

**Assessing the genetic status,
distribution, prey selection and
conservation issues of Himalayan
wolf (*Canis himalayensis*) in Trans-
Himalayan Dolpa, Nepal**

Samundra Ambuhang Subba

Nature Conservation
2012



Biology
Master's Degree Project
60 Higher Education Credits
Lund University

Status of Himalayan wolf in Trans-Himalayan Dolpa, Nepal

Himalayan wolf is one of the least known mammals and studies have confirmed that it is a genetically unique population drifted from the general wolf-dog group for quite some time ago. However the wolf of Nepal was still in ambiguity whether it is Himalayan wolf or Tibetan wolf of China and Northern Mongolia. The canine has also been known to inflict massive livestock depredation attributing retributive killings by the locals. In Nepal, no empirical studies had been implemented so far behind the reason of depredation and solid proof of its distribution in the Trans-Himalaya.

A baseline study was conducted investigating its genetic identity, distribution, prey choice and conflict issues with humans in a remote area in Dolpa, Nepal. The study on taxonomic status was carried out by aligning and comparing the DNA patterns obtained from wolf-scats in Dolpa with published sequences of Himalayan wolf and other known wolf sub species. These scats were also used for prey analysis by comparing the hair patterns obtained from them. The distribution pattern was developed through geographic modeling validated by the wolf signs recorded during the field survey. Lastly the conflict issues were assessed by conducting questionnaire surveys to the locals in the wolf dominated settlements.

The study revealed the wolf of Nepal to be closely associated with Himalayan wolf rather than Tibetan wolf, very much deviated from the general wolf-dog group. Furthermore, we could also suggest that it is a genetically unique population and possibly a species on its own isolated from the common gray wolf since quite some time. The distribution model predicts its occurrence around the upper Dolpo region characterized by alpine steppe and patches of shrublands. Preferably Himalayan wolf tend to occur around six major VDCs (Village Development Committees) of rainshadow upper Dolpo region viz. Bhijer and Dho (Study sites), Saldang, Charka, Tinje and Mukot. A serious conflict was observed between the local community and the wolf with wolf attributing mass livestock depredation more than snow leopard and almost half of the local population acknowledging to have been involved in retributive killings. The principal reasons behind this chaos were the marginal economy of the locals and wolf's diet shift as it preferred domestic ungulates rather than less abundant wild preys assessed from prey analysis. An instantaneous action of extensive researches and conservation campaigns is imperative to preserve this species accentuating its uniqueness and significant role in regulating sparse biodiversity of Himalayan ecosystem.

Advisor: **Richard Ottvall and Kashmira Kakati**
Degree Project 60 credits in Conservation Biology 2012
Department of Biology, Lund University

Abstract

Almost nothing is known about the Tibetan wolf (*Canis lupus chanco*) apart from opportunistic anecdotes, notes in journals and conflict with humans. Taxonomic status of this canine has not been investigated so far for Nepal's wild population to state it as Tibetan wolf or Himalayan wolf. Furthermore, persecution of the wolf has been a major issue in the Himalayan region without any detailed assessment of its prey selection and the reason behind its mass depredation on livestock. Moreover investigations of its occurrence and distribution in the Trans-Himalaya have not been properly documented. In an attempt to collect the baseline quantitative information needed to develop conservation strategies I investigated the wolf's distribution in the Dolpa region, Nepal, by the method of sign survey, interview and GIS modeling software. In addition a prey selection assessment was conducted by the method of non-invasive scat analysis through discerning prey items by studying and comparing the cuticular and medullary hair patterns supplemented by genetic analysis of the scat samples. A questionnaire interview was also conducted during the survey period interviewing local communities to assay the livestock depredation rate by wolf and the effect on their marginal economy attributing their attitude towards the predator. According to the result 76 signs of Himalayan wolf were discovered at study sites Bhijer, en route to Dho valley from Bhijer and Dho valley. The GIS distribution model suggests that Himalayan wolf is found to be distributed along the alpine steppe (68%) at Bhijer, Dho valley, Saldang, Tinje, Charka and Mukot. Two wolf scat samples were closely related to the Himalayan wolf (*Canis lupus himalayensis*). This genetically unique wolf has recently been described as distinct from the Tibetan wolf and found to occur in the Himalayan region. Horse was the principal prey (20.6% Frequency of Occurrence/item and 39.9% of Biomass consumption) among all preys and livestock constituted with 60.7% of frequency per item. In the period 2010-2011, a total of 906 livestock loss ($4.3 \pm$ SD 5.4 per household) were accounted to wolves with an annual loss of 11% attributing an economic livestock loss value of \$1440 per household. 59% of local people agreed that the wolf should be completely exterminated and in contrast 71% agreed on wolf preservation when promised of full compensation measures. Consequently 48% of local people agreed that they have directly/indirectly participated in wolf persecution (cub killings and poisoning).

Background

Wolves (*Canis lupus*) known as the father of dogs are one of the most resilient, intelligent predator that have adapted and flourished in a wide range of habitats from arctic tundra to the Arabian deserts (Mech & Boitani 2003). Tibetan wolf (*Canis lupus chanco*) a sub-species of gray wolf is found to be distributed across central Asia from Trans Himalayan region of India, Nepal and Tibet to the northern parts of Mongolia and Korean Peninsula (Pocock 1941). Recent genetic studies on wolves occurring in the Himalaya have suggested a genetically unique wolf population, also called the Himalayan wolf (*Canis himalayensis*). Together with the Indian wolf (*Canis indica*) they do not seem to belong to the general wolf-dog clade and have been suggested to be distinct species. However the samples examined were mainly collected from non-wild populations of zoological gardens (Agarwal et al 2007). Apart from a few museum specimens (Sharma et al 2004) there has been no specific studies done so far on wolves in Nepal investigating its taxonomic status - a distinct species or a sub-species (*Canis lupus chanco*) - and its phylogenetic status is therefore still obscure. We just know that Tibetan wolves exist in central Asia from anecdotes but nothing is known whether they really exist and are surviving in the Trans-Himalaya as there are no scientific researches to back up its presence. Furthermore, we do not know anything about its population status, ecology and behavior (Sharma et al, 2004). Only in Nanda Devi Biosphere Reserve, India, a written record stated the sighting of Tibetan wolves while conducting wildlife habitat interaction study in 2005-2006 (Bhattacharya & Sathyakumar 2010). In Nepal, no studies have been implemented so far on this canine and its occurrence, distribution, abundance and ecology is still basically unknown.

The Himalayan ecosystem has a sparsely distributed biodiversity with predators like the wolf, Himalayan and/or Tibetan wolf (*Canis himalayensis* & *Canis lupus chanco*), Himalayan black bear (*Ursus thibetanus*), lynx (*Lynx lynx*) and snow leopard (*Panthera uncia*) with very limited natural preys to predate (Mishra 1997; Namgail et al 2007; Bagchi & Mishra 2006). This situation is created by the interspersions of humans into the natural ecosystem along with their livestock interfering with the natural mode of things diminishing the chances of vulnerable wild prey to thrive and flourish. Therefore this disproportionate balance between wild preys and

livestock results in creation of conflicts between predators and local community (Mishra 1997). Tibetan wolves have been known to account for massive livestock depredation, even more than snow leopard, Eurasian lynx and Himalayan Black Bear (Xu et al 2007; Namgail et al 2007; Mishra 1997). In Laddakh, India the livestock losses incurred by Tibetan wolves accounted for 60% compared to 38% and 2% by snow leopard and Eurasian lynx respectively (Namgail et al 2007). They have also been known to kill children in Laddakh where once it was abundant and considered as a plague (Pocock 1941). In Finland, wolves have mainly induced fear and negative attitudes among the local people (Bisi et. al 2007). Communities living in Trans-Himalaya have marginal economy and are strongly dependent on their livestock; hence livestock depredation highly affects their economic condition (Oli et al 1994; Ikeda 2004). Consequently it triggers antagonism towards the predators and wolves suffer intense retaliatory killing where they have been known to be poisoned and hunted by people who are also rewarded for these actions (Namgail et al 2007). Generally, the possible den sites are located where the wolf cubs are exterminated to prevent livestock mortality (Namgail et al 2007).

In relation to relative change in abundance of available prey the diet of carnivore has been shifted (Mishra 1997). Wolves in particular are known to select and target prey which fulfills their biomass consumption per successful attack (Mech and Boitani 2003). Although referring from anecdotes about the prey of the Tibetan wild canine like marmots, woolly hare, pika and blue sheep, the possibility of outcome could be argued (Pocock 1941). In India and China wolf are the main concerned predators which are responsible for more livestock deaths than any other predator (Xu et al 2007; Namgail et al 2006). The ecology of this wolf regarding its selection of prey and its importance in the diet is still not examined in Nepal.

The inadequate scientific data on the rate of livestock depredation and the effects it implicates on local communities and the wolf itself has been a major concern to formulate strong conservation initiatives and effective resolution of heated conflicts. Furthermore we lack a basic understanding about the predator, its distribution in the Trans-Himalaya and choice of prey which will broaden the gap to discover this resilient but ignored predator and could unveil its real identity to rather regard as cardinal element of ecosystem than a pest. This study mainly

attempts to address this lack and assist the conservation biologists and policy makers to develop certain action plans. My objective was to assay the distribution pattern of Tibetan wolves in Trans Himalaya region of Dolpa (SPNP), Nepal by sign survey methods. In addition it also focuses on identifying the potential prey that wolves select by the method of scat analysis. Genetic analysis of the collected scat samples to assay its identity was also taken into consideration. Lastly it focuses on investigating possible human-wolf conflict in Upper Dolpo by examining the local attitudes, livestock depredation rate and monetary losses imposed by the predator.

Study Area

The study sites Bhijer and Dho Valley are the two VDCs (Village Development Committee) in Upper Dolpo region which lies inside the boundary of Shey Phoksundo National Park covering an area of 4904 km² (3555 km²-core zone; 1349 km²- buffer zone) (DNPWC 2006) It is the largest national park of Nepal encompassing most regions of Dolpa district and parts of Mugu District. It has also one of the remote and most difficult terrains which are entirely road less (Jackson 1996). Bhijer (29°25'57" North latitude and 82°57'27" East longitude) lies in the core zone of SPNP flanked by barren cliffs (Mount Kanjirowa) and glaciers in the north and north western region bordering Tibet and *Nengla Bhanjyang* (5368m) in the east bordering Saldang VDC. In the south, Bhijer VDC is the initial point (Shey Gumba) of Upper Dolpo from the trek route of Shey Phoksundo lake (south to north) crossing Mount Kangla (5360m); a physical boundary separating two different vegetation types. Lying in the rain shadow region the site is cold and semi-arid with occasional precipitation of rain on summer-autumn and heavy snowfall during long winter. Furthermore, the region is characterized by undulating alpine steppe with few scattered distribution of shrub lands and barren lands at an altitudinal range of 4200- 5300 meters. The locals in Bhijer are dominantly agro-pastoralists constituting 93 households and utilizing the whole grazing pastures for their livestock over many centuries. Only few people could speak the national dialect with the whole mass of Tibetan ethnicity speaking only Tibetan language.

Dho valley (29°10'35 North and 83°09'42" East) lies in the buffer zone of SPNP on the southeastern region of the park opened up by Thakchu river flowing all along the V-shaped valley. The study sites were conducted around the borders of the valley with altitude ranging from 4400-4900 meters. The climatic conditions and vegetation pattern corresponds more or less with Bhijer, however Dho valley has relatively dry weather patterns with series of heavy wind during day time. Consequently the area meets occasional precipitation of rainfall during late summer and autumn seasons. Steppes are dominant in the bordering hills with *Caragana sp* and *Lonicera sp* pervasive along the banks of river cutting the undulating hills. It is the largest village in Upper Dolpo with 230 households and the locals are mainly farmers, pastoralists, merchants and Buddhist monks. Majority of semi-arid grasslands are used for pastoralism.

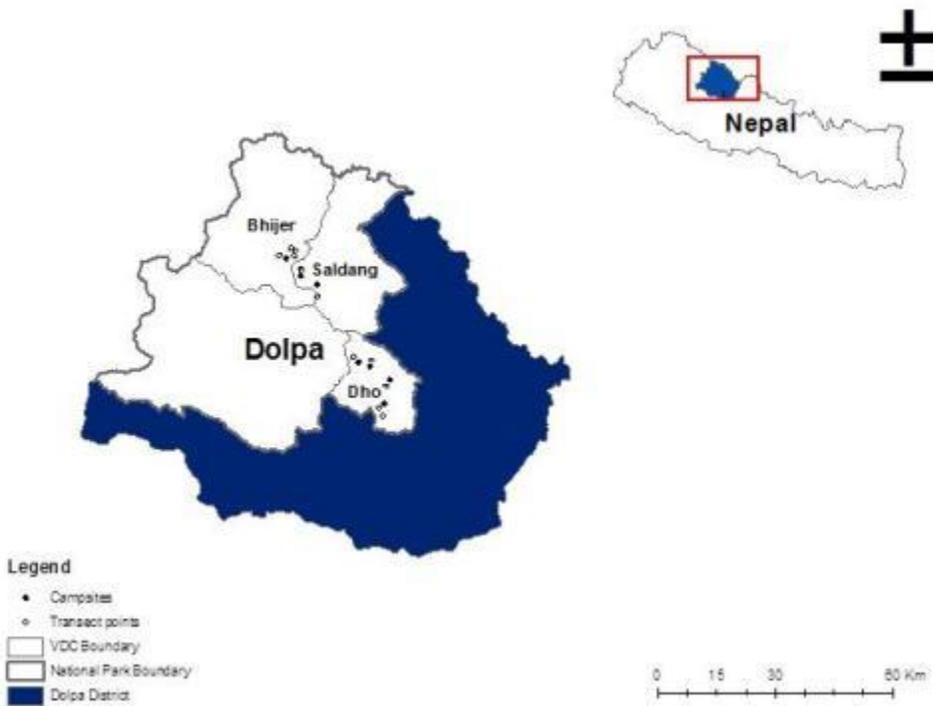


Figure 1: Map depicting the study sites (Bhijer and Dho) conducted inside the national park boundary where few campsites and transects were conducted enroute to Dho when crossing Saldang VDC

Shrubs growing in these regions were *Caragana gerardiana* and its other species, *Lonicera sp* and *Artemisia sp* (Bhijer), *Juniperus indicus*, *Rhododendron sp* and *Abelia triflora* (DNPWC 2006). Herbivorous mammals were Blue sheep (*Pseudois nayaur*), Himalayan marmot (*Marmota himalayensis*), Woolly hare (*Lepus oiostolus*) and Pika (*Ochotona sp*) in this alpine

steppe habitat. The main predators of this ecosystem are Tibetan wolf (*Canis lupus chanco*)/Himalayan wolf (*Canis himalayensis*) and occasional spatial variation with snow leopard (*Panthera uncia*) and moderate probability of Eurasian lynx (*Lynx lynx*). Among the bird species Griffon vulture (*Gyps fulvus*) and ravens (*Corvus corax tibetanus*) were the scavengers of the Himalayan ecosystem with also some galliformes like Chukar partridge (*Alectoris chukar*) and Himalayan snowcock (*Tetrogallus himalayensis*).

Materials and Methods

Taxonomic status by genetic analyses

Genetic identification of Himalayan wolf was carried out in Central Molecular Dynamics Nepal (CMDN), Kathmandu. 20 scat samples were collected from the field and were stored in centrifuge tubes of 50ml as per the protocol of the lab. Ethanol was used as a disinfectant agent which was poured over the chunk of scat with 90% of total tube volume. Out of 20 samples 17 samples were chosen for DNA extraction while the rest were considered unfit due to contamination. Qiagen QIAamp DNA extraction tool's kit was used and followed the protocol developed for snow leopard and common leopard scats. Since this method was implemented for the first time in Nepal exact protocol for the species' DNA extraction couldn't be achieved prior to extraction.

Mitochondrial DNA amplification was carried out focusing on the hypervariable control region and cytochrome *b* sequences. 3 sets of primers were used for control region amplification L16462-H222, MITFOR-MITREV and Lang4F/4R primer pairs. Former primers consisted of a 339 bp fragment of the control region at position 15745-16124 of the mitochondria, mid primers consisted of 192 bp at position 15525-15716 and the latter consisted of a 458 bp fragment at position 15402-15860 (Pilot et al 2010). Universal primers L14724- H15149 were used for amplifying regions of cytochrome *b* consisting of 500 bp fragment (Irwin et al 1991). The primers were provided by Grimso Research Station (SLU), Sweden.

Finally the PCR products were sequenced in ABI PRISM 310 Genetic Analyzer using forward primers and BLASTed in GenBank (<http://ncbi.nlm.nih.gov>) (National Center for Biotechnology Information, U.S National Library of Medicine).

Distribution

Several species of the Himalayan ecosystem are very difficult to spot and capture alive due to difficult access routes and wide geographical terrains having relatively low abundance of animals (Jackson et al 2005). Indirect sign survey and camera trap methods are therefore the best approach in studying these Himalayan species (Jackson et al 2005; McCarthy et al 2008). To begin with, discussions with Nepal's biologists and conservationists, National Park's officials, SLCC members and local people were conducted to locate the ideal study site of the wolf in Dolpa, its prey, habit and habitat. Based on these discussions, Bhijer and Dho valley were selected to conduct an indirect signs survey. A total of 13 transects were walked by foot between July-October 2011, total length of 55.5 km and an average of 4.3 km/transect at elevations ranging from 4200 to 5200m (Table 1). According to the SLCC members and local communities the ideal movements of wolves are around stationed corrals, ridgelines of grassy hills, livestock grazing pastures and foot trails. Thus, the survey was conducted accordingly and campsites were stationed beside the corrals. All 13 transects were initiated by reaching the upper ridgelines of the adjacent hills beside the corrals.

Indirect signs such as pugmarks, scats and prey's carcass were recorded by Garmin (eTrex Vista HCx) GPS device. GPS location, elevation, vegetation type and distance to nearest settlement were recorded to assess the wolves' altitudinal range, habitat type and correlation with settlements. Scent marks were very difficult to identify as the areas were very windy with very few patches of bush grasses (probable scent mark spots). Each pugmark, scat and its age were identified by the help of an expert SLCC ranger, including rocky surfaces and was measured with a measuring scale. The carcasses of the wolf were identified by the bite marks on the prey's hind legs and information through local people. Each scat was collected in a plastic bag for lab analysis recording its age and the type of surface where it was found.

Direct sign survey was also conducted along with the indirect sign survey by opportunistic spotting and broadening the possibility by choosing the routes chosen by the wolf for movement. We also used a method of howling on all transects after reaching an observation point on the ridges and scanned the landscape thoroughly with binocular lens of 400mm.

Data analysis

I used SPSS Statistics 17 to analyze the correlation between wolf occurrences and distance to nearest settlement supplemented by G.P.S field data. The potential distribution area of wolf was designed by GIS modeling dividing the distribution into two zones (Usual wolf range and Occasional wolf range). The geodatabase of Dolpa district were downloaded from ICIMOD and WWF library. Four parameters were used to assess the distribution pattern: Vegetation, Elevation, Distance to nearest settlement and questionnaire interviews with locals. The analysis was further supplemented by the indirect signs survey and direct sign survey. The zones were divided according to two criteria using spatial analyst tool in ArcGIS software 9.3. Criterion 1: Areas which meet all the suitable parameters viz., vegetation (grassland), Elevation (4100-5200 m), distance to nearest settlement/corrals (0.5-8 km) were selected as the usual wolf range. Criterion 2: Areas which meet only the vegetation and elevation range were selected as occasional wolf range. The vegetation type, elevation range and distance to settlements were estimated according to the study done in the field and through discussions with local people. Wolf signs and ideal confirmation of its occurrence by questionnaires were also incorporated into the model to further strengthen the distribution pattern.

Prey Selection

Prey assessments of carnivores are most commonly done by a non-invasive approach of diet analysis through scats collected in the field (Chavez & Gese 2005; Sabrina 2006; Spaulding et al 2000; Jackson 1996; Anwar et al 2011; Chundawat & Rawat 1994). Himalayan ecosystems have wide geographical terrains and low diversity of wild animals, not to mention the hostile physical environment and difficult access routes (Jackson 1996). Diet analysis by scats is the only effective method to examine the predator's food habits in the Trans-Himalaya. Since no study have been attempted so far on Tibetan wolves' prey analysis I followed the method of Jackson

(1996), Oli (1993) and Anwar (2011) on snow leopard's scat analysis which shares the same geographical area having substantial prey species in common. Identification of predated species of Himalayan ecosystem is done by the analysis of hairs available on the scats. Bones, hoofs and feathers are considered to be of little value since no references have been prepared so far for these items (Oli 1993).

The prey analysis of Himalayan wolf was assessed by random collection of scats during the transect survey. The scats were selected by expert rangers who happen to be the local inhabitants of wolf afflicted area and are very much aware of the canine's movement and behavior. Moreover the alpine grassland rarely invites other predators (snow leopard and Eurasian lynx) dominated by Himalayan wolf. The collected scats were dried in the sun for 48 hours and their length, circumference and weight were measured. Then the dried scats were crushed followed by separation of unwanted bones, plant materials and stones from the hairs adhered with other substances of scats. Subsequently, scats were placed in the beaker and washed with ethanol to avoid contamination. After that, the samples were washed in tap water on a mesh sieve of 100 μm separating the hair tufts from the unwanted excreted substances with the help of glass rod and tweezers. The samples were then dried in the incubator at 60°C for 12-24 straight hours.

The scat samples were cleaned with ethanol before examining the hairs. Henceforth 20 hairs were selected randomly from each scat sample and their necessary cuticular scale patterns and medullary portions were studied under a binocular microscope (Geotech BMS 2008) with 400x and 640x magnifications. The slides were prepared according to Sahajpal et al (2008) as their methods were simpler and materials were readily accessible. For studying the cuticular scale patterns a saturated gelatin solution with distilled water was prepared. Then a thin film of the solution was placed over the microscopic glass slide. Immediately, the hair samples were mounted over the film and left to dry for an hour. Longer hairs were cut into two pieces and placed adjacently with each other. Finally the hairs were gently plucked out from the slides and observed under the microscope. To make the observation easier and quicker I drew a borderline around the hair impressions. For studying the medullary portions the hairs were

placed on paraffin wax and cut into smaller pieces (0.5 cm) with a cutting blade and dipped in xylene overnight. After dipping in xylene the cross sections were obtained and viewed under the microscope for comparisons. The patterns were then compared with the reference collections gathered from the field and photographic slides prepared by Oli (1993).

As recommended by Klare et al (2011) and mentioned in Sabrina (2006) I calculated both biomass and frequency based methods. The frequency based method illustrates how often a prey is eaten and the biomass calculation method shows the importance of the prey in the predator's diet (Sabrina 2006). Frequency of occurrence/scat (F%/S) is the frequency percentage of prey item occurring in the whole scat sample and frequency of occurrence/items (F%/I) is the item frequency occurred in the whole items. Moreover Whole Scat Equivalent (WSE) was calculated for each prey items by assigning its value according to the percentage of prey contents found in the scat. Henceforth biomass consumption and biomass percentage was calculated by using Weaver's equation for gray wolves ($y = 0.439 + 0.008x$, $y =$ biomass consumed per scat; $x =$ assumed live weight of the prey) (Weaver 1993). The assumed live weight of the prey's and livestock were obtained from Anwar et al (2011).

Questionnaire survey

The attitude towards wolves among people living within wolf habitats was investigated by a questionnaire. Two villages, Bhijer and Dho VDCs were selected for the interview in August 2010. Random samples of 31 out of 93 households were interviewed for Bhijer and 46 out of 230 households for Dho valley representing $1/3^{\text{rd}}$ and $1/5^{\text{th}}$ of Bhijer and Dho respectively. People from houses, stationed corrals (*goaths*) and Tibetan monasteries were selected for the interview.

Most of the villagers at Bhijer were pastoralists and farmers while at Dho there were even businessmen running hotels and trading goods. The Nepalese government has introduced 5% compensation towards snow leopard killings but since the villagers have to travel 3-7 days to report their losses it would be very difficult to assess the cause of death. Moreover villagers usually give up as a 5% compensation measure is considered not to be worth the efforts of reporting the losses. No compensation schemes are introduced in the villages of Bhijer and

Dho, therefore there were no livestock depredation records available. Rate of livestock depredation were instead assessed by interviewing the villagers of their livestock quantity and livestock lost due to wolves and snow leopards. Species and age of the livestock lost by wolves were recorded.

In Nepal the principle reason behind retributive killing of predators are mainly due to economic losses implicated by wolves, snow leopard and Eurasian lynx (Oli et al 1994; Jackson 1996; Ikeda 2004). Attitudes of local people towards the wolf were recorded at four different levels on the basis of compensation percentages (100, 50, 5 and 0).

Results

Genetic analyses

Out of 17 scats 8 scats produced successful DNA nucleotides while repeating the DNA extraction process twice and PCR of the mitochondrial portion thrice. The rest could have undergone degeneration or unavailability of suitable wolf DNAs. From the 8 successful samples two samples tested positive of having wolf sequences when blasted with primers MITFOR/MITREV and L16462-H222. Only one sample tested positive with wolf sequences in cytochrome *b* region while the last primer Lang4F/4R was unsuccessful for blast operation as all samples appeared to be domestic dog.

Of the two scat samples sequences of about 600 bp of the control region and one sequence of about 300 bp of the cytochrome *b* were successfully sorted out. These sequences were imported to the software BioEdit and manually edited and aligned with a selection of other wolf sequences imported from GenBank. The alignment comprised of about 330 bp of the control region and 300 bp of the cytochrome *b*, but no valuable information was lost due to the shortening of sequences. The two wolf sequences from this study were identical and therefore one haplotype was used in the analyses. When running a phylogenetic tree in the software MEGA 5.0 the haplotype from Upper Dolpo clustered in the group of haplotypes of the Himalayan Wolf. This cluster is well separated from other wolf haplotypes (Figure 2). Also a 300 bp cytochrome *b* sequence from this study was closely related to Himalayan Wolf (Figure 3).

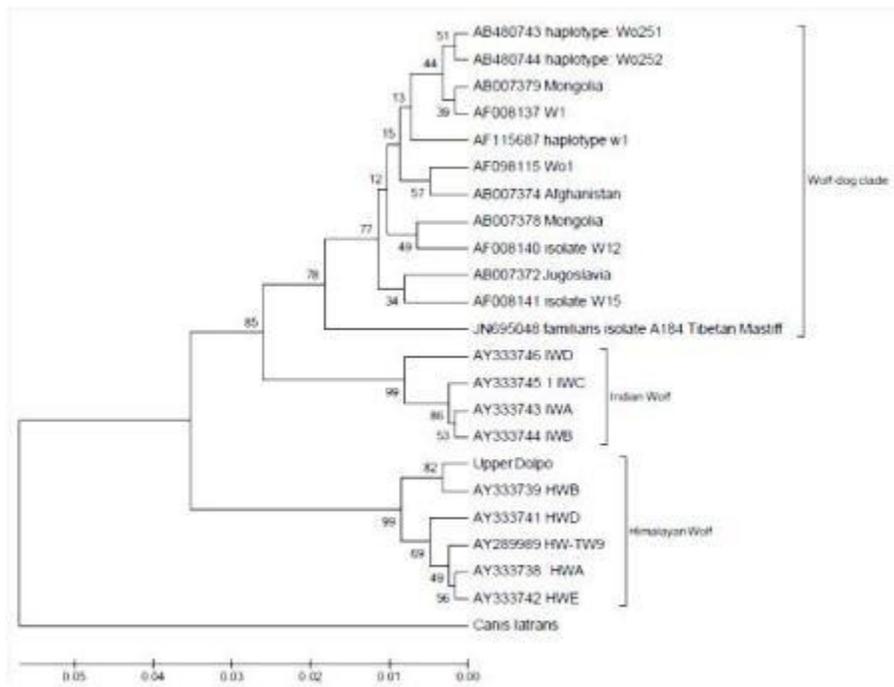


Figure 2. A UPGMA tree based on 500 bootstraps (software MEGA 5.0) of about 330 bp of the control region. The sequence from this study is called Upper Dolpo.

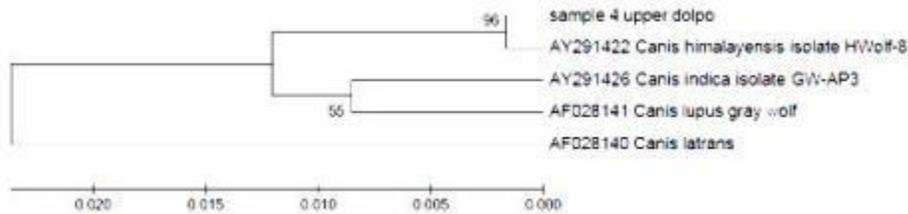


Figure 3. A UPGMA tree based on 500 bootstraps (software MEGA 5.0) of about 300 bp of the cytochrome *b*. The sequence from this study is called Upper Dolpo.

Distribution

During our transect surveys wolves were spotted three times in Bhijer and once in Dho valley. All wolves were spotted on the ridgeline of grassland hills. The method of howling failed to

produce any result as the wolves apparently were completely unresponsive towards our impersonating howl.

Indirect wolf signs (n=76) were found on all transects. During the survey 68% of wolf signs were observed in grassland habitat with 17% and 15% of signs found in barren land and shrub land respectively. In addition 65% of wolf scats were discovered on the foot trails followed by 33% on bush grasses of ridgelines and 2% on grazing pastures. These signs were observed at an elevation range of 4200-5200 meters. The correlation between wolf occurrence and distance to nearest settlement appeared insignificant (P value= 0.13; R square= 0.3; Slope= -0.2). However the density of indirect and direct signs decreased gradually as the distance increased from the settlement. A maximum of 22 signs were recorded on the range of 2-3 km from the nearest village. Three pugmarks of wolves were successfully recorded with a mean size of 13X10 cm.

Table 1: Himalayan wolf signs recorded in Dolpa region at Bhijer, en route from Bhijer to Dho (Saldang) and Dho valley.

Area	Transect	Live spot	Scat	Pugmark	Prey kill	Total	(%)
Bhijer	6	3	35	4	2	44	58
En route to Dho	1		6	3		9	12
Dho	6	1	17		5	23	30
Total	13	4	58	7	7	76	100

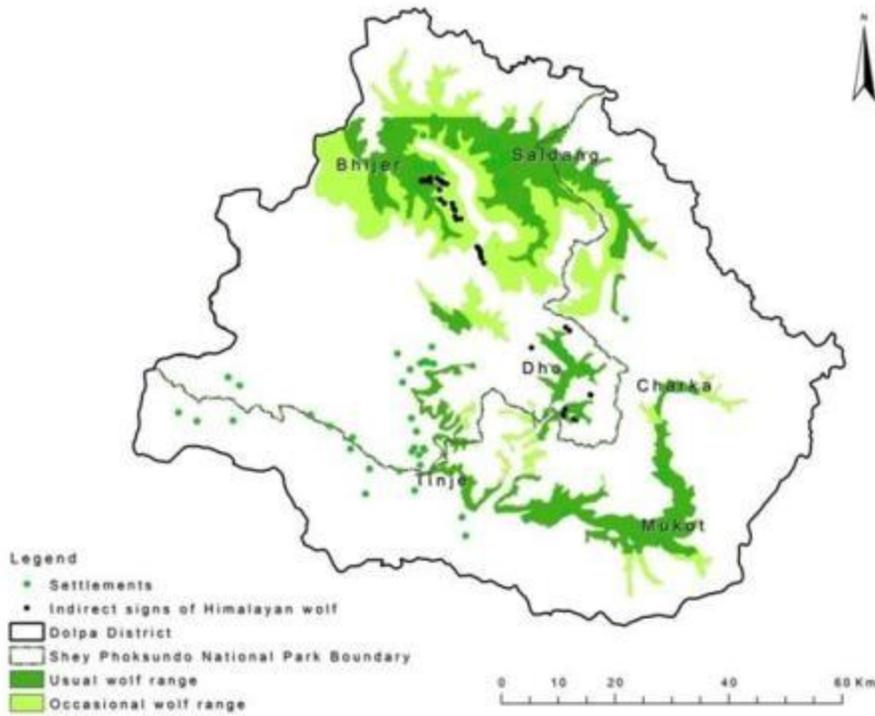


Figure 4: Predicted autumn distribution of Himalayan wolf in Trans-himalayan Dolpa district in six major VDCs labeled in the map. Areas representing usual wolf range are shown in dark green color and areas with occasional wolf range are shown in light green color.

The distribution model of Himalayan wolf in Dolpa district attempts to highlight the most likely regions where they can be occurring (Figure 4). Apparently Himalayan wolves were distributed in the Upper Dolpo region which lies in the rain shadow area of alpine steppe vegetation with no single tree. The usual (core) zone in dark green color is distributed along the six main VDCs of Upper Dolpo having similar livestock composition and more or less similar livelihood. The occasional wolf range in light green are those ranges which have the same vegetation type and elevation range. However since wolf movements were generally observed around/near stationed corrals/settlements (sky green) this region could be chosen by wolves for other modes of behavioral activity such as denning, breeding, resting and territorial markings. These regions in Bhijar and Dho were less preferred by shepherds to station their corrals and are used only for travel purpose. According to the questionnaire 99% of local people have seen the Himalayan wolf in their lifetime and 48% of these people have spotted the wolf more than once in 2010-2011. The local people who have seen the Himalayan wolf in their lifetime reported

that wolves are known to occur in 6 main VDCs (Bhijer, Dho, Saldang, Charka, Tinje and Mukot). Apparently these VDCs are in the usual wolf range in the designed distribution map (Figure 4).

Prey Selection

During the survey we collected 20 wolf scats from Bhijer and Dho valley out of which 17 were suitable for diet analysis with the remaining 3 got degraded. The genetic analysis confirmed only two scat samples with positive ID of Himalayan wolf. Two samples collected near the village on the bank of a river were tested positive with dog (Tibetan mastiff) while the other 15 samples were collected from ridgelines of alpine grassland. These 15 samples were assumed to come from wolves. Thus only 15 samples were used for prey assessment.

According to the prey analysis, Himalayan wolf's diet comprised domestic goat, sheep, yak, dzo, horse, Himalayan marmot, pika, blue sheep and woolly hare (Table 2). Horse was the predominant prey with 26.7 % of F%/S and 22.9 % of F%/I. Wild preys constituted only about 34.7 % and 29.8% of F%/S and F%/I respectively in contrast to livestock attributing 65.3 % and 56.2 % of F%/S and F%/I (Figure 5). According to biomass estimation, horse escalated among others as the preferred prey attributing 42.3 % of biomass consumption followed by Yak (26.8 %) and goat (6.6 %) compared to wild blue sheep (6.6 %) and Himalayan marmot (6 %) (Table 2). Heavier preys were the most preferred prey with 91.7 % of biomass consumption of which 93.3% are domestic species.

Table 2: Biomass estimate and frequency of occurrence per scat and item consumed by Himalayan wolf in Trans Himalaya Dolpa, Nepal, ($Y=0.439+0.008X$; $B= Z \times Y$)

Prey	Assumed live weight (X)	Biomass consumed per scat (Y)	WSE (Z)	Biomass consumed (B)	Biomass consumption (%)	No. of items found (n)	F%/S	F%/I
Domestic								
Horse	225	2.2	4	9	42.3	80	26.7	22.9
Yak	300	2.8	2	5.7	26.8	40	13.3	11.5
Dzo	140	1.6	1	1.6	7.4	20	6.7	5.7
Goat	25	0.6	2.2	1.4	6.6	44	14.7	12.6
Sheep	30	0.7	0.6	0.4	1.9	12	4	3.4
Wild								
Blue sheep	65	1	1.5	1.4	6.6	29	9.7	8.3
Himalayan marmot	4.5	0.5	2.9	1.4	6.4	57	19	16.3
Pika	0.2	0.4	0.7	0.3	1.5	14	4.7	4
Woolly hare	1	0.4	0.2	0.1	0.4	4	1.3	1
Plant materials	-	-	-	-	-	14	4.7	4
Unidentified remains	-	-	-	-	-	35	11.7	10
Total	790.7	10.3	15	21.1	100	349	116.3	100

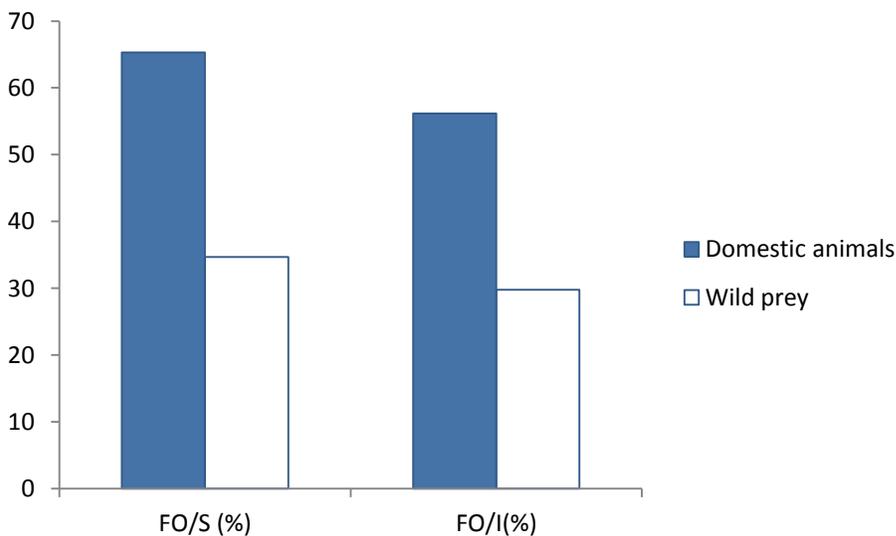


Figure 5: Frequency of occurrence per scat and item of wolf's diet between domesticated livestock and natural prey.

On the period of our survey (July-October 2011) we came across 5 wolf kills, all horses, and remaining 5 were reported from locals. Of the reported ones 3 domestic goat and 1 sheep were killed during the same day at the same time. Only 3 out of 10 wolf kills were from Dho valley.

Conflict with local community and their attitudes

323 households of Bhijer and Dho hold a total of 7569 livestock with an average of 33 (± 21.0) livestock per household. Mass of livestock comprised of goat and sheep (68%), followed by yak (20%), horse (8%) and dzomo cow/yak-dzomo hybrid (4%). In the two villages the number of alleged livestock predated by Himalayan wolf in 2009-2010 was accounted to be 906 which attributed to an annual loss of 11%. The number of livestock predated varied significantly between Bhijer and Dho ($\chi^2 = 53.95$; $df = 3$; $P < 0.001$). Bhijer comprised 64% of reported deaths although household size and population were smaller than Dho valley. An average of 4.3 (SD ± 5.4) livestock was lost per household with maximum number of goat and sheep (65%) were recorded to be predated by wolf. However, according to their respective livestock proportion, wolves relatively prey on more horses (18%) and lesser dzomo cows (2%) than other domestic species ($\chi^2 = 67.76$; $df = 3$; $p < 0.001$).

Table 3: Livestock surplus with livestock losses to Himalayan wolf in the year 2010 of two VDCs in Shey Phoksundo National Park, Dolpo

	Livestock quantity					No. of Livestock lost				
	Goat	Yak	Horse	Dzomo	Total	Goat	Yak	Horse	Dzomo	Total
VDC										
Bhijer	2424	459	153	63	3099	420	93	57	6	576
Dho	2745	1025	470	230	4470	170	80	80	0	330
Total	5169	1484	623	293	7569	590	173	137	6	906
%	68	20	8	4		65	19	15	1	
	Livestock quantity per household (S.D)					Loss of livestock per household (S.D)				
	Goat	Yak	Horse	Dzomo	Total	Goat	Yak	Horse	Dzomo	Total
Bhijer	28.9 (± 17.2)	5.5 (± 3.4)	1.7 (± 0.7)	0.8 (± 1.6)	36.7	5 (± 5.2)	1.1 (± 1.2)	0.6 (± 0.7)	0.1 (± 0.3)	6.8
Dho	18 (± 18.5)	7.1 (± 5.2)	2.1 (± 1.6)	2.3 (± 1.6)	36.9	1 (± 2.4)	0.6 (± 1.2)	0.4 (± 0.7)	0	2.1
Total	23.7 (± 18.2)	6.3 (± 4.5)	1.9 (± 1.3)	1.2 (± 3.5)	33.1	3 (± 4.4)	0.8 (± 1.2)	0.4 (± 0.7)	0.1 (± 0.2)	4.3

Table 4: Annual monetary losses incurred by Himalayan wolf per household at Bhijer and Dho VDCs of Upper Dolpo, Nepal

Monetary loss (US Dollar)					
Village	Goat/Sheep	Yak	Horse	Dzomo Cow	Total
Bhijer	623.4	690.2	764.2	44.5	2122.4
Dho	146.2	344	464	0	954.1
Upper Dolpa	374.1	505.2	539.2	21.5	1439.9

* Figures indicate loss of livestock value per household in US Dollars at an exchange rate of 1\$= 80.2 NRs

Himalayan wolf attributed to the highest number of livestock depredation (71%) followed by snow leopard (29%). No depredation was attributed to Eurasian lynx (0%). The estimated monetary loss incurred by Himalayan wolf was approximately \$ 356,000 and \$ 1440 per household in 2010. 7 % of the total livestock value was significantly affected by the wolf with more monetary losses at Bhijer (\$ 2122 or 18%) excessively higher than Dho (\$ 954 or 8%) per household (Table 4). Although goat and sheep were predated more than other livestock, horses and yak attributed a higher loss of value each amounting approximately \$ 540 and \$ 505 per household (Table 4).

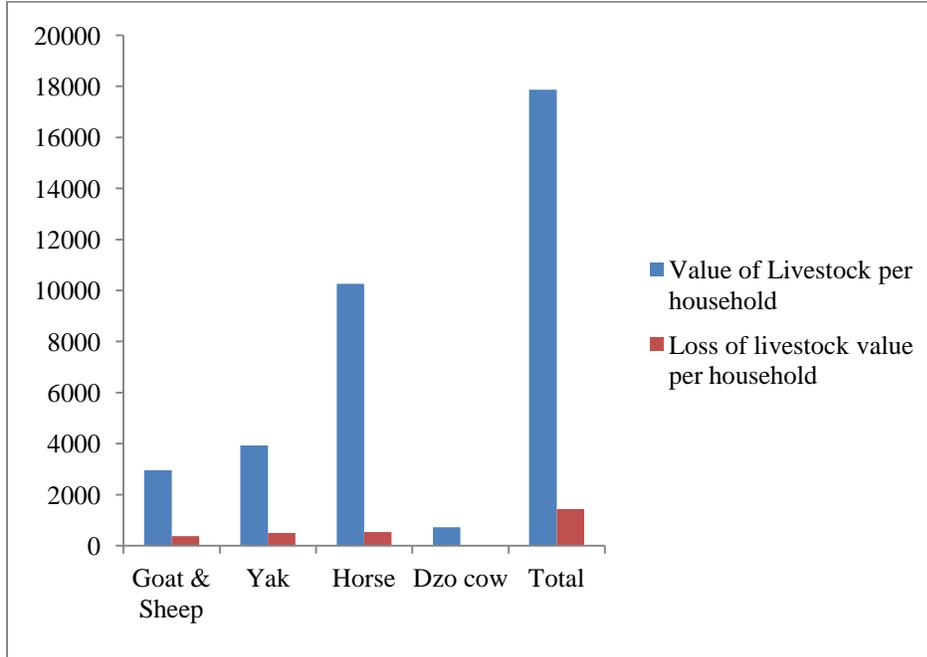


Figure 6. Value of livestock in comparison with loss of livestock value incurred by Himalayan wolf in US\$.

Poaching in Upper Dolpo according to the questionnaires was unanimously unsupported and 96% of the total respondents haven't sighted any incidents of illegal killings. The only threat to Himalayan wolf is considered to be retaliatory killing by the local people with 46% of the total respondents agreeing that they have directly/indirectly participated in hunting wolf cubs during breeding season and sometimes brought to villages to collect ransoms from local people as a reward of exterminating their most bothersome pest.

The attitudes of local people toward the wolf was dominantly negative as 59% agreed that it should be completely exterminated from Dolpa with only 35% agreeing that it should be conserved. Those people who agreed that the wolf should be conserved were mostly businessmen, farmers, Buddhist monks and livestock herders who had not reported any of their livestock predated this year. The attitudes of local people varied significantly between Bhijer and Dho valley ($\chi^2= 7.15, df= 2, P<0.05$) with 68% acknowledging the wolf should be eradicated at Bhijer and 52% at Dho . However when interviewed by mentioning a full compensation of their livestock losses the attitude changed drastically with 71% of local people with positive attitude and 24% negative (Figure 7).

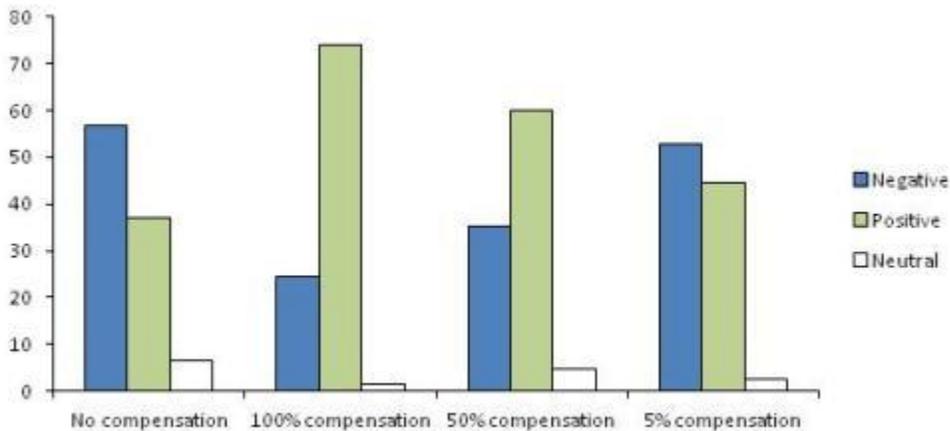


Figure 5: Attitudes of Local people towards Himalayan wolf in Upper Dolpo with respect to amount of compensation

Management of Domestic mammals in Upper Dolpo

99% of local people are pastoralists in Bhijer and 54% in Dho valley. Management of domesticated mammals is similar in all regions of Upper Dolpo. The method is still conventional and ineffective towards the ambush predator snow leopard and resilient Himalayan wolf. In the early spring when the cold and harsh winter ends, the villagers assemble their whole livestock into one single collective herd and lead them in nearby stationed corrals. Only selected representatives of the whole village live in the camped corrals built with stones and clothed roofs. This system is followed by rotational shifting from one available pasture to the next until winter arrives. In the early morning, the livestock are led by the herder into the pastures and left them to graze unguarded freely during the day. The animals are then brought to the stationed corrals and goats are kept inside a round shaped corral built by stones of 1-2 feet high. Yaks and Dzo cows are generally left around the corrals. Tibetan mastiffs are chained beside the stone built corral during the night to guard and alert the livestock herders against any threat. However in some stationed corrals, the dogs are unfettered during the night and chained during the day time.

Horse is the only mode of human carrying transportation which is tied during the night near their individual houses or sometimes in corrals or self built camps when locals are travelling. However during the day it is left open to graze and is most prone to be attacked by predators as it constantly moves alone to the far ridges and slopes for greener pastures. It is also the most expensive property compared to livestock and almost every villager possesses them.

During late autumn (September-October), the villagers collect firewood and fodder from adjacent hills (mostly shrubs and bushes). At Dho valley, villagers use Yak to load and carry firewood and fodders from subalpine and temperate forests in the south which are easier to access in terms of cost and time. Progressively, they assemble their livestock and bring them to the village where each household with their respective stock moves them inside the inbuilt pens of their houses and thus stores and feed them with the collected fodders until the harsh freezing winter passes away. Horses are also kept within the houses. However, yaks are left open around the village during winter as they are well adapted to the severe climate conditions

posing an easy prey for the predators. Moreover the houses with pens on the ground floor are built only for goats/sheep and horses.

Discussion

Genetic analysis

The result clearly reveals that the canine found in Upper Dolpo is Himalayan wolf (*Canis himalayensis*), distinct from the common gray wolf (*Canis lupus*). With 99% grouping of Himalayan wolf alone and more or less of 6% divergence from the general wolf-dog clade suggests that it is a genetically unique group which have been isolated from the common wolf by quite some time. Himalayan wolf have been known to occur in western trans-himalayan region of India (Agarwal et al 2007) and thus from this analysis we can confirm that both wolf found in Nepal and India are similar distributed over homogeneous trans-himalayan landscapes, climate and vegetation types. Agarwal (2007) have also hypothesized that this species is one of the oldest wolf lineages and the Indian subcontinent could be the centre of wolf evolution. Fossil records of gray wolves have also indicated that the wolves of North America migrated from Eurasia over 500 thousand years ago (Weckworth et al 2010). With two samples predicting the origin of wolves and its radiation to other sub species is difficult, however with further more extensive analysis of substantial samples tracing of wolf lineages could be initiated.

Universal primer of cytochrome *b* L14724- H15149 was ineffective in producing viable wolf sequences. This could be due to the primer being non-species/genera specific as it could fragment and amplify other species' DNA available in the scats (prey and plant materials). Lang4F/4R produced sequences positive to domestic dog (*Canis lupus familiaris*) even of the two positive wolf samples. It could be due to a shorter bp fragment which amplifies the portion of control region less variant to wolf-dog clade. Although only two wolf samples were analyzed in this study it is very intriguing to understand that the two/single wolves of Nepal belongs outside of the general wolf-dog clade and are a genetically unique population or possibly even a distinct species.

Distribution

According to the GIS modeling, the distribution of Himalayan wolf ranged from north western Bhijer to south eastern Mukot covering six main VDCs of Dolpa district. Apparently all these regions lie in Upper Dolpo where the vegetations are characterized by dry alpine grasslands, barren lands and shrub lands distinct from rain shadow coniferous regions of lower Dolpo. Although sign survey was not implemented at Saldang, Tinje, Charka and Mukot, these regions were incorporated in the distribution pattern by extrapolating the data recorded at Bhijer and Dho. These regions have similar vegetation pattern (grasslands), elevation range, livestock diversity and livestock management system. A detailed raster map of elevation was unavailable defining only layers of regions with an elevation gradient. Consequently some of the signs recorded in the field lied outside the peripheral distribution range. In addition the range could be more precise and clear with a comprehensive geodatabase and field data of the whole range.

In the course of this study we verified from wolf sign locations that wolf prefer grassland habitat and choose the ridgelines and foot trails for movement. However, a glance overview of these regions apprehends mostly grassland pastures with scattered regions of few barren land and shrub land. From the genetic analysis wolf tend to prefer patches of bush grasses on ridgelines to defecate their feces and could also be a behavior of territorial marking as the habitat is characterized by periodic heavy wind and precipitation where urine marking could not only be the sole way to defend their territory. Moreover Mech and Boitani (2003) have stated that gray wolves choose a conspicuous object to defecate their faeces in order to mark their territory. Tibetan wolves appeared to be more active around the settlements/corrals in Dolpa than away from it. This could be due to disproportionate balance between wild and domestic ungulates which is not uncommon in these regions.

During our study period we spotted only blue sheep, in total an estimated 90-100 individuals as the medium sized natural prey and most of the grazing pastures have been occupied by livestock with stationed corrals only couple of kilometers away. Additionally wolves prefer denning sites away from human habitation in afflicted regions. Therefore on July – October

season wolf pups are generally 4-5 months old, and are capable of travelling with their parents to hunt (Mech & Boitani 2003). A survey during the winter period when the environment is too hostile could open up our understanding towards its occurrence and movement more in depth. According to the locals wolves tend to wonder around the village even more closely than in summer as the shepherds move their livestock inside their homes and keep them until the winter passes in April-May. However yaks are left open in the snow as they are adapted to survive in harsh winter conditions.

Prey Selection

Horse (*Equus ferus caballus*) was the most preferred prey both in terms of frequency of occurrence and biomass consumption. It was also the most predated domestic animal with respect to proportion size from the questionnaire analysis. Likewise in India, horse was the most frequent animal to be predated by snow leopard and Tibetan wolves (Mishra 1997; Namgail et al 2004). Horse is one of the least managed domestic animals in Upper Dolpo, Nepal which fulfills all the criteria to be selected by wolf as principal prey. Horse is used as a medium of transport for people travelling around the Himalayan terrain and is generally left free during the day and often gets lured by the lush grasses in the wild increasing its chances of being devoured by wolf.

Livestock (Yak, Dzo Cow, Goat and Sheep) were the most consumed mammals compared to few natural preys available. It is not surprising to see that wolves prey more upon domestic preys than natural preys. We have accounted from many researches in trans-Himalaya about the low abundance of natural preys and poor livestock management techniques resulting depredation by snow leopard (Anwar 2011; Mishra 1997; Jackson 1996; Ole et al 1993) and Himalayan wolf (Namgail et al 2007). Nowell and Jackson (1996) suggested that these predators attack on livestock because they are incompetent to escape quickly or defend themselves against wild predators. However, this might not be the only reason why wolf attack them in Trans-Himalaya regions. Researchers have affirmed that wolves prefer medium to large sized ungulates in their diet which saves them fuel and time (Meriggi et al 1996; Sabrina 2006). Furthermore, Wolves are also known to attack on prey which is easier to locate and have the largest biomass on each

successful attack (Sabrina 2006). All domesticated mammals in Dolpa are medium to large sized animals unlike natural preys where blue sheep are the only medium sized prey. Since there is a probable misbalance between livestock and wild prey abundance wolf select and attack what the ecosystem holds. The reason could also be attributed towards the traditional system of livestock management.

Smaller preys such as marmots, hare, pika and birds are of seasonal importance (Mech and Boitani 2003). Since this study was carried out in autumn, it would be interesting to implement further researches during the winter period. As mentioned earlier about locals securing the livestock (goats, sheep and horses) inside their pens built under houses, diet composition of the canine could be contradicting from the analysis done in this study. In addition villagers have stressed about their loss of yak to wolves in winter. For a robust analysis of diet composition the sample size should be at least more than 130 (Trites & Joy 2005; Klare 2011). I was fortunate to get most animals in my small sample analysis but it might not always be the case. Hence the diet analysis will be much better with larger sample size and further study during the winter period giving a stronger assessment and variation of prey selection seasonally. However, Dolpa is one of the most remote and isolated Trans-Himalayan regions of Nepal (Jackson 1996) and therefore with limited time and resources I could only collect available scats but I hope in the future projects researchers will choose to survey during April-May which is the best time to study Himalayan species and are well equipped with available resources and diligent attitude to conduct in winter season as well.

Conflict issue

One of the most serious threats to wild predators in Himalayan ecosystem is the conflict with humans for one principle reason i.e. livestock depredation (Oli et al 1993; Mishra et al 2004; Ikeda 2004; Xu et al 2007; Bagchi & Mishra 2005; Namgail et al 2007). The findings from my study implicated a significant effect on the marginal economy of the local villagers threatened by Himalayan wolf. Compared to Namgail et al (2007) of 4% livestock predated by Himalayan wolf in India my study revealed 11%. As mentioned in Mishra (2004) and Oli et al (1993), locals do tend to exaggerate the actual number of killings due to the antagonism created by the

events. However interpreting the data and the number of kills observed during the survey, the rate of wolf depredation is high. I estimated monetary loss by Himalayan wolf with respect to livestock proportion value and it can be interpreted from the result that it is one of the critical reasons to agitate hostility between pastoralists and the canine. This is evident from the result where the attitudes of local people changed drastically when interviewed about the full compensation measures.

We have encountered from many studies on snow leopard's conflict issue with humans and the consequences it brings for the charismatic feline (Xu et al 2007; Oli et al 1993; Namgail 2007). However we tend to forget the fact that Himalayan wolf is the main responsible predator attributing maximum livestock deaths than snow leopard, Himalayan black bear and Eurasian lynx (Namgail et al 2007; Mishra 2004). As a consequence the wolf is in a serious threat more than any other Himalayan predator resulting from retributive killing. To be more clear, I have compared the past depredation losses imposed by Himalayan wolf and snow leopard including the attitudes of local people towards them in Nepal, India and China (Table 5).

Table 5: Comparison of two main predators (snow leopard and Himalayan wolf) on livestock depredation rate with local perceptions done in different regions of Trans-Himalaya

Categories	Oli et al (1993)	Ikeda (2004)	Bagchi & Mishra (2005)	Xu et al (2007)	Namgail et al (2007)	Current study	
Study period	1989-1990	1998-2001	2002-2003	2006	2001-2003	2010-2011	
Study Area	Manang district (ACA) Nepal	Taplejung District (KCA), Nepal	Kiber, Spiti, India	Pin Valley, Spiti India	Gouli, Region, China	Laddakh, India	Dolpa, Nepal
Predator species	Snow leopard	Snow leopard	Snow leopard	Snow leopard	Snow leopard	Himalayan wolf	Himalayan wolf
Households with livestock lost (%)	34	56	43	41	4	n/a	71
Livestock lost per household	2	2.2	1.1	0.6	n/a	2.8	4.3

Monetary loss/household (\$)		56	168	n/a	n/a	n/a	190*	1440
Positive attitude (%)		n/a	n/a	22	5	n/a	n/a	35
Negative attitude (%)		n/a	n/a	30	45	n/a	n/a	59

*indicates the value lost by all predators combined

The table attempts to compare the effects of snow leopard and Himalayan wolf towards livestock and eventually the local people. Although the studies were conducted in different periods and could differ little with certain parameters (abundance of natural prey, livestock quantity, livelihoods), we can see that Himalayan wolf inflicts maximum number of livestock deaths inducing the cause for locals to retaliate. In Gouli region, China 85% believed that wolf was the main predator of livestock rather than snow leopard, although the livestock loss by wolves was not estimated in that particular study (Xu et al 2007). Furthermore Namgail et al (2007) affirmed that livestock depredation is caused more by Himalayan wolf (60%) than Snow leopard (38%) which was more or less similar with my study; Himalayan wolf (71%) and snow leopard (29%). Thus Himalayan wolf is facing more persecution in Upper Dolpo with 48% agreeing that they have participated in wolf-cubs killing, which is evident even in India (Mishra 1997, Namgail et al 2004).

The number of livestock predated varied significantly between Bhijer and Dho with lower depredation rate at Dho. As a result, the attitudes were also significantly different with more positive attitudes at Dho valley. The livestock management techniques are similar in both VDCs, however 99% of local inhabitants at Bhijer are pastoralists with 54% at Dho. Dho valley is the most urban village in Upper Dolpo and is considered to be a place of trade, business and commerce. The attitudes towards the wolf have probably changed due to the change of livelihood (business, trade or farming) and education. A local hotel businessman said that, people believe that it is impossible to exterminate Himalayan wolf as they have attempted a lot in the past in many ways (poisoning and cub killing) and now they have compromised their medium of income exchange with other profitable occupations available. It would be

interesting to study the relative abundance of natural preys in Dho valley with other remote villages as Dho could have more grazing pastures available for wild ungulates attributing relatively higher abundance. This could be a factor in reducing wolf-human conflict.

Conflict management

Observing the situation in Upper Dolpo with distinct attitudes between two areas, change of livelihood from animal husbandry to other kind of income generating activities could perhaps alter the negative perception towards the wolf. In Taplejung (KCA), Nepal WWF Nepal has engaged local people in various types of income generating programs like sewing, accounting and kitchen gardening (WWF Nepal 2001). In the long run this would enhance their socio-economic conditions and the ecosystem as well, allowing enough space for wild ungulates to populate and restore the wolf's natural prey numbers in order. However this is only possible over a long period of time. In specific perspective, improved livestock management techniques, compensation measures and incentive strategies will help to mitigate the conflict. In Upper Dolpo, domestic animals are most vulnerable during the daytime which they are frequently attacked. Rigg et al (2009) conducted a research in Slovakia to lessen the depredation rate by introducing guarding dogs which resulted as the most effective method and stressed that it is one of the best ways to safeguard economic losses and in areas of unfenced pastures. With appropriate training and breeding Tibetan mastiffs which guards the corral during night could be a successful initiative in protecting livestock during day time. Apparently a national newspaper article had published the similar story about the ability of Tibetan mastiffs in Jumla (Kantipur 2012).

This campaign will be most effective if the whole community and other stakeholders take the responsibility and lead from the very beginning for a successful conservation approach (Jackson and Wangchuk 2004; WWF Nepal 2001). In Nepal, conservation campaigns have been most successful with community involvement (e.g KCA) and therefore with technical help/education and initiative funds from donors/NGOs/government the ecosystem could be restored in near future.

Conclusion

We now know that the wolf which was assumed to be Tibetan wolf (*Canis lupus chanco/Canis lupus laniger*) appeared to be completely different relative of wolf and dog known as Himalayan wolf (*Canis himalayensis*). We also know that Himalayan wolf is distributed over the alpine grasslands of Dolpa and is threatened by the ecological imbalance created by the interspersed distribution of humans and its livestock. Further research on its abundance and ecology is critical to understand the species in depth and quick conservation initiative is imperative as Dolpa is probably the hot spot area for Himalayan wolf in Nepal. Meanwhile only one wolf-core region (Bhijer) lies inside the boundary of Shey Phoksundo National Park core zone while all (Charka, Tinje, Mukot and Dho-Buffer Zone) lies outside. The status of this unique wolf and the whole natural ecosystem is in jeopardy which could be alleviated with synergetic action of researches and conservation campaigns. Furthermore, this species has the right to be enlisted in the higher conservation valuable species as it is unique and could also be the ancestor of all the wolf lineages.

References

- Agarwal, R.K., Kivisild, T., Ramadevi, J., Singh, L. 2007. Mitochondrial DNA coding region sequences support the phylogenetic distinction of two Indian wolf species. *J Zool Syst Evol Res* 45: 163-172.
- Anwar, M.B., Jackson, R., Nadeem, M.S., Janecka, J.E., Hussain, S., Beg, M.A., Muhammad, G., Qayyum, M. 2011. Food habits of snow leopard *Panthera uncial* (Schreber, 1775) in Baltistan, Northern Pakistan. *Eur J Wildl Res* 57: 1077-1083.
- Bagchi, S., Mishra, C. 2006. Living with large carnivores: predation on livestock by the snow leopard (*Uncia uncia*) *Journal of Zoology* 268: 217-224.
- Bhattacharya, T., Sathyakumar, S. 2010. Sighting of Tibetan wolf (*Canis lupus chanco*) in the Greater Himalayan range of Nanda Devi Biosphere Reserve, Uttarakhand, India: a new record. *Journal of Threatened Taxa* 2(12): 1345-1348.
- Bisi, J., Kurki, S., Svensberg, M., Liukkonen, T. 2007. Human dimensions of wolf (*Canis lupus*) conflicts in Finland. *Eur J Wildl Res* 53:304-314.

- Chavez, A.S., Gese, E.M. 2005. Food Habits of Wolves in Relation to Livestock Depredations in Northwestern Minnesota. *The American Midland Naturalist* 154(1): 253-263.
- Chavez, A.S., Gese, E.M., Krannich, R.S. 2005. Attitudes of Rural Landowners toward wolves in Northwestern Minnesota. *Wildlife Society Bulletin* 33(2):517-527.
- Chundawatt, R.S. & Rawat, G.S. 1994. Indian cold deserts: a status report on biodiversity. Wildlife Institute of India, Dehradun, Uttaranchal.
- Ciucci, P., Boitani, L. 1998. Wolf and Dog Depredation on Livestock in Central Italy. *Wildlife Society Bulletin* 26(3): 504-514.
- Ikeda, N. 2004. Economic impacts of livestock depredation by snow leopard *Uncia uncia* in the Kanchenjunga Conservation Area, Nepal Himalaya. *Environmental Conservation* 31(4): 322-330.
- Irwin, D., Kocher, T., Wilson, A. 1991. Evolution of the Cytochrome *b* Gene of Mammals. *Journal of Molecular Evolution* 32(2): 128-144.
- Jackson, R.M., Roe, J.D., Wangchuk, R., Hunter, D.O. 2005. Surveying Snow Leopard Populations with emphasis on camera trapping: A Handbook. The Snow Leopard Conservancy, California.
- Jackson, R.M., Wangchuk, R. 2004. A Community-Based Approach to Mitigating Livestock Depredation by Snow leopards. *Human Dimensions of Wildlife* 9: 307-315.
- Jackson, R.M., Ahlborn, G.G., Gurung, M., Ale, S. 1996. Reducing livestock depredation losses in the Nepalese Himalaya. In: Timm, R.M & A.C. Crabbs, (Eds), *Proceedings of the 17th Vertebrate Pest Conference*, University of California, Davis, California pp. 241-247
- Jackson, R.M. 1996. Home range, movements and habitat use of Snow leopard (*Uncia uncia*) in Nepal. Ph.D thesis. University of London (External Programme), London, U.K, 233 pages.
- Jedrzejewski, W., Jedrzejewska, B., Okarma, H., Schmidt, K., Zub, K., Musiani, M. 2000. Prey selection and Predation by wolves in Bialowieza Primeval Forest, Poland. *Journal of Mammalogy* 81(1):197-212.
- Kantipur. 2012. A Tibetan mastiff of Jumla. Kantipur publications pp 3.
- Klare, U., Kamler, J.F., Macdonald, D.W. 2011. A comparison and critique of different scat-analysis methods for determining carnivore diet. *Mammal Review* 41(4): 294-312.
- Kubarsepp, M., Valdmann, H. 2003. Winter Diet and movements of Wolf (*Canis lupus*) in Alampedja Nature Reserve, Estonia. *Acta Zoologica Lituonica* 13(1): 28-33.

- Liberg, O., Andren, H., Pedersen, H., Sand, H., Sejberg, D., Wabakken, P., Akesson, M., Bensch, S. 2005. Severe inbreeding depression in a wild wolf (*Canis lupus*) population. *Biol. Lett* 1:17-20
- Llaneza, L., Ordiz, A., Palacios, V., Uzal, A. 2005. Monitoring wolf populations using howling points combined with sign survey transects. *Wildl. Biol. Pract* 1(2): 108-117
- Maheshwari, A., Sharma, D. 2010. Snow leopard Conservation in Uttarakhand and Himachal Pradesh. WWF-India, New Delhi.
- Marucco, F., Pletscher, D.H., Boitani, L. 2008. Accuracy of scat sampling for carnivore diet analysis: wolves in the Alps as a case study. *Journal of Mammalogy* 89(3): 665-673.
- McCarthy, K.P., Fuller, T.K., Ming, M., McCarthy, T.M., Waits, L., Jumabaev, K. 2008. Assessing estimators of Snow leopard abundance. *Journal of Wildlife Management* 72(8):1826-1833.
- Mech, L.D & Boitani, L. 2003. *Wolves: Behavior, Ecology and Conservation*. The University of Chicago Press, Chicago.
- Meriggi, A., Brangi, A., Matteucci, C., Sacchi, O. 1996. The feeding habits of wolves in relation to large prey availability in northern Italy. *Ecography* 19(3): 287-295
- Mishra, C., Allen, P., McCarthy, T., Madhusudan, M.D., Bayarjargal, A., Prins, H.H.T. 2003. The Role of Incentive Programs in Conserving the Snow Leopard. *Conservation Biology* 17(6): 1512-1520.
- Mishra, C. 1997. Livestock depredation by large carnivores in the Indian trans-Himalaya: conflict perceptions and conservation prospects. *Environmental Conservation* 24(4): 338-343.
- Muhly, T.B., Musiani, M. 2009. Livestock depredation by wolves and the ranching economy in the Northwestern U.S. *Ecological Economics* 68:2439-2450.
- Namgail, T., Fox, J.L., Bhatnagar, Y.V. 2007. Carnivore- Caused Livestock Mortality in Trans-Himalaya. *Environ Manage* 39:490-496.
- Neff, R. 2007. Devils in the Darkness. *Ohmynews*.
- Nowak, R.M. 2002. The Original Status of Wolves in Eastern North America. *Southeastern Naturalist* 1(2): 95-130.
- Oli, M.K., Taylor, I.R., Rogers, M.E. 1994. Snow leopard *Panthera uncia* Predation of Livestock: An assessment of local perceptions in the Annapurna conservation area, Nepal. *Biological conservation* 68:63-68.

Oli, M.K. 1993. A key for the identification of the hair of mammals of a snow leopard (*Panthera uncia*) habitat in Nepal. The Zoological Society of London 231: 71-93.

Oli, M.K., Taylor, I.R., Rogers, M.E. 1993. Snow leopard *Panthera uncia* Predation of livestock: An assessment of local perceptions in the Annapurna Conservation Area, Nepal. Biological Conservation 68: 63-68.

Olsen, S.J., Olsen, J.W. 1977. The Chinese wolf, Ancestor of New world dogs. Science 197(4303):533-535.

Pandey, M.R., Chetri, M. 2005. Nomads and Pastoralism: Linkage with Biodiversity Conservation in Upper Mustang, Nepal. Out Nature 3:42-49.

Petersen, U.M. 2011. Insular and disjunct distribution of the Arctic wolf in Greenland, 1978-1998. Polar Biol 34: 1447-1454.

Pilot, M., Branicki, W., Jedrejewski, W., Goszczynski, J., Jedrzejevska, B., Dykyy, I., Shkvrya, M., Tsingarska, E. 2010. Phylogeographic history of grey wolves in Europe. BMC Evolutionary Biology 10: 104.

Pocock, R.I. 1941. Fauna of British India: Mammals Volume 2. Taylor and Francis, London. Sharma, D.K., Maldonado, J.E., Jhala, Y.V., Fleischer, R.C. 2004. Ancient wolf lineages in India. The Royal Society 271:S1-S4.

Proffitt, K.M., Gude, J.A., Hamlin, K.L., Garrott, R.A., Cunningham, J.A., Grigg, J.L. 2011. Elk distribution and spatial overlap with livestock during the brucellosis transmission risk period. Journal of Applied Ecology 48: 471- 478.

Rigg, R., Findo, S., Wechselberger, M., Gorman, M.L., Zubiri, C.S., Macdonald, D.W. 2009. Mitigating carnivore-livestock conflict in Europe: lessons from Slovakia. Fauna & Flora International, Oryx 45(2): 272- 280.

Sabrina, M. 2006. Diet composition of wolves (*Canis lupus*) on the Scandinavian peninsula determined by scat analysis. Master thesis. School of Forest Science and Resource Management, Technical University of Munich, Munich.

Sahajpal, V., Goyal, S.P., Jayapal, R., Yoganand, K., Thakar, M.K. 2007. Hair characteristics of four Indian bear species. Science and Justice 48:8-15.

Sharma, D.K., Maldonado, J.E., Jhala, Y.V., Fleischer, R.C. 2004. Ancient wolf lineages in India. Proceedings: Royal Society London Biological Science (Supplement) 271: S1-S4.

Spaulding, R., Krausman, P.R., Ballard, W.B. 2000. Observer bias and analysis of Gray wolf diets from scats. Wildlife society Bulletin 28(4):947-950.

- Srivastav, A. and Nigam, P. 2009. National Studbook of Tibetan wolf (*Canis lupus chanco*). Wildlife Institute of India, Dehradun and Central Zoo Authority, New Delhi.
- Trites, A.W., Joy, R. Dietary Analysis from fecal samples: How many scats are enough? *Journal of Mammalogy* 86(4): 704-712.
- Wayne, F. W. 2008. The lost wolves of Japan. *The Journal of Japanese studies* 34(1): 230-233 (Review)
- Weaver, J.L. 1993. Refining the Equation for Interpreting Prey Occurrence in Gray Wolf Scats. *The Journal of Wildlife Management* 57(3): 534-538.
- Weaver, J.L., Hoffman, S.W. 1979. Differential Detectability of Rodents in Coyote Scats. *The Journal of Wildlife Management* 43(3): 783-786.
- Weckworth, B.V., Talbot, S.L., Cook, J.A. 2010. Phylogeography of wolves (*Canis lupus*) in the Pacific Northwest. *Journal of Mammalogy* 91(2): 363-375.
- Zabel, A., Holm-Muller, K. 2007. Conservation performance payments for carnivore conservation in Sweden. *Conservation Biology* 22(2):247-251.
- WWF Nepal. 2001. Kanchenjunga Conservation Area Project: Annual Technical Progress Report July 1, 2000- June 30, 2001. Unpublished report. WWF Nepal Program. Kathmandu, Nepal: 32pp
- Xu, A., Jiang, Z., Li, C., Guo, J., Da, S., Cui, Q., Yu, S., W, G. 2007. Status and conservation of the snow leopard *Panthera uncia* in the Gouli Region, Kunlun Mountains, China. *Fauna & Flora International, Oryx* 42(3): 460-463
- Encyclopedia Britannica 2012. Mammal: Blue sheep
 [<http://www.britannica.com/ludwig.lub.lu.se/EBchecked/topic/1486485/blue-sheep>] Accessed May 11th 2012.
- Department of National Parks and Wildlife Conservation. 2006. National Parks:Shey Phoksundo National Park
 [<http://dnpwc.gov.np/national-parks-shey.asp>] Accessed May 5th, 2011.
- IUCN. 2010. IUCN Red list of threatened species: Mammals
 [<http://www.iucnredlist.org/initiatives/mammals>] Accessed April 18th 2011.

Acknowledgement

I would like to thank my supervisors Dr. Richard Ottvall and Dr Kashmira Kakati for their invaluable support and technical advices. I sincerely express my gratitude to Rufford Funds for Nature and Lund University for the financial support without which this project couldn't have been possible. I would also like to thank my senior advisers Prof Jan- Ake Nilsson, Dr. Ghana Gurung, Dr. Mikael Akesson, Mr. Kamal Thapa, Mr. Gokarna J. Thapa for believing in me and guiding through the whole research period. I greatly appreciate the support of Snow Leopard Conservation Committee President, Dawa Lama, my assistants Yuming Nembang and Sneha Pandey and my rangers for all the field support. I am very grateful to CMDN laboratory: Miss Priya Joshi, Dibesh Kishor Bajracharya and the hardworking staffs for carrying out the genetic analysis. Finally I express my deep gratitude to my family and God for helping me in lots of ways.

Photographs of the research study

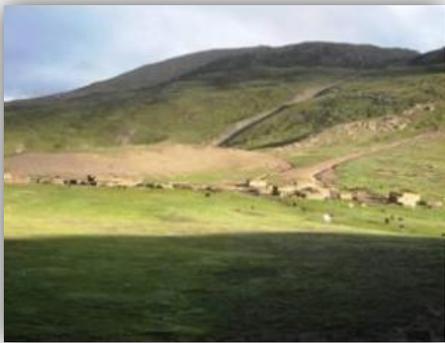


Plate 1: Campsite at Bhijer's corral



Plate 2: Campsite at Thakse's corral (Dho VDC)



Plate 3: Two Himalayan wolves on the ridgeline, adjacent to Bhijer's corral



Plate 4: Pugmark of Himalayan wolf en route to Dho valley



Plate 5: Scat sample 1 of the wolf confirmed by genetic analysis at Tata village (Bhijer VDC) near horse's carcass



Plate 6: Another confirmed scat sample 2 of the wolf on the ridgelines of Bhijer's corral



Plate 7: Scat sample collected on the centrifuge tube for mitochondrial DNA analysis at Bhijer



Plate 8: Measuring the pugmark of Himalayan wolf on the ridgeline of Kaltyak (Bhijer VDC).



Plate 9: Recent wolf kill (3-5 days) of a horse around Thakse village (Dho VDC)



Plate 10: Domestic goats and sheep guarded by stone built corral at Thakse 'corral (Dho VDC).



Plate 11: Questionnaire interviews to local people at Bhijer VDC

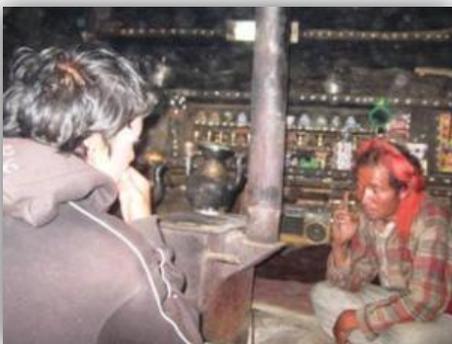


Plate 12: Questionnaire interviews with local pastoralist and farmer at Kalang village (Dho VDC).