.*, *Cryptosporidium sp.* and *Cyclospora sp.* have been reported from various other wild mammals including Raccoons (Taylor *et al.*, 2007) and Red Panda (Lama *et al.*, 2015). *Isospora* spp and *Cryptosporidium spp* are also absent in livestock.

Besides the Coccidian parasites, the Red Panda were found to be infected with two other protozoan parasites, *Entamoeba sp*. and *Balantidium sp*. Amoebic dysentery, an intestinal disease caused by infection with the protozoan parasite *Entamoeba*. spp. is an important disease of man and animals throughout the world. *Entamoeba sp*. had also been reported from Red Panda of RNP (Shrestha *et al.*, 2015). In both RNP and Ilam, Red Panda were infected with more than 50% by *Entamoeba species*. *Entamoeba* is causative agent of amoebiosis among livestock it has been reported from cattles of Kenya (Kanyari *et al.*, 2009). In the present study, this parasite is also reported in cattle with prevalence rate 21.95% which was found to be less than 87% and 77% in Sheep and Goat by Kanyari *et al.*, (2009) and 83% by Paul *et al.*, (2010) in cattle. The prevalence of *Entamoeba* in Red Panda was higher than livestock it was may be due to behavioral and genetic factors associated in Red Panda and livestock (Gillespie *et al.*, 2005).

Balantidium coli is the ciliate zoonotics protozoan parasites. Non-human primates have been considered the most important reservoirs for human infection (Walzer and Healy 1982; Nakauchi, 1999). *Balantidium sp*. has been reported from White-handed gibbon (*Hylobates lar*), squirrel monkey (*Saimiri sciurea*), Japanese macaque (*Macaca fuscata*), wild boar (*Sus scrofa*) and chimpanzee (*Pan troglodytes*) from Japan (Nakauchi 1999). This is the first case to report the *Balantidium sp.* in Red Panda in the global context with prevalence rate 14.28%. The prevalence of *Balantidium sp.* in livestock during the present study was lowest (7.31%) than other protozoans which is similar to Kanyari *et al.*, (2009) who reported 2% and 3% prevalence in sheep and goat, 1.6% and 6.6% in cattle by Uysal et al .(2009) and Paul *et al.*, (2010) respectively. *B. coli* was reported in pig by Ismail *et al.*, (2010) and Weng *et al.*, (2005) and observed 64.7% and 47% prevalence which was greater than prevalence of this study. The greater prevalence may be due to Many food or water resources can become polluted because pandas defecate while feeding (Zhang and Wei 2006).

The first report on isolation and maintenance of *B. coli* was done by Barrett and Yarbroug (1921) in animals. *B. coli* are a ciliated and a normal inhabitant of intestine of wild and domestic animals, probably capable of becoming somewhat pathogenic under favorable condition. It has been identified by Varadharajan and Kandasamy (2000) from India. The infection of *B. coli* may be due to the contamination of water or food with cyst in the grazing area (Schuster and Ramirez 2008).

Generally wild animals become infected with Nematode, Cestode and Trematode helminth parasites. To compared the life cycle of cestode and trematode, a suitable intermediate host is required but not for most of the nematode parasites. Interestingly, Red Panda of Ilam were found infected with only nematode parasites but livestock were infected by cestode and trematode too.

However the trematode, *Ogmocotyle ailuri* was previously described from Red Panda at a zoo in the America (Price 1954 and 1960). *O. ailuri* also isolated from the small intestine of Taiwanese monkey, Macaca cyclopis (Yoshimura *et al.*,1996) and Japanises monkey, Macaca fuscata (Iwaki *et al.*, 2012).

Another trematode, *Heterobiharzia americana* also recorded in Archer and Wichita countries of north contra Texa and overall prevalence was 47.2% (Kelley 2010) and other trematodes *Alaria sp*, *Digenea sp* and *Eurytrema procyonis* were observed in Raccoon (Wright and Gompper 2005). Prevalence of trematode were found 13% in Red Panda from KBCA (Lama *et al.*, 2015) but genera was unidentified. Absent of trematodes in present study might be due to absent of suitable intermediate host in Ilam. Trematode infection was most common among livestock (Bandyopadhaya *et al.*,, 2010, Yadav and Tando 1989, Byanju *et al.*, 2011).

Cestode infection in Red Panda (Lama *et al.*, 2015) had shown from KBCA similarly the Red Panda of RNP have been reported to be infected with Moniezia, a common herbivore cestode parasite but none of the Red Panda samples collected from Ilam were positive. However three genera of cestode had been reported from Raccoon in Archer and Wichita countries of North Central Taxas including *Atrioenia procyonis, Mesocestoides spp., Taenia pisiformis* (Kelley and Horner 2008). The cestode spp. described from cattle includes *Moniezia* (Horak *et al.*, 2004, Kanyari *et al.*, 2009, Laha *et al.*, 2012) and *Taenia* (Biu *et al.*, 2009). In the present study, only one cestode, *Moniezia* was recorded in cattle with prevalence rata 14% which was almost similar with 21% and 16% observed by Kanyari *et al.*, (2009) in sheep and goat 10% and 11% infection reported by Laha *et al.*,, (2012) and Farooq *et al.*,, (2012) in cattle and higher than 0.65% and 0.48% revealed by Rafiullah *et al.*, (2011) in male and female cattle respectively.

Altogether seven genera of nematodes in Red Panda and six genera of nematodes in livestock were observed from Ilam community forest. Among them *Oxyuris sp.* showed the 100% prevalence rate in Red Panda which was highest than 58.14% recorded by Shrestha *et al.*, (2015). The highest prevalence rate of *Oxyuris* may be due to the cool climate of the area. Cool climate is suitable for the development of *Oxyuris* larva. The parasite was observed equally high prevalence (87.8%) in livestock too.

Generally round worm of Red Panda are considered as *Balyascaris* but due to the indistinguishable shape and size, they are simply consider here as ascaris. *Balyascaris* is an important intestinal nematode of Red Panda as well as Raccoon. This parasite had been recorded from Spain in white-headed lemurs (*Eulemur albifrons*) (Martinez *et al.*,, 2015), North America (Kazacos, 2001) Germany (Bauer *et al.*, 2011). In this study the prevalence of ascaris was found 57.14% which was higher than 38.88% and 13.04% prevalence rate of *Balyascaris* reported in Red Panda from Rara National Park, Mugu, Nepal (Shrestha *et al.*,2015) and Kothi Bhir Community area, Rolpa, Nepal (Lama *et al.*,2015) respectively. *Balyascaris* is found in Red Panda, Gient Panda, Raccoon, Cat, Dog etc. Nematodes like, *B. procyonis, Capillaris acrophili, C. plica, C. procyonis, C. putorii and Placoconus lotoris* had been reported in Raccoons from Southern New york (Wright and Gompper 2005). Similarly, *B. procyonis* was also reported in Raccoons from Western North Carolina (Hernandez *et al.*, 2012). The highest prevalence rate were recorded from North-Eastern, mid-western ,mid-Atlantic, some western states (California, Washington, Oregon and Coloradol and some region of Taxas (Kazacos 2001, long et at. 2006, Chavez *et al.*, 2012). In more than 85% of cases Infections have no symptoms, especially if the number of worms is small. During the study, the prevalence of *Ascaris* in livestock was 57.14% which agree with 57% recorded by Awraris *et al.*, (2012) in Ethiopia and greater than 1.5%, 25.9%, 17.6%, 14.7% and 3.7% recorded by Chaoudhary *et al.*, (1993), Tomass *et al.*,(2013), Ismail *et al.*, (2010), Matsubayashi et al . (2009) and Uysal *et al.*, (2009). Almost equal prevalence rate was observed in between Red Panda and livestock but it is indistinguishable between *Ascaris* found in Red Panda and livestock.

During the study, *Trichostrongylus sp*. was recorded for first time from Red Panda in the global context. The prevalence of *Trichostrongylus sp.* and *Strongyloides sp.* in Red Panda was found 41.46% and 50% respectively. *Strongyloides sp*. had been recorded in Red Panda by Shrestha (2015) and prevalence was low compared to present study. The paraste was already recorded in American Raccoons from New york (Wright and Gompper 2005). In case of livestock, the parasite has been reported in Kenya (Kanyari *et al.*, 2009), India (Laha *et al.*, 2012), Pakistan (Rafiullah *et al.*, 2011), Tanzania (swai *et al.*,, 2006) and Nigeria (Bui *et al.*, 2009) with prevalence rate 13%, 25.13%, 4.21%, 20% and 11% respectively. The prevalence rate of *Strongyloides* sp. was higher (53.65%) as compare to all previous finding rate. The prevalence of *Trichostrongylus sp*. was found 41.46% which was higher than 13.83% and 16.24% infection recorded by Rafiullah *et al.*, (2011) in male and female cattle respectively and lower than 55.8% recorded in goat and higher than 28.8% and 9.7% observed in sheep and cattle by Ntonifor *et al.*, (2013). This result showed that Ilam community forest was highly contaminated with *Strongyloides* and *Trichostongyloides* eggs both from Red Panda and livestock.

Trichuris is another common nematode parasite prevalent in both wild and domestic animals. The parasite has been reported from RNP (Shrestha 2015) and KBCA (lama *et al.*, 2015). The prevalence of *Trichuris* was 42.85% which was higher than 4.65% and 26.08% recorded by Shrestha *et al.*, (2015) and Lama *et al.*, (2015) respectively. High worm load may cause growth retardation, anemia and hemorrhagic diarrhea (Hale and Stewart, 1979). *Trichuris spp*., have been infecting large number of livestock (Horak *et al.*, 2004, Yadav and Tendon 1989, Byanju *et al.*,, 2011, Kanyari *et al.*, 2009, Bui *et al.*,, 2009, Laha *et al.*, 2012, Rafiullah *et al.*, 2011). In the present study, *Trichuris sp.* (31.70%) was isolated from cattle. The prevalence of *Tichuris sp*. was comparatively higher than 2%, 13.08%, 5.27%, 2.6% recorded by Kanyari *et al.*, (2009), Laha *et al.*, (2012), Rafiullah *et al.*, (2011) and Bui *et al.*, (2009) respectively.

Crenosoma spp. is the Metastrongylus lungworm infecting wild and domesticated canids in Europe (Morgan *et al.*, 2005, Traversa *et al.*, 2010). Recently, emergence of this parasite was observed in several European countries (Traversa *et al.*, 2010) due to population increase and urbanization of Red foxes (*Vulpes vulpes*) (Deplazes *et al.*, 2004) which is the major reservoir hosts of this parasite in Europe. The prevalence of *Crenosoma* was found 42.85%, in Red Panda which was almost similar 34.88% by Shrestha *et al.*, 2015 Rara National Park, Mugu, Nepal and higher than 4.3% from European zoos (Bertelsen *et al.*,2010). Hook Worm infection has been reported from Red Panda of RNP (Shrestha *et al.*, 2015) with prevalence rate 44.19% which was almost similar with present study which revealed 35.7%. Hookworms are cosmopolitan in distribution (Bowman *et al.*,, 2003) and can be transmitted orally but also by cutaneous penetration and cause high mortality in animals and human (Hotez *et al.*, 2004). *Crenosoma spp* was not observed in cattle because it is the parasites of carnivore.

Angiostrongyloid vasorum was recorded in Red Panda from different countries. It is a most important lungworm which causes pathogenic pneumonia to Red Panda. *A. vasorum* was recorded from Denmark, U.K and European zoos by Bolt *et al.*,1992, Janet *et al.*, 2009, Bertelsen *et al.*, 2010) respectively. *Angiostrongylus sp* was also reported in Nepal (Lama *et al.*, 2015 and Shrestha 2015) but during this study *A. vasorum* was not recorded. *Aelurostrongyloid spp.* have been reported in carnivora by different researchers in global and national context. In the present study, none of these nematodes were isolated in the faecal matter of Red Panda of Ilam community forest, Nepal. Some reports have been indicated the presence of *Toxocara spp*, *Bunostomum spp*, *Haemonchus spp*, *Oesophagostomum spp*, *Cooperia spp*, *Ostertagia spp.* etc from cattle (Hoorak *et al.*, 2004, Bandyopadhyay *et al.*, 2010, Bilal *et al.*, 2009).

In conclusion, GIT parasites are major problems of animals in the study area. Therefore; comprehensive study on GIT parasites, cost effective strategic treatment and awareness creation to the farmers should be instituted and for the conservation of Red Panda grazing system of livestocks in the habitat of Red Panda should be stop to break the transmission processes.

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