Prevalence of intestinal parasites in small ruminants and their sensitivity to treatments with ethnobotanical remedies in Cholistan, Pakistan

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HH	Households
LF	Livestock farmers
LH	Local healers
MVM	Modern veterinary medicine
EVM	Ethno-veterinary medicine
MR	Mixed remedies (EVM + MVM)
LA	Large animal (cattle, buffalo, camel, horse)
SA	Small animal (sheep, goat)
G	Goat
S	Sheep
N	Nematodes
T	Trematodes
C	Cestodes
PBS	Phosphate buffered saline
A	Aqueous extract
M	Methanol extract
AM	Aqueous-methanol extract

Dedication to

The sweet memories of my grandparent (late),

My Parents

&

My loved ones

Your sacrifices, patience, love and understanding will always be cherished.

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Summary

Livestock production contributes substantially to the livelihoods of poor rural farmers in Pakistan; strengthening pastoral communities plays an imperative role in the country's thrive for poverty alleviation. Intestinal helminths constitute a major threat for pastoral livestock keepers in the whole country because chronic infestation leads to distinct losses in livestock productivity, particularly the growth of young animals. Synthetic anthelmintics have long been considered the only effective way of controlling this problem but high prices, side effects and chemical residues/toxicity problems, or development of resistance, lead to their very limited use in many pastoral systems. Additionally, poor pastoralists in remote areas of Pakistan hardly have access to appropriate anthelmintic drugs, which are also relatively expensive due to the long routes of transportation.

The search for new and more sustainable ways of supporting livestock keepers in remote areas has given rise to studies of ethno-botanicals or traditional plant-based remedies to be used in livestock health care. Plant-based remedies are cheap or free of cost, environmentally safe and generally create no problem of drug resistance; they thus might substitute allopathic drugs. Furthermore, these remedies are easily available in remote areas and simple to prepare and/or administer.

Cholistan desert is a quite poor region of Pakistan and the majority of its inhabitants are practicing a nomadic life. The region's total livestock population (1.29 million heads) is almost twice that of the human population. Livestock husbandry is the primordial occupation of the communities and traditionally wealth assessment was based on the number of animals, especially goats and sheep, owned by an individual. Fortunately, about 60% of this desert region is richly endowed with highly adapted grasses, shrubs and trees. This natural flora has a rich heritage of scientifically unexplored botanical pharmacopoeia.

Against this background, the present research project that was conducted under the umbrella of the International Center for Development and Decent Work at Kassel University, focused on a development aspect: in the Cholistan desert region it was firstly examined how pastoralists manage their livestock, which major health problems they face for the different animal species, and which of the naturally occurring plants they use for the treatment of animal diseases (Chapter 2). For this purpose, a baseline survey was carried out across five locations in Cholistan, using a structured questionnaire to collect data from 100 livestock farmers (LF) and 20 local healers (LH).

Most of LF and LH were illiterate (66%; 70%). On average, LH had larger herds (109 animals) than LF (85 animals) and were more experienced in livestock husbandry and management. On average LF spent about 163 Euro per year on the treatment of their livestock, with a huge variability in expenditures. Eighty-six traditional remedies based on 64 plants belonging to 43 families were used. Capparaceae was the botanical family with the largest number of species used (4), followed by Chenopodiaceae, Poaceae, Solanaceae and Zygophyllaceae (3). The plants *Capparis decidua* (n=55 mentions), *Salsola foetida* (n=52), *Suaeda fruticosa* (n=46), *Haloxylon salicornicum* (n=42) and *Haloxylon recurvum* (n=39) were said to be most effective against the infestations with gastrointestinal parasites. Aerial parts (43%), leaves (26%), fruits (9%), seeds and seed oils (9%) were the plant parts frequently used for preparation of remedies, while flowers, roots, bulbs and pods were less frequently used (<5%). Common preparations were decoction, jaggery and ball drench; oral drug administration was very common. There was some variation in the doses used for different animal species depending on age, size and physical condition of the animal and severity of the disease.

In a second step the regionally most prevalent gastrointestinal parasites of sheep and goats were determined (Chapter 3) in 500 animals per species randomly chosen from pastoral herds across the previously studied five localities. Standard parasitological techniques were applied to identify the parasites in faecal samples manually collected at the rectum. Overall helminth prevalence was 78.1% across the 1000 animals; pure nematode infestations were most prevalent (37.5%), followed by pure trematode (7.9%), pure cestode (2.6%) and pure protozoa infestations (0.8%). Mixed infestations with nematodes and trematodes occurred in 6.4% of all animals, mixed nematode-cestode infestations in 3.8%, and all three groups were found in 19.1% of the sheep and goats. In goats more males (81.1%) than females (77.0%) were infested, the opposite was found in sheep (73.6% males, 79.5% females). Parasites were especially prevalent in suckling goats (85.2%) and sheep (88.5%) and to a lesser extent in young (goats 80.6%, sheep 79.3%) and adult animals (goats 72.8%, sheep 73.8%). *Haemonchus contortus*, *Trichuris ovis* and *Paramphistomum cervi* were the most prevalent helminths.

In a third step the *in vitro* anthelmintic activity of *C. decidua*, *S. foetida, S. fruticosa, H. salicornicum* and *H. recurvum* (Chapter 2) was investigated against adult worms of *H. contortus*, *T. ovis* and *P. cervi* (Chapter 3) via adult motility assay (Chapter 4). Various concentrations ranging from 7.8 to 500 mg dry matter/ml of three types of extracts of each plant, i.e. aqueous, methanol, and aqueous-methanol (30:70), were used at different time intervals to access their anthelmintic activity. Levamisol (0.55 mg/ml) and oxyclozanide (30 mg/ml) served as positive and phosphate-buffered saline as negative control.

All extracts exhibited minimum and maximum activity at 2 h and 12 h after parasite exposure; the 500 mg/ml extract concentrations were most effective. Plant species ($P<0.05$), extract type ($P<0.01$), parasite species ($P<0.01$), extract concentration ($P<0.01$), time of exposure ($P<0.01$) and their interactions ($P<0.01$) had significant effects on the number of immobile/dead helminths. From the comparison of LC_{50} values it appeared that the aqueous extract of *C. decidua* was more potent against *H. contortus* and *T. ovis,* while the aqueous extract of *S. foetida* was effective against *P. cervi.* The methanol extracts of *H. recurvum* were most potent against all three types of parasites, and its aqueous-methanol extract was also very effective against *T. ovis* and *P. cervi*. Based on these result it is concluded that the aqueous extract of *C. decidua*, as well as the methanol and aqueous-methanol extract of *H. recurvum* have the potential to be developed into plant-based drugs for treatment against *H. contortus*, *T. ovis* and *P. cervi* infestations.

Further studies are now needed to investigate the *in vivo* anthelmintic activity of these plants and plant extracts, respectively, in order to develop effective, cheap and locally available anthelmintics for pastoralists in Cholistan and neighboring desert regions. This will allow developing tangible recommendations for plant-based anthelminthic treatment of sheep and goat herds, and by this enable pastoralists to maintain healthy and productive flocks at low costs and probably even manufacture herbal drugs for marketing on a regional scale.

Zusammenfassung

Die Tierhaltung in Pakistan trägt wesentlich zum Lebensunterhalt von armen Bauern bei; die Stärkung der pastoralen Gemeinschaften spielt dabei eine unerläßliche Rolle bei der Bekämpfung von Armut. Gastrointestinale Helminthen sind ein wichtiges gesundheitliches Problem für pastorale Nutztierherden im ganzen Land und können daher die Lebensgrundlage von Tierhaltern wesentlich beeinträchtigen. Ein chronischer Befall der Tiere mit Helminthen führt zu deutlichen Verlusten der Produktivität, besonders des Wachstums von Jungtieren. Synthetische Entwurmungsmittel wurden lange als die einzig wirksamen Mittel betrachtet um den Befall zu kontrollieren. Hohe Produktpreise, unerwünschte Nebenwirkungen, chemische Rückstände/Toxizität oder die Entwicklung von Resistenzen haben jedoch zu einer begrenzten Verwendung dieser Anthelmintika in vielen pastoralen Systemen geführt. Darüber hinaus haben arme Hirten in abgelegenen Gebieten Pakistans kaum Zugang zu geeigneten Medikamenten, welche zudem aufgrund langer Transportwege auch relativ teuer sind.

Auf der Suche nach neuen und nachhaltigeren Ansätzen zur Unterstützung von Tierhaltern bei der Erhaltung der Herdengesundheit in abgelegenen Gebieten wurde daher in den vergangenen 20 Jahren die wissenschaftliche Aufmerksamkeit verstärkt auf ethno-botanische oder traditionell genutzte pflanzliche Heilmittel gelenkt. Pflanzliche Heilmittel sind billiger oder gar kostenlos, umweltfreundlich und rufen in der Regel keine Resistenzprobleme hervor; somit können diese allopathische Medikamente ersetzen. Darüber hinaus sind diese Mittel in abgelegenen Gebieten leicht verfügbar und einfach zuzubereiten und zu verabreichen.

Die Wüstenregion Cholistan ist eine sehr arme Region in Südwest Pakistan; die Mehrheit ihrer Bewohner führt ein Nomadenleben. Der Gesamtnutztierbestand (1.29 Millionen) in der Region ist fast doppelt so hoch wie die Zahl der Einwohner. Nutztierhaltung ist die traditionelle Tätigkeit der Bevölkerung und Reichtum wird anhand der Anzahl an Tieren (v.a. Ziegen und Schafe) bemessen, die sich im Besitz einer Person befinden. Erstaunlicher Weise sind etwa 60% dieser Wüstenregion mit sehr angepaßten Gräsern, Sträuchern und Bäumen bewachsen. Diese natürliche vorkommende Flora dürfte ein reiches Spektrum an wissenschaftlich unerforschten pflanzlichen Arzneimitteln beinhalten.

Die vorliegende Doktorarbeit wurde unter der Schirmherrschaft des Internationalen Zentrums für Entwicklung und menschenwürdige Arbeit (international Center for Development and Decent Work – ICDD – *www.icdd.uni-kassel.de*) an der Universität Kassel durchgeführt, wobei der Entwicklungsaspekt im Mittelpunkt der Arbeit stand.

Zunächst wurde in der Wüstenregion Cholistan untersucht, wie Hirten ihre Tiere managen, welches die häufigsten Gesundheitsprobleme der verschiedenen Tierarten sind und welche

natürlich vorkommenden Pflanzen sie für die Behandlung von Tierkrankheiten verwenden (Kapitel 2). Zu diesem Zweck wurde eine Erhebung in fünf Ansiedlungen durchgeführt; mit Hilfe eines strukturierten Fragebogens wurden 100 Viehzüchter (VZ) und 20 lokalen Heiler (LH) individuell befragt.

Die meisten VZ und LH waren Analphabeten (66% bzw. 70%). Im Durchschnitt hatten LH größere Herden (109 Tiere) als VZ (85 Tiere) und mehr Erfahrung in der Tierhaltung. Durchschnittlich investierten VZ 163 Euro pro Jahr in die Behandlung ihres Viehs, jedoch mit starken individuellen Abweichungen. Sechsundachtzig traditionelle Heilmittel wurden aus 64 Pflanzenarten hergestellt und genutzt; die Arten konnten 43 Pflanzenfamilien zugeordnet werden. Capparaceae stellten die größte Anzahl der verwendeten Arten (4), gefolgt von Chenopodiaceae, Poaceae, Solanaceae und Zygophyllaceae (jeweils 3). Die Pflanzenarten *Capparis decidua* (n=55 Nennungen), *Salsola foetida* (n=52), *Suaeda fruticosa* (n=46), *Haloxylon salicornicum* (n=42) und *Haloxylon recurvum* (n=39) sollten nach Angaben der VZ und LH am effektivsten gegen den Befall mit Magen-Darm-Parasiten wirken. Oberirdische Pflanzenteile (43%), Blätter (26%), Früchte (9%), Samen und Samenöl (9%) waren die am häufigsten verwendeten Pflanzenteile für die Heilmittelherstellung, während Blüten, Wurzeln, Zwiebeln und Hülsen weniger häufig verwendet wurden (jeweils <5%). Die übliche Art der Zubereitung bestand aus Abkochen, Kandieren und Drehen von kleinen Pillen; die orale Verabreichung der Medikamente war die häufigste. Die Dosierung eines Heilmittels variierte zwischen Tierarten, Alter, Größe und Kondition der Tiere und Schwere der Erkrankung.

In einem zweiten Schritt wurden die regional am häufigsten vorkommenden Magen-Darm-Parasiten von Schafen und Ziegen bestimmt (Kapitel 3). In den Herden an den fünf bereits untersuchten Standorten wurden insgesamt 500 Tiere pro Art zufällig ausgewählt. Es wurden Standardtechniken angewendet, um die Parasiten in rektal gewonnenen Kotproben zu identifizieren.

Insgesamt lag die Prävalenz gastrointestinaler Parasiten bei 78,1% aller 1000 Tiere; reiner Fadenwurmerbefall war am häufigsten verbreiteten (37,5%), gefolgt von reinem Trematoden- (7,9%), reinem Cestoden- (2,6%) und reinem Protozoenbefall (0,8%). Ein Mischbefall mit Nematoden und Trematoden fand sich bei 6,4% aller Tiere, ein Mischbefall mit Fadenwürmern und Cestoden bei 3,8%. Ein Befall mit allen drei Helminthentaxa wurde bei 19,1% der Schafe und Ziegen nachgewiesen. Bei Ziegen waren eher die männlichen (81,1%) als die weiblichen Tiere (77,0%) befallen, im Gegensatz zu den Schafen (männlich: 73,6%; weiblich: 79,5%). Parasiten wurden besonders häufig bei Kitzen (85,2%) und Lämmern (88,5%), in geringerem Maße bei Jungtieren (Ziegen: 80,6%; Schafe: 79,3%) und adulten Tieren (Ziegen: 72,8%;

Schafe: 73,8%) gefunden. *Haemonchus contortus*, *Trichuris ovis* und *Paramphistomum cervi* waren die am weitesten verbreiteten Helminthenarten.

In einem dritten Schritt wurde die *in vitro* Aktivität von *C. decidua, S. foetida*, *S. fruticosa*, *H. salicornicum* und *H. recurvum* (Kapitel 2) gegen adulte *H. contortus*, *T. ovis* und *P. cervi* (Kapitel 3) getestet, mit Hilfe des Adult Motility Assays (Kapitel 4). Pro Pflanze wurden unterschiedliche Konzentrationen an Pflanzenmaterial (zwischen 7.8 und 500.0 mg Trockensubstanz pro ml) mit drei unterschiedlichen Lösungsmitteln erstellt (Wasser, Methanol, und Wasser-Methanol 30:70). Diese wurden mit unterschiedlich langer Wirkzeit eingesetzt (2 – 24 Stunden), um ihre Helminthentoxizität zu bestimmen. Levamisolhydrochlorid (0,55 mg/ml) und Oxyclozanid (30,00 mg/ml) dienten als Positivkontrolle, phosphatgepufferte Kochsalzlösung als Negativkontrolle.
Alle Extrakte zeigten minimale und maximale Toxizität nach 2 und 12 Stunden der Exposition; die Extraktkonzentration von 500 mg/ en war dabei jeweils am effektivsten. Pflanzenart ($P<0,05$), Extraktionsmittel, Parasitenart, Extraktkonzentration, Zeit der Exposition und deren Wechselwirkungen hatten einen signifikanten Einfluß (jeweils $P<0,01$) auf die Anzahl an immobilen/toten Helminthen. Der Vergleich der LC_{50}-Werte zeigte, dass wässrige Extrakte von *C. decidua* stärker gegen *H. contortus* und *T. ovis* wirkten als jene von *S. foetida* gegen *P. cervi*. Die Methanolextrakte von *H. recurvum* waren am wirkungsvollsten gegen alle drei Arten von Parasiten wobei auch der Methanol-Wasser-Extrakt sehr effektiv gegen *T. ovis* und *P. cervi* wirkte. Aus diesen Ergebnissen kann geschlossen werden, dass das wässrige Extrakt von *C. decidua* sowie das Methanol- und Wasser-Methanol-Extrakt von *H. recurvum* das Potential besitzen, als Entwurmungsmittel gegen *H. contortus*, *T. ovis* und *P. cervi* zu wirken.

Weitere Studien sind nun erforderlich, um die anthelmintische Wirkung dieser Pflanzen bzw. Pflanzenextrakte *in vivo* zu untersuchen. Darauf aufbauend können dann effektive, kostengünstige und lokal verfügbare Anthelmintika für die regelmäßige Entwurmung der kleinen Wiederkäuer von Viehhaltern in Cholistan und den angrenzenden Wüstenregionen entwickelt werden. Viehhalter könnten so die Gesundheit und Produktivität ihrer Herden mit geringen Kosten sicherstellen und eventuell sogar selbst zubereitete, effektive pflanzliche Medikamente auf regionaler Ebene vermarkten um zusätzliches Einkommen zu schöpfen.

CHAPTER 1

GENERAL INTRODUCTION AND STUDY OBJECTIVES

1.1. General introduction

1.1.1. Role of livestock in Pakistan's economy

Agriculture is the mainstay of Pakistan's economy and contributes 21 percent to national GDP. Agriculture generates productive employment opportunities for 45 percent of the country's labour force and 60% of the rural population depends upon this sector for its livelihood. The agricultural sector is dominated by livestock, which provides approximately 55% of the agricultural value added and 11.6% of the national GDP. It has a vital role in ensuring food security, generating overall economic growth, reducing poverty and the transformation towards industrialization. The gross value added of this segment at constant factor cost has increased by 4% from 5.29 billion Euro (2010-2011) to 5.51 billion Euro (2011-2012; Economic Survey of Pakistan, 2012).

Livestock is central to the livelihood of the rural poor in the country and can contribute substantially to poverty alleviation by strengthening the socio-economic conditions of pastoralists (Gadahi et al., 2009). Historically the livestock sector was subsistence-oriented and dominated by smallholders, and even today livestock are considered a secure source of income for small farmers and landless poor, and a source of employment generation at the rural level (Gadahi et al., 2009; Khajuria et al., 2012). It also helps to reduce income variability, especially in cases of crop failure due to a variety of causes. This is particularly true for sheep and goats (Khajuria et al., 2012), of which numbers have doubled in Pakistan during the past 15 years (Iqbal and Jabbar, 2005). This can be ascribed to the relatively low inputs needed such as startup capital, feedstuffs and maintenance expenditures as compared to large ruminants (Terefe et al., 2012).

1.1.2. Helminthiasis: constraint to livestock development

Sheep and goat production depends on feed supplies, good health practices and appropriate animal husbandry management. Various climatic and casual factors are leading to a high degree of parasitic infestation resulting in decreased longevity, fertility and productivity of infested animals (Khajuria et al., 2012). Helminthiasis is, however, of high economic significance in view of its insidious nature and easy transmissibility due to underfeeding, availability of a wide variety of hosts and vectors, inadequate/low level of awareness of farmers and high medication costs (Garedaghi et al., 2011; Terefe et al., 2012). Several authors (Raza et al., 2007, 2012; Khan et al., 2010; Farooq et al., 2012) have explored various aspects of helminth infestation in different localities of Pakistan and reported prevalence ranges of 25 - 92%.

Helminth infestation lowers the animal's immunity and renders it susceptible to other pathogenic infections; finally this may result in heavy economic losses (Garedaghi et al., 2011).

The problem is however much more severe in developing countries due to very favorable environmental conditions for helminth transmission (Zeryehun, 2012), poor nutrition of the host animal (Mbuh et al., 2008) and poor sanitation in rural areas (Badran et al., 2012). As a result diseases caused by helminths remain a major impediment to small ruminant production in the tropics and elsewhere (Kumsa et al., 2011), and up to 95% of small ruminants are reported to show helminth infestation in these latitudes (Mbuh et al., 2008; Terefe et al., 2012). However, the majority of animals infested with helminths do not show clinical signs owing to the chronic nature of the disease.

1.1.3. Failure of allopathic anthelmintics

Synthetic anthelmintics have long been considered the only effective way of controlling the dilemma of helminth infestation but nowadays the allopathic anthelmintics available in the market often are not effective or have induced resistance resulting in recurrence of parasitic infestations (Jabbar et al., 2006a; Saeed et al., 2007). Additionally, high prices, unavailability and scarcity in remote areas, side effects and chemical residues / toxicity problems, or development of resistance (Jabbar et al., 2006a; Saeed et al., 2007) and inaccessibility especially to the low income communities lead to the very limited use of allopathic anthelmintics in many pastoral systems (Gilleard, 2006). The resistance to anthelmintics not only constitutes a major problem in developing countries, but is also a serious threat to livestock in the rest of the world (Waller, 1987) because of frequent treatment failures. Vaccination may be an alternative way to control the parasitic infestations, but antigenic complexity and antigenic variation at various developmental stages of the parasites has slowed the process of vaccine development.

1.1.4. Plant remedies: an alternative to chemical drugs

The search for new and more effective remedies for controlling the diseases of livestock has given rise to the study of plant based remedies (Mathias, 2004). Plants not only grant food and shelter to human beings but have served, through centuries, as a constant source of medication for the treatment of a variety of diseases. The history of ethno-botanicals is almost as old as human civilization (Sarojini et al., 2012) and most of the population in the Indo-Pakistan subcontinent, and in as other developing countries elsewhere, has relied on plants for curing their animals and as well themselves (McCorkle, 1995; Jabbar et al., 2006b). This has also been acknowledged by the World Health Organisation that estimated that 80% of the people in the developing world or 60% of the human race depend largely on the plants, for the control and treatment of various human and animal diseases (World Health Organisation, 2010).

Medicinal plants run the pedestal of health care systems and in many poor rural areas, ethno-botanicals can play an important role in animal production systems and livelihood development, and often become the only available means for curing the ailments (Jabbar et al., 2005). All parts of plants may serve as source of medicinally useful components, and many plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides (Iqbal et al., 2005; John et al., 2006).

Medicinal plants and ethno-botanical remedies are economical, safe and generally have no problem of drug resistance; they might thus be unmatched substitutes of allopathic anthelmintics. Furthermore, these remedies are easily available, simple to prepare and/or administer, at minute or free of cost to the farmer (Jabbar et al., 2005) and even considered as the best healing agents for the treatment of parasitic diseases (Kone et al., 2012).

1.1.5. Ethno-botanicals as anthelmintics

Today many of the allopathic anthelmintics available in the market are either not effective or have induced resistance, resulting in recurrence of parasitic infestations (Jabbar et al., 2006a; Saeed et al., 2007). Plant derived drugs serve as a prototype to develop more effective and less toxic medicines. The medicinal properties of plant species have made an exceptional input to the beginning and improvement of many conventional health care systems. Thus, there has recently been a resurgence of interest in the development of drugs from the plants, especially from those of developing countries that have a rich heritage of botanical ethnopharmacopoea (Schoen and Wynn, 1998). The search for new agents to treat diseases of bacterial, viral, fungal or parasitic origin from medicinal plants is even more urgent in the context of countries like Pakistan, because the country has an agriculture-based economy, a large proportion of (poor) rural dwellers, and is bestowed with a unique biodiversity. About 600 of the country’s 6000 known plant species are considered of therapeutic value (Hamayun, 2003; Khan et al., 2012).

1.1.6. Documentation and scientific validation of traditional remedies

The traditional knowledge systems have started to vanish with the passage of time due to low or no investment, least interest of researchers and lack of written credentials in this sector (Ahmad et al., 2009). Knowledge on ethno-veterinary/ traditional veterinary medicine, like other traditional knowledge systems, is being passed on orally from generation to generation and it may disappear because of hasty technological and socioeconomic changes, and loss of cultural heritage (Mathias-Mundy and McCorkle, 1989). Documentation and recording information regarding the use of ethno-botanicals may be the only solution to preserve this precious knowledge for future generations before it is lost forever.

In Pakistan, rural dwellers are using a large variety of plants for various therapeutic purposes. Yet, only a few studies investigated usage and effectiveness of traditional remedies for treating livestock diseases (Jabbar et al., 2006; Hussain et al., 2008; Babar et al., 2012). All the plant species documented in previous studies are region specific, and knowledge gained in one region cannot be transferred in all cases to another one. Therefore, for the present study, a relatively poor and remote area, Cholistan desert, was selected, which is geographically and climate wise a quiet different county. The Cholistan desert region, with its northern border located about 270 km south of Lahore in the eastern part of Punjab Province, Pakistan, covers an area of 16,000 km². It is one of the least developed areas of Punjab Province, inhabited by livestock nomads of different ethnic groups (Khan, 1992; Akhter and Arshad, 2006). These groups have their distinct ways of life, beliefs and tradition, and have been utilizing local plants for various purposes over generations. In 2006, the region hosted about 1.3 Mio head of livestock, more than twice as much as the local human population (Livestock Census of Pakistan, 2006). Ceremonies like weddings, funerals and tribal celebrations include slaughtering and exchange of animals, and traditionally wealth is assessed from the number of animals, especially cattle, sheep and goats, owned by the individual (Farooq et al., 2008). Although a significant area of Cholistan is covered with sand dunes, about 60% is inhabited by highly adapted grasses, shrubs and trees. These plants are capable of surviving extended drought periods and produce nutritious fodder during favorable seasons (Khan, 1992); they are furthermore used as vegetables, fruits, fuels, and to fabricate agricultural implements. Due to their poverty and remoteness, livestock keepers cannot easily access veterinary services; therefore they use the local plant species as traditional remedies for the treatment of their animals' and their own ailments (Arshad et al., 2003). Many of these plants, of course, have a prolonged and uneventful use that may serve as indirect testimony of their efficacy. However, in the absence of an objective proof of efficacy, and since their scientific evaluation as compared to commercial anthelmintics is limited, the validity of these remedies is questionable and their use remains locally restricted.

1.2. Objective and conceptual framework of the study

In view of the above discussed considerations, but especially due to the constraint to livestock production in general, and in search for alternative plant based medication against helminthiasis in particular, this PhD project was designed to achieve the following objectives:

1- To record the ethno-botanical/ plant-based remedies used by the pastoralists of Cholistan desrt, Pakistan, for the control and healing of different livestock disorders.

2- To investigate the prevalence of helminthiasis in sheep and goat herds of pastoralists of Cholistan desert, Pakistan.

3- To explore and evaluate the effectiveness of most frequently used plants against major helminth species prevalent in sheep and goat herds.

4- To development of recommendations for effective and cheap treatment of pastoral herds with local plant remedies, which will help pastoralists to maintain healthy and productive flocks at low costs and maybe even manufacture herbal anthelmintic drugs which can be marketed.

To achieve these objectives, the following methodological approach was developed (Figure 1.1): in a first step a baseline survey was conducted to collect data regarding the usage of traditional remedies from 120 pastoralists (100 livestock farmers and 20 local healers; chapter 2) of Cholistan desert, Pakistan. Secondly, a large-scale survey was conducted to determine the prevalence of helminth infestation in sheep and goats herds (chapter 3) from previously visited households. Finally, five plants recommended against the helminths by a maximum number of households (chapter 2) were evaluated for their anthelmintic activity (chapter 4) against three most prevalent parasites of sheep and goat flocks (chapter 3).

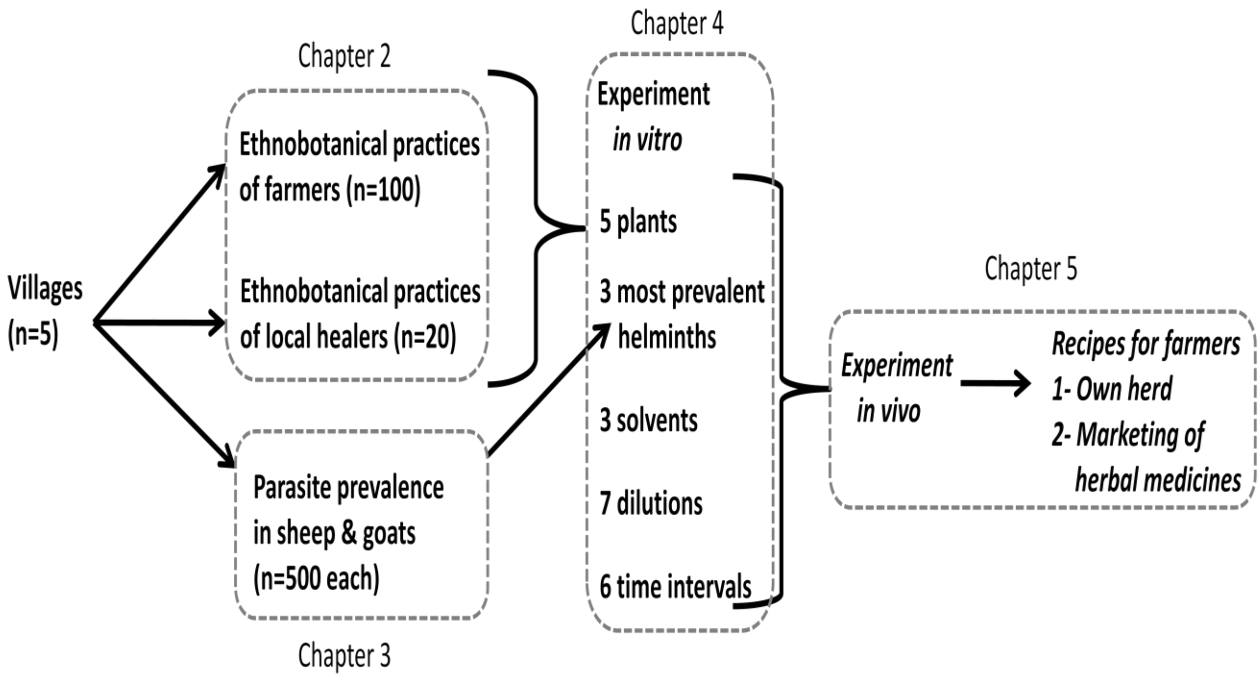

Figure 1.1: Methodological approach of the study

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CHAPTER 2

ETHNO-BOTANICAL REMEDIES USED BY PASTORALISTS FOR THE TREATMENT OF LIVESTOCK DISEASES IN CHOLISTAN DESERT, PAKISTAN

Ethno-botanical remedies used by pastoralists for the treatment of livestock diseases in Cholistan desert, Pakistan

Abstract

Ethno-pharmacological relevance: Account of the traditional plant based *viz*. ethno-botanical remedies used by the pastoralists of Cholistan desert, Pakistan, for the control and treatment of livestock diseases and ailments.

Materials and methods: The study was conducted across five locations in Cholistan desert, Pakistan, using a structured questionnaire to collect data from 100 livestock farmers (LF) and 20 local healers (LH). From correlation analyses 3 least correlated variables were identified among 5, which were representative of LFs. Cluster analysis was performed on the basis of these 3 variables and LFs were grouped into 3 logically different clusters. Kruskal-Wallis test and crosstab analyses were used to detect significant differences between clusters and effects of various variables on their use of ethno-botanical remedies.

Results: Most of the male (100%) interviewees were married (LF 78%; LH 70%) and illiterate (LF 66%; LH 70%). LH had larger herds (average 109 animals) than LF (average 85 animals) and were more experienced in livestock husbandry and management. LF spent about 162.5 Euros annually on the treatment of their livestock, but there was great variability in expenditures. Average animal treatment experience of LH was 29 years; all were experts in treatment of all types of diseases (100%) and animal species (70%).

Eighty-six traditional remedies based on 64 plants belonging to 43 families were used. Capparaceae was the botanical family with the largest number of used species (4), followed by Chenopodiaceae, Poaceae, Solanaceae and Zygophyllaceae (3). Aerial parts (43%), leaves (26%), fruits (9%), seeds and seed oils (9%) were frequently used parts, while flowers, roots, bulbs and pods were less frequently used (<5%). Common preparations were decoction, jaggery and ball drench; oral drug administration was very common and doses were estimated using lids, spoons, cups and handfuls. Doses used for different animal species varied depending on animal age, size and physical condition and severity of the disease.

Conclusions: Pastoralists are practicing traditional plant-based livestock medication without scientific validation as they cannot afford allopathic drugs. Therefore, efficacy of documented medicinal plants against the most prevalent livestock diseases should be evaluated, in order to recommend effective preparations and treatments to this poor population group.

Keywords: Ethno-veterinary medicine, Livestock farmers, Local healers; Traditional remedies.

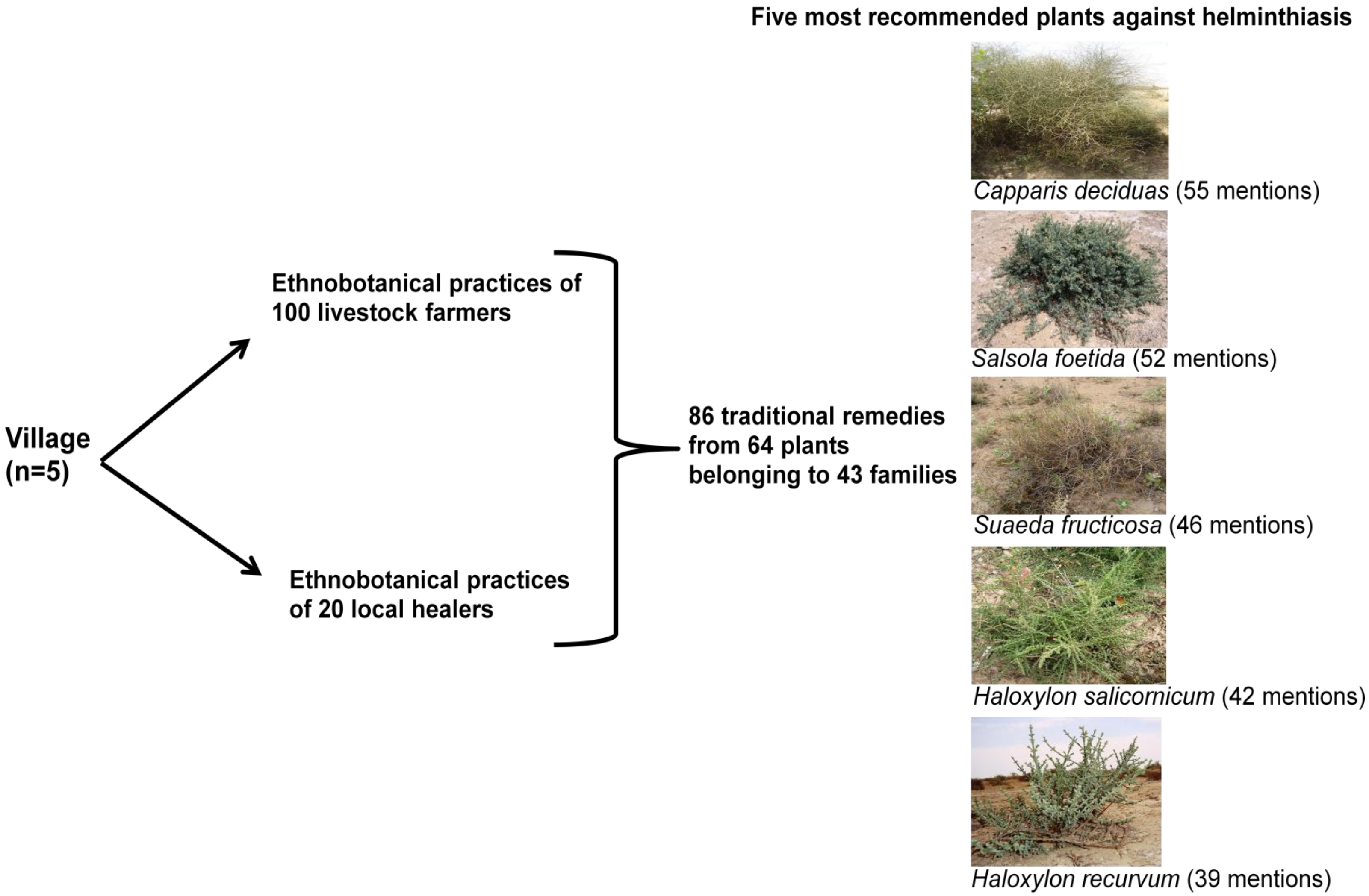

Figure 2.1: Graphic abstract of the participatory study.

2.1. Introduction

In many rural areas of developing countries the use of medicinal plants is the mainstay of primary healthcare. The World Health Organisation estimated that 60% of the global population largely depends on traditional medicine for the control and treatment of various diseases (World Health Organization, 2010). Reasons for the still important use of traditional plant-based remedies by rural communities are (i) the strong relation of communities with local flora, (ii) the easy accessibility of local plants and (iii) their lack of side effects, (iv) the simple mode of their use, and (v) poor access of rural dwellers to allopathic drugs and their high costs. Ethno-veterinary medicine (EVM) is a specialized area of ethno-botany that deals with the study of traditional knowledge, methods, practices and beliefs of people about health care, remedies and clinical practices for disease treatment and prevention, husbandry and production of livestock (McCorkle, 1986). EVM knowledge, like other traditional knowledge systems, is transmitted orally from generation to generation (McCorkle, 1986; McCorkle et al., 1995), and is disappearing because of rapid socio-economic, environmental and technological changes (Mathias-Mundy and McCorkle, 1989). However, in developing as well as in developed countries the use of traditional plant-based remedies is regaining attention (Uprety et al., 2012; Monigatti et al., 2013; Motlhanka and Nthoiwa, 2013).

In Pakistan, rural dwellers are using a large variety of plants for various therapeutic purposes. Yet, only a few studies investigated usage and effectiveness of traditional remedies for treating livestock diseases in district Muzaffar Garh (Jabbar et al., 2006) and of helminth infestations in Sahiwal and Bhakkar districts of the Punjab, respectively (Hussain et al., 2008; Babar et al., 2012). The plant species documented in these studies are region-specific, and knowledge gained in one region can therefore not be transferred in all cases to another one. The present study concentrated on a quite poor and remote area, Cholistan desert, which in terms of geography and climate is quite different from the above-mentioned regions. The Cholistan desert region, with its northern border located about 270 km south of Lahore in the eastern part of Punjab Province, covers an area of 16,000 km^2. It is one of the least developed areas of Punjab Province, inhabited by livestock nomads of different ethnic groups (Khan, 1992; Akhter and Arshad, 2006). These groups have their distinct ways of life, beliefs and tradition, and have been utilizing local plants for various purposes over generations. In 2006, the region hosted about 1.3 Mio head of livestock, more than twice as much as the local human population (Livestock Census of Pakistan, 2006). Ceremonies like weddings, funerals and tribal celebrations include slaughtering and exchange of animals, and traditionally wealth is assessed from the number of animals, especially cattle, sheep and goats, owned by the individual (Farooq et al., 2008). Although a significant surface of Cholistan is covered with sand dunes, about 60% is covered by highly adapted grasses, shrubs and trees. These plants are capable of surviving extended drought periods and produce nutritious fodder during favorable seasons (Khan, 1992); they are furthermore used as vegetables, fruits, fuels, and to fabricate agricultural implements. Due to their poverty and remoteness, livestock keepers cannot easily contact veterinary personnel; therefore they use the local plant species as traditional remedies for the treatment of their animals' and their own ailments (Arshad et al., 2003).

In view of the above, we discussed, with pastoralists and local animal healers in the Cholistan region, their general livestock husbandry practices, recurrent livestock diseases and traditional plant-based disease treatments, thereby aiming at identifying those remedies that are considered most effective.

2.2. Material and methods

2.2.1. Study area

Cholistan desert is about 480 km long and 32 km to 192 km wide, located between latitudes 27°42′ - 29°45′ N and longitudes 69°52′ - 75°24′ E (FAO, 1993). Average annual rainfall is only 128 - 175 mm; the ground water is brackish and 25 - 90 m deep (Akbar et al., 1996). Mean summer temperatures range from 34 - 37°C and maxima reach 50°C in May and June; mean winter temperatures range between 14 - 16°C with minima below zero during December and January (Khan, 1992; Arshad et al., 2007).

The people of Cholistan lead a semi-nomadic life, moving from one place to another in search of water and fodder for their animals. The local tribes store rain water in man-made ponds in the ground or between sand hills, called 'tobas', for the consumption of their livestock and themselves. Habitations are small and extremely scattered around the tobas. Various locations within the desert are named after the owners of tobas or historical forts. For this study 5 locations, at least 30 km apart, were selected (Figure 2.2). Each location comprised 3 - 9 villages or tobas.

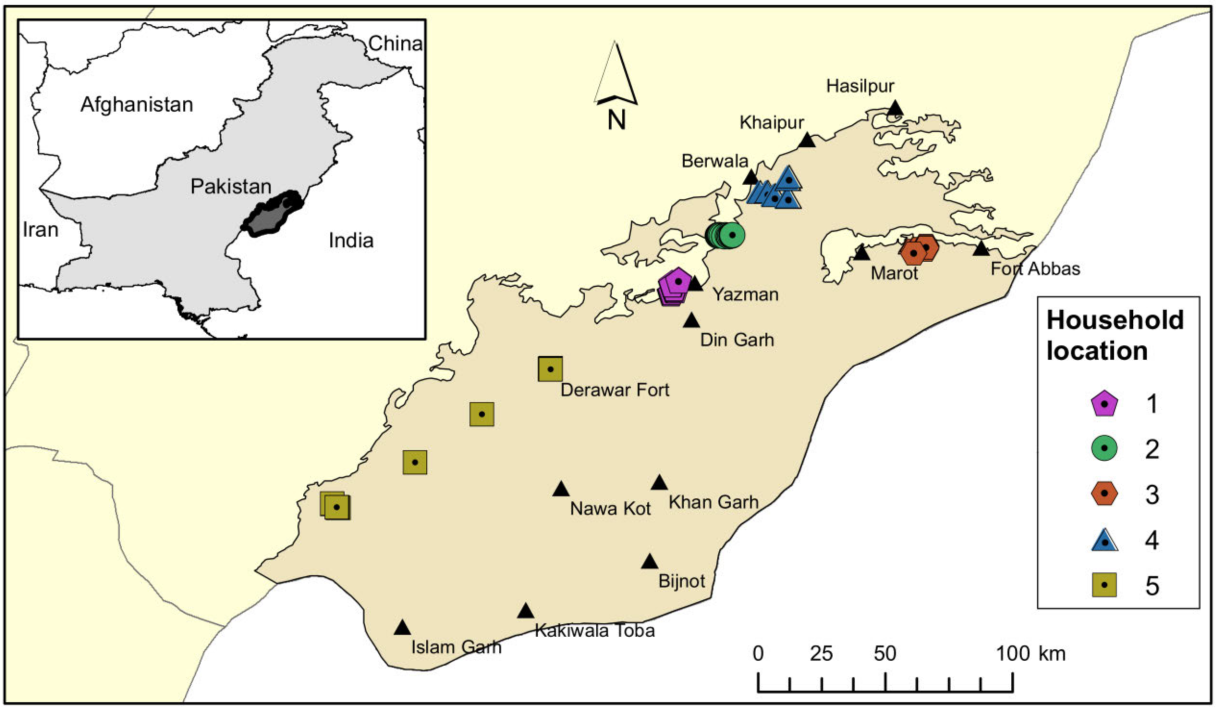

Figure 2.2: Map of the study area in the Cholistan desert, Pakistan, with the homesteads of interviewed pastoralists at the five locations.

2.2.2. Data collection

From July 2010 until January 2011 a structured questionnaire with open-ended and closed questions was used for face-to-face interviews of 100 livestock farmers (LF) plus 20 local healers (LH) keeping flocks dominated by sheep and goats. As a basis for selecting respondents, data on locations, population, animal species and route maps was obtained from Cholistan Development Authority Bahawalpur, Pakistan. The questionnaire was pre-tested with 12 farmers and modified as required; it covered the following major areas:

1- Demographic household aspects (age, education, marital status);
2- Livestock herd size and composition, feeding aspects;
3- Detailed animal health care practices;
4- Detailed use of traditional remedies and medicinal plants; respondents were asked to show the plant species (except in the case of commonly known species) in the field and describe in detail their use.

With the help of a local guide the first household (HH) that visibly kept sheep and goats was chosen. The respondent was informed about the motives for the survey and was interviewed immediately if he agreed upon. After the interview, he was requested to provide names and addresses of three other HHs in his locality that were rearing small ruminants. From these three names one was randomly selected for the next interview. For the selection of local healers, information was collected from the livestock farmers. On the basis of mentioned names and reported competency, 20 local healers were selected across the 5 locations.

The questions were orally translated from English into the local language *Saraiki*. The answers of the respondents were translated into English and noted down by the interviewer. One interview lasted 25 to 70 minutes. After the interview, the geographical location of each HH was recorded with a Trimble Geo-explorer II GPS (Sunnyvale, CA, USA).

2.2.3. Statistical analysis

The data were tabulated, coded into numerical values and subjected to statistical analyses with SPSS 17.0 (SPSS Inc., Chicago, Illinois). Initially the five most important variables that were characterizing LF were subjected to Pearson correlation analyses. Three key variables that were least correlated with each other were identified. Two-step Cluster analysis was performed on the basis of these 3 variables and LF were grouped into 3 logically different clusters. To detect significant differences and evaluate the effect of various variables on the clusters EVM practices, the Kruskal-Wallis test and crosstab analyses were used.

2.3. Results

Due to the social norms in the area, the male interviewer could not talk to women, so only knowledge held by males can be reported here; yet, males still retain the bulk of information concerning the use of medicinal remedies for treating livestock.

2.3.1. Household characteristics

Males were responsible for family income. Most of them were married (LF 78%; LH 70%) and illiterate (LF 66%; LH 70%) or had only primary education (LF 30%; LH 10%; Table 2.1 and 2.2). The average age of LF and LH respondents was 46.3 years (range 18 - 86) and 52.4 years (range 27 - 71), respectively (Table 2.1 and 2.2). LHs had more animals (109 ±50.5) than LF (85 ±60.0) and had also more years of experience in livestock husbandry and management (LH 29 ±13.1 years; LF 25 ±14.3 years). The average number of sheep, goats, cattle, buffalo and camels in LH flocks (Table 2.3) was 43.1 (range 0-115), 43.9 (range 12-135), 14.9 (range 0-70), 4.7 (range 0-20), and 2.1 (range 0-20), respectively, whereas LF kept 34.2 sheep (range 0-170), 32.6 goats (range 0-250), 14.4 cattle (range 0-170), 2.2 buffaloes (range 0-15) and 2.1 camels (range 0-30).

Among LF, 39% kept buffalo, 91% cattle, 99% goat, 97% sheep and 43% camels, while among LH, 50% kept buffalo, 75% cattle, 100% goat, 95% sheep and 45% camels. Against different viral and bacterial diseases, 48.7% of the LF were vaccinating their buffaloes, 60.4% their cattle, 69.7% their goats, 72.2% their sheep and 9.3% their camels; while 35.9% of the LF were also deworming the buffaloes, 65.9% the cattle, 45.4% the goats, 62.9% the sheep and 32.6% the camels against parasitic infestations.

Livestock farmers were spending on average 162.5 Euro per year on the treatment of their livestock with however a huge variability in the expenditures (range 21-2208 Euro). Average experience in treating animals of local healers was 29 years (range 8-52) and all healers were very familiar with the symptomatic diagnosis of all kinds of diseases and experts in treating these (100%) in the different animal species (70%; Table 2.2).

Table 2.1: Household (HH) characteristics of 100 livestock farmers in Cholistan, Pakistan, interviewed during 07/2010 –01/2011.

HH characteristics	**Category**	**Frequency (n)[T]**
Marital status	Single	8
	Married	78
	Widowed	10
	Divorced	4
Education	Illiterate	66
	Primary school (5 years)	30
	Secondary school (10 years)	4
Age HH head (years)	≤ 25	6
	26 –60	74
	>60	20
Animal keeping experience (years)	≤ 10	15
	11 – 30	55
	>30	30
Livestock herd size (animals)	≤ 50	24
	51 – 100	52
	101 – 200	21
	> 200	3
Medication expenses (€, during last 12 months)	≤ 100	32
	101 – 200	30
	201 – 300	18
	301 – 500	8
	> 500	12
Member of cluster*	MVM user	15
	EVM user	43
	MR user	42

[T]Frequency and percentages are the same as the total number of livestock farmers is 100;

* MVM= Modern veterinary medicine; EVM= Ethno-veterinary medicine; MR= Mixed remedies.

Table 2.2: Household (HH) characteristics of 20 local healers in Cholistan, Pakistan, interviewed during 07/2010 –01/2011.

HH characteristics	Category	Frequency (n)	Percentage (%)
Marital status	Single	1	5
	Married	14	70
	Widowed	5	25
Education	Illiterate	14	70
	Primary school (5 years)	2	10
	Secondary school (10 years)	1	5
	Intermediate (12 years)	2	10
	Master	1	5
Occupation	Local-healer	12	60
	Businessman	1	5
	Job (government/ private sector)	7	35
Age HH head years	≤ 40	3	15
	41–60	12	60
	>60	5	25
Animal keeping experience (years)	≤ 10	3	15
	10 – 40	12	60
	>40	5	25
Livestock herd size (animals)	≤ 100	6	30
	101 – 200	13	65
	> 200	1	5
Field of expertise	All species	14	70
	Herbivores	5	25
	Small ruminants	1	5
EVM* training source	Got diploma in herbal medicine	3	15
	Self-study of books	1	5
	Forefathers/ tradition	7	35
	Professional trainer	9	45

*EVM = Ethno-veterinary medicine.

Table 2.3: Composition (n) of pastoralist herds in Cholistan, Pakistan.

	Cattle	Sheep	Goats	Camels	Buffaloes
LF (n=100)	1404	3085	3014	200	217
Adult	916	1918	1892	144	124
Young	203	473	462	34	45
Suckling	285	694	660	22	48
LH (n=20)	300	864	879	59	93
Adult	186	528	553	33	50
Young	51	137	127	10	21
Suckling	63	199	199	16	22

2.3.2. General livestock management

The pastoralists could be divided into three clusters based on their main animal medication practices and numbers of animal kept: (i) pastoralists using allopathic drugs for the treatment of their animals, further referred to as 'modern veterinary medicine' users (MVM) accounting for 15% of LF; (ii) pastoralists completely relying on plant-based remedies for curing their animals, further referred to as 'ethno-veterinary medicine' users (EVM) comprising 43% of LF; (iii) pastoralists combining the use of medicinal plants with allopathic drug use ('mixed remedies' users; MR), accounting for 42% of LF (Table 2.4).

Average herd size of MVM was 96.3 (±124.92), while EVM kept 86.7 (±32.17) and MR 79.7 (±47.14) animals. MR were older (51.3 ±14.87 years); and had a longer animal keeping experience (28.1 ±14.68 years) than EVM (age: 42.5 ±14.11 years; experience: 22.2 ±14.28 years) and MVM (age 43.3 ±10.93 years; experience 21.0 ±11.50 years), with differences in age but not in experience being significant ($P \leq 0.01$). EVM were annually spending 99 Euro (±77.5) on treating their animals, while MR and MVM spent significantly more ($P < 0.001$), namely 215 Euro (±130.1) and 890 Euro (±589.6), respectively. All MVM (100%) and 88.1% of MR were stall-feeding their animals, whereas only 18.6% of EVM were practicing this ($P < 0.001$); the remainder LF sent their animals on pasture for year-round grazing (Table 2.4). Poor cleanliness at farm prevailed in MVM users (53.3%) as compared to EVM (51.2%) and MR (23.8%); 64.3% and 11.9% of MR users had very good and good cleanliness conditions at their farm; in contrast to EVM (very good: 44.2%; good: 4.6%,) and MVM users (good: 46.7%; $P \leq 0.05$).

Table 2.4: Means (±SD) and frequencies (%), respectively, of major variables that distinguish three clusters of pastoralist households (HH) in Cholistan, Pakistan.

Variable		**MVM* user** (n=15)	**EVM* user** (n=43)	**MR* user** (n=42)	χ^2	***P**≤*
Age of HH head (years)		43.3 ±10.93	42.5 ±14.11	51.3 ±14.87	8.26	0.016
Animal keeping experience (years)		21.0 ±11.50	22.2 ±14.28	28.1 ±14.68	4.92	0.085
Herd size (animals)		96.3 ±124.92	86.7 ±32.17	79.7 ±47.14	5.25	0.072
Spending on medication (Euro/year)		890 ±589.60	100 ±77.50	215 ±130.10	57.79	0.001
Stall feeding (%)	Yes	100	18.6	88.1	54.52	0.001
	No	0	81.4	11.9		
Grazing (%)	Yes	33.3	86.1	28.6	31.29	0.001
	No	66.7	13.9	71.4		
Farm cleaning (%)	Poor	53.3	51.2	23.8	9.34	0.053
	Good	46.7	44.2	64.3		
	Very good	0	4.6	11.9		
MVM treatment (%)	Yes	100	0	100	100.00	0.001
	No	0	100	0		
EVM treatment (%)	Yes	0	100	100	100.00	0.001
	No	100	0	0		
Reasons for using EVM						
MVM not available (%)	Yes	-	53.5	38.1	2.03	0.114
	No	-	46.5	61.9		
MVM expensive (%)	Yes	-	100	100	86.00	0.001
	No	-	0	0		
MVM has side effects (%)	Yes	-	67.4	52.4	2.01	0.116
	No	-	32.6	47.6		
EVM more effective (%)	Yes	-	79.1	45.2	10.36	0.001
	No	-	20.9	54.8		
Other reasons (%)	Yes	-	34.9	23.8	1.26	0.189
	No	-	65.1	76.2		
Origin of plants (%)						
	Local collection	-	95.3	80.9	17.24	0.001
	From market	-	4.7	19.1		
Knowledge on EVM effectiveness (%)						
	Self-experience	-	11.6	4.8	2.01	0.571
	Tradition	-	25.6	28.6		
	Recommendation	-	58.1	57.1		
	Other	-	4.7	9.5		

* MVM= Modern veterinary medicine; EVM= Ethno-veterinary medicine; MR= Mixed remedies.

** Kruskal-Wallis test for continuous variables (above); Chi-square test for categorical variables (below).

2.3.3. Knowledge of medicinal plants

Information regarding the usage of medicinal plants was collected from both LF and LH, and later on all the data was incorporated collectively (Appendix 2.1). On average LF reported the use of 4 - 7 while LH described the use of 11 - 15 traditional plant-based remedies. Most LF knew at least some conventional recipes, dosages and modes of preparations. However, usage and knowledge of traditional plants varied greatly between respondents. Sometimes LF were also advised traditional formulations by their fellows based upon their past recommendations by LH.

LH had a rich history of successful usage of medicinal plants which reflected their experience of controlling and curing of different diseases. Traditional knowledge was gained by LH from trained persons (45%), fathers and grandfathers (35%). Furthermore, 15% of LH got a diploma in herbal medicine (*Unani Tibb* medicine) from Tibbyah Collage, Bahawalpur, Pakistan, whereas 5% claimed to have obtained their EVM knowledge from books and own practice (Table 2.4). Most LH (70%) were expert in treatment of all animal species, 25% were specialized in herbivores and 5% were experts in the medication of small ruminants. All LH were treating all kinds of diseases, such as skin problems, digestive tract disorders, respiratory infections, and parasitic infestations.

The interviews provided information about 86 traditional remedies in which 64 plants belonging to 43 families were used. Capparaceae was the dominant botanical family with the largest number of cited species (4), followed by Chenopodiaceae, Poaceae, Solanaceae and Zygophyllaceae (3). Two plant species were reported each from Amaryllidaceae, Amaranthaceae, Apiaceae, Asclepiadaceae, Brassicaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae and Malvaceae families (Appendix 2.1).

Most of the traditional remedies relied on one single plant but sometimes a mixture of different plants was used, for example to treat indigestion and gastric problems (Appendix 2.1). Some plants were also used to treat more than one disorder – as an example, LF reported to use *Calotropis procera* for curing three different diseases; another 20 plants, among them *Acacia nilotica* subsp. *indica, Azadirachta indica, Capparis decidua, Haloxylon salicornicum, Prosopis cineraria, Ricinus communis* and *Zyziphus spina-christi,* were used to treat two different diseases each (Appendix 2.1).

2.3.4. Dosages, preparations and applications of plants

Of the 86 recorded plant-based remedies, a majority was used to treat helminthiasis (18.6%), followed by skin problems and itching (9.3%), respiratory tract infections and coughing (8.1%), indigestion and digestive tract disorder (8.1%), diarrhea and dysentery (5.8%). The use as laxative substance (5.8%), antipyretic (5.8%), nerve tonic and cooling agent (4.7%), and for wound and injury healing (4.7%) was also mentioned frequently (Appendix 2.1). Less important were uses as diuretic substance, fly repellent, anti-inflammatory agent, and for treating the expulsion of placenta (each 3.5%).

Oral route of drug administration was the most common, and dosages were estimated using lids, spoons, cups and handfuls. There was however a variation in the doses used for different animal species, depending on age, size and physical condition of the animal and the intensity of disease. Sheep and goats generally received half of the dose used for large animals (cattle, buffaloes, camels, horses). As far as the duration of treatments was concerned, normally a single dose of remedy was given on a daily basis; in severe cases remedies were applied twice a day. The medication continued 5 - 11 days until the disease symptoms had disappeared.

To prepare the 86 remedies, the use of an admixture of aerial parts dominated (43.1%), followed by leaves (25.6%), fruits (9.3%), seeds and seed oils (9.3%) and flowers (3.5%). The use of whole plants, leaf and flower admixtures, roots, bulbs and pods was less frequent (<3%). The way of preparing the remedies also varied greatly: smoke was used for external application (3.5%) along with pastes (9.3%) and poultice (3.5%), while decoction (27.9%), ball drench (5.8%), jaggery (17.4%) and boiled infusions (4.7%) were used for oral application. In addition to this, medicinal plants were also used as feedstuff (12.8%) and sometimes seed oil of plants were used for topical application (2.3%). Eventually additional substances were added to the plant-based remedies - these included *Ferula assa-foetida* L., wheat flour, milk whey, salt and kerosene oil which served as vehicles for active ingredients.

2.4. Discussion

2.4.1. General characteristics of pastoral households

In Cholistan, people are predominantly practicing a nomadic life style and own small to large size herds of camels, cattle, sheep and goats, while buffalo farming is done in peripheral areas. Livestock is the backbone of the regional economy and inhabitants completely depend on livestock production for their livelihood (Akbar et al., 1996). Pastoralists are living quite below the poverty line – often basic human needs like clean drinking water, sufficient food, health and education are lacking (Khan, 1992; Akhter and Arshad, 2006). There are no roads into the interior of the desert and mostly local people use camels as a mode of transportation on sandy tracks (Akbar et al., 1996; Arshad et al., 1999).

The household heads in our study were exclusively male; the average age of LF and LH respondents of 46 and 52 years corresponds well to an average age of 48 years of male pastoralist family heads in Cholistan (Farooq et al., 2009). However, while the average livestock farming experience of LH and LF was 29 and 25 years, Farooq et al. (2009) reported an average livestock keeping experience of 32 years. At 58%, overall literacy rate is very low in Pakistan (male: 69%, female: 45%; Federal Ministry of Education, 2009), but the values we found for Cholistan were even lower and similar to those reported by Farooq et al. (2009). Due to the generally very difficult environmental and infrastructural conditions of the Cholistan region, and the high illiteracy rate, pastoralists are not well aware of modern livestock production techniques.

As far as keeping different animal species is concerned, the present data is in accordance with that of Farooq et al. (2009) who also reported high numbers of small ruminants; this is explained by the relatively low inputs needed such as startup capital, feedstuffs and maintenance expenditures as compared to large ruminants. Another reason is the sale of small ruminant meat – especially mutton of indigenous breeds is very popular in Pakistan for the Eid al-Adha festival. In other areas of Pakistan, cattle and buffalo are the preferred species because these are major milk producing animals; according to Afzal and Naqvi (2004), small-holders with up to 6 animals keep 60% buffaloes and 56% cattle, large-holders with up to 20 animals keep 6% buffalo and 10% cattle across the country.

2.4.2. Animal health care practices

The fact that 43% and 42% of the interviewed LF were classified as EVM and MR users, indicates that the majority of pastoralists in Cholistan rely on traditional health care practices. Partly, this may be due to the remoteness and poverty of the region, lack of access to veterinary services, or expensiveness of allopathic medicines. Whatever the exact reason is, EVM and MR users reportedly spent less money on animal medication and had a longer livestock-keeping experience than MVM users. This situation indirectly testifies that plant-based remedies are the main available source of medication in the community, and their use is considered safe, effective and trustworthy.

In the studied locations, different species of animals and herds share the same water sources and grazing areas, therefore free mixing of diseased animals with healthy ones expose livestock to various types of diseases. The most common diseases in large ruminants are parasitic infestations (ecto-parasitism, helminthiasis and other endo-parasitism), hemorrhagic septicemia, black quarter, foot and mouth disease, anthrax, mange, trypanosomiasis (*Trypanosoma evansi*) and camel pox. Small ruminants are also suffering from various parasitic infestations as well as enterotoxaemia, pleuropneumonia, sheep and goat pox, and anthrax (Food and Agriculture Organization, 1993; Akhtar and Arshad, 2006). Especially helminth infestations seem to be a major threat for the livestock herds in Pakistan, with a prevalence of gastrointestinal parasites of 25 - 92% across different regions (Raza et al., 2007; Khan et al., 2010). Helminthiasis can especially persist in tropical countries with favorable environmental conditions for helminth transmission (Mohanta et al., 2007), poor nutrition of the host animals (Mbuh et al., 2008) and poor sanitation facilities (Badran et al., 2012). The preoccupancy of LF with this specific health problem was evident from the fact that on average every pastoralist recommended at least one traditional remedy for the medication of helminth infestations, and overall 18.6% remedies were recorded for the treatment of helminthiasis. The five plants *Capparis decidua* (n=55 mentions), *Salsola foetida* (n=52), *Suaeda fruticosa* (n=46), *Haloxylon salicornicum* (n=42) and *Haloxylon recurvum* (n=39) were mentioned as the most effective ones for treatment of infestations with gastrointestinal parasites.

Since Pakistan comprises of different climatic zones, its natural botanical biodiversity is high; out of approximately 6000 plant species about 600 are considered as therapeutic agents and medicaments (Hamayun et al., 2003; Khan et al., 2012). Cholistan desert, from an ethno-botany point of view, is one of the least investigated areas of Pakistan with the exception of a few contributions made by Arshad et al. (2003), Farooq et al. (2008) and Khan (2009). Arshad et al. (2003) documented the medicinal plants for human medication and described their possible conservational strategies; Farooq et al. (2008) reported ethno-veterinary practices for the treatment of parasitic livestock diseases and Khan (2009) worked on wild medicinal plants for curing animal aliments in the northern part of Cholistan.

Although for the whole Cholistan desert 118 indigenous plant species are reported (Arshad et al., 2000) our respondents used only 64 of these to treat one or several animal health problems. Whether the remaining plant species are not used in traditional EMV practices or only occur outside our study region remains to be investigated. Some of the plants mentioned as remedies by our respondents, such as *Azadirachta indica, Brassica campestris*, *Calotropis procera, Calligonum polygonoides, Capparis decidua, Capparis spinosa, Citrullus colocynthis, Cyperus rotundus, Eruca sativa, Ferula assa-foetida, Haloxylon salicornicum, Lamium amplexicaule, Neurada procumbens* and *Solanum surattense* have also been reported for the same or similar medical uses in Qassim Region, Saudi Arabia (Abbas et al., 2002), Muzaffar Garh, Pakistan (Jabbar et al., 2006), Cholistan desert, Pakistan (Farooq et al., 2008), northern part of Chloistan, Pakistan (Khan, 2009), northern part of Nara desert, Pakistan (Qureshi et al., 2010), and Marmaris, Turkey (Gurdal and Kultur, 2013). However some variation exists in the mode of preparation and dosage.

The effectiveness of *Azadirachta indica,* mentioned as an anthelmintic by LF, has been scientifically validated *in vivo* by Costa et al. (2006) and Iqbal et al. (2010) against gastrointestinal nematodes of sheep. While Costa et al. (2006) could not validate its effectiveness, Iqbal et al. (2010) reported an anthelmintic activity of the methanol extract of this plant. These are the only two studies that tested the effectiveness of any of the plants mentioned by LF and LH of our study.

2.5. Conclusions

Among the long list of reported livestock diseases, infestation of small ruminants with gastrointestinal parasites seems to be a major concern of pastoralists in Cholistan, given the high numbers of sheep and goats in their herds and the numerous remedies reported for the treatment of diarrhea and dehydration. Since the majority of livestock keepers are relying on plant-based remedies for animal health care, they would benefit from an in-depth study of (i) the prevalence of gastrointestinal parasites in their flocks, and (ii) an *in vitro* and/or *in vivo* study on the effectiveness of promising plant-based remedies against major parasites. The combined outcomes of such studies would allow scientists, local healers and pastoralists alike to target and fine-tune the use of certain low cost, locally available plant-based remedies in order to improve animal health and performance and thus the livelihoods of their owners.

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Appendix 2.1: Traditional plant-based remedies used by pastoralists in Cholistan desert, Pakistan, and their related information.

No.	Botanical name	Family	Local name	Voucher no.	Respon-dents (n)	Medicinal use/ Ailment	Preparations†	Dosage *
1	*Acacia nilotica* subsp. *Indica*	Mimosaceae	Kikar	Ch-05	11	Infertility	Powder[8] of pods mixed in milk (1 L) and drench	500g LA 250g SA
2	*Acacia nilotica* subsp. *Indica*	Mimosaceae	Kikar	Ch-05	3	Diarrhoea, dysentery	Decoction[3] of leaves	250g LA 50g SA
3	*Aerva javanica* (Burm. f.) Juss. Ex Schult.	Amaranthaceae	Bhoie	Ch-12	5	Laxative	Decoction[3] of aerial parts	500g LA 200g SA
4	*Aerva javanica* (Burm. f.) Juss. Ex Schult.	Amaranthaceae	Bhoie	Ch-12	9	Skin problems	Poultice[6] made from leaves	Sufficient quantity
5	*Alhagi camelorum* Fisch.	Papilionaceae	Jawaan	Ch-14	2	Indigestion, digestive tract disorders	Decoction[3] of leaves	250g LA 100g SA
6	*Allium cepa* L.	Amaryllidaceae	Peyaz	Ch-33	42	Indigestion, digestive tract disorders	Bulb mixed with *Zingiber officinale* (100g) plus common salt (50g)	250g LA 100g SA
7	*Allium sativum* L.	Amaryllidaceae	Lehsin	Ch-43	15	Indigestion, digestive tract disorders	Bulb mixed with salt (50g) in jaggery[1]	100g LA 50g SA
8	*Azadirachta indica* A. Juss.	Meliaceae	Neem	Ch-21	18	Myiasis wounds	Leaves boiled[4] in water; wash affected area with this	Sufficient quantity
9	*Azadirachta indica* A. Juss.	Meliaceae	Neem	Ch21	23	Helminthiasis	Decoction[3] of aerial parts	1kg LA 500g SA
10	*Boerhavia procumbens* Roxb.	Nyctaginaceae	Bash khera	Ch-20	4	Respiratory tract infections, cough	Mix root powder[8] in honey	100g LA 50g SA
11	*Boerhavia procumbens* Roxb.	Nyctaginaceae	Bash khera	Ch-20	8	Skin problems, itching	Mashed leaves mixed with milk used to bandage topically	Sufficient quantity
12	*Bombax ceiba* L.	Bombacaceae	Sumbal	Ch-58	1	Laxative	Flowers as feedstuff	250g LA 100g SA
13	*Brassica campestris* L.	Brassicaceae	Sarson	Ch-49	4	Mange, lice and tick infestation	Seed oil mixed with equal quantity of kerosene oil applied topically	Sufficient quantity

No.	Botanical name	Family	Local name	Voucher no.	Respon-dents (n)	Medicinal use/ Ailment	Preparations†	Dosage *
14	*Brassica campestris* L.	Brassicaceae	Sarson	Ch-49	7	Laxative	Seed oil mixed with milk whey (1 L) and drenched	500g LA 150g SA
15	*Calligonum polygonoides* L.	Polygonaceae	Phoog	Ch-13	5	Skin problems	Decoction[3] of aerial parts	200g LA 100g SA
16	*Calligonum polygonoides* L.	Polygonaceae	Phoog	Ch-13	10	Fly repellent	Aerial parts burnt to create smoke	200g LA 100g SA
17	*Calotropis procera* (Aiton) Dryand	Asclepiadaceae	Aak	Ch-15	4	Anti-inflammatory	Milky juice[7] of plant applied to affected areas	Sufficient quantity
18	*Calotropis procera* (Aiton) Dryand	Asclepiadaceae	Aak	Ch-15	13	Indigestion, digestive tract disorders	Leaves and flowers crushed and mixed in jaggery[1]	250g LA 150g SA
19	*Calotropis procera* (Aiton) Dryand	Asclepiadaceae	Aak	Ch-15	26	Helminthiasis	Aerial parts mixed in jaggery[1]	500g LA 250g SA
20	*Capparis decidua* (Forssk.) Edgew.	Capparaceae	Kari	Ch-32	55	Helminthiasis	Aerial parts used as feedstuff	500g LA 250g SA
21	*Capparis decidua* (Forssk.) Edgew.	Capparaceae	Kari	Ch-32	12	Diarrhoea, dysentery	Fruit used as feedstuff	500g LA 250g SA
22	*Capparis spinosa* L.	Capparaceae	Kabar	Ch-31	10	Respiratory tract infection, cough	Aerial parts mixed with *Foeniculum vulgare* fruits (100g) for ball drench[2]	250g LA 100g SA
23	*Capparis spinosa* L.	Capparaceae	Kabar	Ch-31	4	Analgesic	Decoction[3] of aerial parts	500g LA 150g SA
24	*Cassia senna* L.	Caesalpiniaceae	Layie	Ch-64	1	Skin problems	Powder[8] of leaves mixed with oil to apply on affected parts	Sufficient quantity
25	*Citrullus colocynthis* (L.) Schrader	Cucurbitaceae	Tumma	Ch-03	19	Indigestion, digestive tract disorders	Powder[8] of dried fruits mixed with *Foeniculum vulgare* (250g) fruits and common salt (100g) for ball drench[2]	100g LA 50g SA
26	*Citrullus colocynthis* (L.) Schrader	Cucurbitaceae	Tumma	Ch-03	24	Helminthiasis	Dry fruit powder in jaggery[1]	250g LA 100g SA
27	*Citrus limon* (L.) Burm.f.	Rutaceae	Lemon	Ch-44	13	Nerve tonic, cooling agent	Fruit juice[7] mixed with sugar and drenched	150g LA 50g SA

No.	Botanical name	Family	Local name	Voucher no.	Respon-dents (n)	Medicinal use/ Ailment	Preparations†	Dosage *
28	*Cleome brachycarpa* Vahl ex DC.	Capparaceae	Noli	Ch-45	2	Maggots in nostrils	Powder[8] of dried leaves blown into nostrils to eliminate maggots	Sufficient quantity
29	*Cocculus hirsutus* (L.) W.Theob.	Menispermaceae	Jhoti	Ch-01	1	Nerve tonic, cooling agent	Decoction[3] of aerial parts	1kg LA 500g SA
30	*Cocculus hirsutus* (L.) W.Theob.	Menispermaceae	Jhoti	Ch-01	3	Laxative	Aerial parts as feedstuff	3kg LA 1kg SA
31	*Convolvulus arvensis* L.	Convolvulaceae	Wan wehri	Ch-34	5	Helminthiasis	Aerial parts as feedstuff	2-3kg LA 1kg SA
32	*Corchorus depressus* L.	Malvaceae	Bo phali	Ch-36	1	Diuretic	Decoction[3] of aerial parts	500g LA 200g SA
33	Crotalaria *burhia* Benth.	Fabaceae	Khip	Ch-35	8	Skin problems, itching	Paste[5] of aerial parts	Sufficient quantity
34	*Cucumis melo* L. subsp. *agrestis* var. *agrestis*	Cucurbitaceae	Chabar	Ch-16	13	Burns, skin abrasions	Leaves paste[5] on affected area	Sufficient quantity
35	*Cucumis melo* L. subsp. *agrestis* var. *agrestis*	Cucurbitaceae	Chabar	Ch-16	2	Respiratory tract infections, cough	Flowers plus *Trachyspermum ammi* (100g) mixed in jaggery[1]	250g LA 100g SA
36	*Curcuma longa* L.	Zingiberaceae	Haldi	Ch-47	1	Wounds, skin injury	Paste[5] of seeds applied on affected area	Sufficient quantity
37	*Cuscuta reflexa* Roxb.	Cuscutaceae	Loot buti	Ch-02	5	Helminthiasis	Aerial parts as feedstuff	2kg LA 1kg SA
38	*Cymbopogon jwarancusa* (Jones) Schult.	Poaceae	Katran	Ch-59	1	Respiratory tract infections, cough	Decoction[3] of leaves and flowers	250g LA 100g SA
39	*Cyperus rotundus* L.	Cyperaceae	Motha	Ch-60	4	Diuretic	Decoction[3] of roots	100g LA 50g SA
40	*Dipterygium glaucum* Decne.	Capparaceae	Phail	Ch-63	5	Antibacterial	Decoction[3] of aerial parts	250g LA 150g SA
41	*Eruca sativa* Mill.	Cruciferae	Asoon	Ch-46	3	Skin problems, scabies	Seed oil applied topically	Sufficient quantity

No.	Botanical name	Family	Local name	Voucher no.	Respon-dents (n)	Medicinal use/ Ailment	Preparations†	Dosage *
42	*Euphorbia granulata* Forssk.	Euphorbiaceae	Dhokhal	Ch-17	9	Scorpio & snake bites	Milky juice[7] of leaves applied on affected area	Sufficient quantity
43	*Farsetia hamiltonii* Royle	Brassicaceae	Gawala takri	Ch-50	4	Nerve tonic, cooling agent	Aerial parts used as decoction[3]	500g LA 150g SA
44	*Ferula assa-foetida* L.	Umbelliferae	Heeng	Ch-51	18	Helminthiasis	Fruit mix in jaggery[1] or wheat flour	50g LA 20g SA
45	*Foeniculum vulgare* Mill.	Apiaceae	Sonnef	Ch-37	30	Respiratory tract infections, cough	Fruit powder[8] mixed with *Trachyspermum ammi* (100g) and given	150g LA 50 SA
46	*Gisekia pharnaceoides* L.	Ficoidaceae	Baloka sagh	Ch-40	3	Antipyretics	Aerial parts as feedstuff	500g LA 250g SA
47	*Grewia villosa* Willd.	Tiliaceae	Jalidar	Ch-57	1	Diarrhoea, dysentery	Whole plant as feedstuff	1kg LA 500g SA
48	*Haloxylon recurvum* Bunge ex Boiss.	Chenopodiaceae	Khar	Ch-52	39	Helminthiasis	Aerial parts mixed in jaggery[1]	150g LA 50g SA
49	*Haloxylon salicornicum* (Moq.) Bunge	Chenopodiaceae	Gora lana	Ch-04	42	Helmintniasis	Aerial parts mixed in jaggery[1]	250g LA 150g SA
50	*Haloxylon salicornicum* (Moq.) Bunge	Chenopodiaceae	Gora lana	Ch-04	17	Fly repellent	Aerial parts burnt to create smoke	250g LA 150g SA
51	*Heliotropium crispum* Desf.	Boraginaceae	Kali boti	Ch-22	5	Otitis	Fresh leaves mash is inserted into the ear canal to relieve ear pain	Sufficient quantity
52	*Hibiscus cannabinus* L.	Malvaceae	Patsan	Ch-24	1	Antipyretic	Decoction[3] of aerial parts	500g LA 150g SA
53	*Hibiscus cannabinus* L.	Malvaceae	Patsan	Ch-24	4	Skin problem, itching	Paste[5] of seeds applied topically	Sufficient quantity
54	*Lamium amplexicaule* L.	Lamiaceae	Phatokan boti	Ch-18	12	Helminthiasis	Leaves mixed in jaggery[1]	250g LA 100g SA
55	*Lasiurus hirsutus* (Forssk.) Boiss.	Poaceae	Gorakh pan	Ch-41	7	Tympany, bloat	Decoction[3] of aerial parts	1kg LA 250g SA

No.	Botanical name	Family	Local name	Voucher no.	Respon-dents (n)	Medicinal use/ Ailment	Preparations†	Dosage *
56	*Launaea nudicaulis* (L.) Hook. f.	Compositeae	Bhatal	Ch-42	1	Helminthiasis	Aerial parts used as feedstuff	2-3kg LA 1kg SA
57	*Lawsonia inermis* L.	Lythraceae	Mehndi	Ch-61	2	Wounds, skin injury	Paste[5] of leaves applied on affected area	Sufficient quantity
58	*Leptadenia spartium* Wight. (*Gymnema spartium* Wall.)	Asclepiadaceae	Chag	Ch-25	6	Expulsion of placenta	Decoction[3] of aerial parts after parturition	1kg LA 250g SA
59	*Leptadenia spartium* Wight. (*Gymnema spartium* Wall.)	Asclepiadaceae	Chag	Ch-25	1	Analgesic	Paste[5] of leaves on affected area	Sufficient quantity
60	*Mollugo nudicaulis* Lam.	Molluginaceae	Gandi boti	Ch-09	1	Wound and injury healing	Aerial parts boiled[4] in water to wash wounds	Sufficient quantity
61	*Neurada procumbens* L.	Neuradaceae	Chapri buti	Ch-30	4	Nerve tonic, cooling agent	Decoction[3] of aerial parts	500g LA 150g SA
62	*Oligochaeta ramosa* Roxb.	Asteraceae	Barm dendi	Ch-10	7	Antipyretic	Decoction[3] of aerial parts	500g LA 200g SA
63	*Panicum antidotale* Retz.	Poaceae	Bansi ghah	Ch-26	2	Respiratory tract infections, cough	Aerial parts mixed in jaggery[1]	100g LA 50g SA
64	*Peganum harmala* (L.) St.-Lag.	Zygophyllaceae	Hermal	Ch-27	10	Fly repellent	Leaves burnt to create smoke	Sufficient quantity
65	*Piper nigrum* L.	Piperaceae	Piper	Ch-28	5	Indigestion, digestive tract disorders	Seed powder[8] used for ball drench[2]	100g LA 50g SA
66	*Polygonum plebium* R.Br.	Polygonaceae	Hikhthra/ Chirri hatha	Ch-38	1	Antipyretic	Decoction[3] of aerial parts	1-2kg LA 500g SA
67	*Prosopis cineraria* (L.) Druce	Fabaceae	Kandi boti	Ch-54	6	Mastitis	Decoction[3] of flowers	500g LA 150g SA
68	*Prosopis cineraria* (L.) Druce	Fabaceae	Kandi boti	Ch-54	2	Anti-inflammatory	Poultice[6] of leaves applied on affected area	Sufficient quantity
69	*Ricinus communis* L.	Euphorbiaceae	Hernoli	Ch-11	28	Laxative	Seed oil drenching	500g LA 200g SA

No.	Botanical name	Family	Local name	Voucher no.	Respon-dents (n)	Medicinal use/ Ailment	Preparations†	Dosage *
70	*Ricinus communis* L.	Euphorbiaceae	Hernoli	Ch-11	8	Expulsion of placenta	Decoction[3] of leaves	1kg LA 500g SA
71	*Salsola foetida* Del. ex Spreng.	Amaranthaceae	Lani	Ch-55	52	Helminthiasis	Aerial parts mixed in jaggery[1]	250g LA 150g SA
72	*Salvadora oleoides* Decne.	Salvadoraceae	Bheal buti/ Jhal	Ch-56	1	Expulsion of placenta	Dried fruit mix in jaggery[1] after parturition	500g LA 150g SA
73	*Solanum surattense* Burm. f.	Solanaceae	Khandyri	Ch-62	12	Wounds, skin injury	Fruits boiled[4] in water to wash wounds	Sufficient quantity
74	*Suaeda fruticosa* (L.) Forssk.	Chenopodiaceae	Kali lani	Ch-19	46	Helmintniasis	Aerial parts used for ball drench[2]	250g LA 150g SA
75	*Suaeda fruticosa* (L.) Forssk.	Chenopodiaceae	Kali lani	Ch-19	15	Diuretic	Decoction[3] of leaves	250g LA 150g SA
76	*Syzygium cumini* (L.) Skeels	Myrtaceae	Jaman	Ch-23	7	Indigestion, digestive tract disorders	Powder[8] of seeds for ball drench[2]	250g LA 150g SA
77	*Syzygium cumini* (L.) Skeels	Myrtaceae	Jaman	Ch-23	2	Anti-inflammatory	Poultice[6] of leaves	Sufficient quantity
78	*Tamarix aphylla* (L.) Karst.	Tamaricaceae	Wan boti/ Ukan	Ch-39	1	Diarrhoea (chronic), dysentery	Decoction[3] of aerial parts	1kg LA 250g SA
79	*Trachyspermum ammi* (L.) Sprague ex Turril	Apiaceae	Ajwain	Ch-53	3	Helminthiasis	Seed powder[8] mixed in jaggery[1]	250g LA 100g SA
80	*Tribulus terrestris* L.	Zygophyllaceae	Bakhra boti	Ch-48	1	Colic	Decoction[3] of leaves	1kg LA 250g SA
81	*Tribulus terrestris* L.	Zygophyllaceae	Bakhra boti	Ch-48	2	Respiratory tract infections, cough	Leaf mixed in jaggery[1]	250g LA 100g SA
82	*Withania coagulans* Dunal.	Solanaceae	Peener	Ch-07	2	Helminthiasis	Aerial parts mix in jaggery[1]	250g LA 100g SA
83	*Withania somnifera* (L.) Dunal.	Solanaceae	Aakri boti	Ch-08	1	Helminthiasis	Aerial parts as feedstuff	1kg LA 250g SA
84	*Zygophyllum simplex* L.	Zygophyllaceae	Lonak	Ch-06	2	Skin problems, itching	Paste[5] of leaves on affected area	Sufficient quantity

No.	Botanical name	Family	Local name	Voucher no.	Respon-dents (n)	Medicinal use/ Ailment	Preparations[†]	Dosage *
85	*Ziziphus spina-christi* (L.) Willd.	Rhamnaceae	Beri	Ch-29	10	Diarrhoea, dysentery	Aerial parts used as feedstuff	1kg LA 500g SA
86	*Ziziphus spina-christi* (L.) Willd.	Rhamnaceae	Beri	Ch-29	1	Antipyretic	Decoction[3] of aerial parts	2kg LA 1kg SA

* LA = large animal (cattle, buffalo, camel, horse), SA = small animal (sheep, goat)

[Ƭ] Normally a single dose of traditional remedy was given on daily basis; in case of severe conditions two applications per day. Medication continued until the symptoms of disease disappeared (on average 5-11 days).

[1] Jaggery: plant is mixed in concentrated product of cane juice or date juice.

[2] Ball drench: plant is coated/covered inside a ball of wheat flour or molasses.

[3] Decoction: plant parts are boiled in water for several hours and the extract is used for drenching.

[4] Boiling: plant parts are plunged in hot water for several hours. Then this water is allowed to cool down and used topically.

[5] Paste: fresh plant is crushed to obtain a semi-solid paste used externally.

[6] Poultice: plant material is crushed to obtain a soft moist mass and applied topically to treat swellings, pain, inflamed or infected body parts.

[7] Juice: plant material is squeezed, filtered through cloth and sometimes requires addition of freshwater or other liquid for dilution.

[8] Powder: grinding the dried plant parts.

CHAPTER 3

PREVALENCE OF GASTROINTESTINAL HELMINTHS IN PASTORAL SHEEP AND GOAT FLOCKS IN THE CHOLISTAN DESERT OF PAKISTAN

Prevalence of gastrointestinal helminths in pastoral sheep and goat flocks in the Cholistan desert of Pakistan

Abstract

Small ruminants are a major source of cash for many rural populations, especially in semi-arid and arid regions of developing countries. Extensively managed animals often host gastrointestinal parasites, and even chronic infestations lead to economic losses. We evaluated the prevalence of gastrointestinal helminths in sheep and goats of the Cholistan desert, Pakistan, where livestock is the backbone of the regional economy. Fresh faeces (10 - 15 g) were collected from 500 sheep and 500 goats across five different localities. Standard parasitological techniques served to identify parasite eggs, and copro-culture enabled larval determination of specific nematodes.

Overall helminth prevalence was 78.1% across the 1000 animals; pure nematode infestations were most prevalent (37.5%), followed by pure trematode (7.9%), pure cestode (2.6%) and pure protozoa infestations (0.8%). Mixed infestations with nematodes and trematodes occurred in 6.4% of all animals, mixed nematode-cestode infestations in 3.8%, and all three groups were found in 19.1% of the sheep and goats. In goats more males (81.1%) than females (77.0%) were infested, the opposite was found in sheep (73.6% males, 79.5% females). Parasites were especially prevalent in suckling goats (85.2%) and sheep (88.5%) and to a lesser extent in young (goats 80.6%, sheep 79.3%) and adult animals (goats 72.8%, sheep 73.8%).

Given the high infestation rates, particular attention should be paid to management of suckling animals. A general means of reducing infestation rates might be the systematic testing of traditional plant-based remedies against helminths for cheap and regular deworming of the herds.

Keywords: Cestodes; Extensive grazing system; Gastrointestinal parasites; Small ruminants; Nematodes; Trematodes.

3.1. Introduction

Livestock production contributes substantially to the livelihoods of resource-poor rural farmers in Pakistan and plays an imperative role in poverty alleviation by strengthening the socio-economic conditions of pastoralists (Gadahi *et al*., 2009). Pakistan's livestock sector provides approximately 55.1% of the agricultural value added and 11.6% of the national GDP. The gross value added of this segment at constant factor cost has increased by 4% from 5.29 billion Euro (2010-2011) to 5.51 billion Euro (2011-2012; Economic Survey of Pakistan, 2012). Historically the livestock sector was subsistence-oriented and dominated by smallholders, and even today livestock are considered a secure source of income for small farmers and landless poor, and a source of employment generation at the rural level (Gadahi *et al*., 2009; Khajuria *et al*., 2012). This is particularly true for sheep and goats (Khajuria *et al*., 2012), of which numbers have doubled in Pakistan during the past 15 years (Iqbal and Jabbar, 2005). This can be ascribed to the relatively low inputs needed such as startup capital, feedstuffs and maintenance expenditures as compared to large ruminants (Terefe *et al*., 2012).

Small ruminants are under sober coercion of clinical and sub-clinical gastrointestinal helminth infestation in developing countries, which reduces their productive and reproductive potential (Zeryehun, 2012; Ayaz *et al*., 2013) due to decreasing voluntary feed intake and/or feed conversion efficiency (Kanyari *et al.,* 2009). Especially the ineffective use of absorbed nutrients leads to retarded growth (Sykes, 1994; Terefe *et al*., 2012) and provokes anemia and even mortality at heavy infestation (Hassan *et al.,* 2011). In addition to these threats, a helminth infestation lowers the animal's immunity and renders it susceptible to other pathogenic infections; finally this may result in heavy economic losses (Garedaghi *et al.,* 2011). The problem is however much more severe in tropical countries due to very favorable environmental conditions for helminth transmission (Mohanta *et al*., 2007; Zeryehun, 2012), poor nutrition of the host animal (Mbuh *et al*., 2008) and poor sanitation in rural areas (Badran *et al*., 2012). As a result diseases caused by helminths remain a major impediment to small ruminant production in the tropics (Kumsa *et al*., 2011), and up to 95% of small ruminants are reported to show helminth infestation in these latitudes (Opara *et al*., 2005; Mbuh *et al*., 2008; Terefe *et al*., 2012). However, the majority of animals infested with helminths do not show clinical signs owing to the chronic nature of the disease.

Three classes of helminths are distinguished, namely nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes). Several authors (Raza *et al.,* 2007, 2012; Khan *et al*., 2010; Farooq *et al*., 2012) have explored various aspects of helminth infestation in different localities of Pakistan and reported prevalence ranges of 25 - 92%. There are still numerous geographical regions in Pakistan where the livestock population needs to be screened for the presence of gastrointestinal helminths in view of their high economic significance.

Apart from Farooq et al. (2012) who investigated helminth infestation in domesticated and wild ruminants, no further report is available on the prevalence of gastrointestinal helminths in the Cholistan desert of Pakistan, where livestock husbandry is the primordial occupation of the pastoralist communities and where traditionally wealth has been assessed based on the number of animals, especially goats and sheep, owned by an individual. Since the resource-poor pastoralists of this area have very limited access to veterinary services, an analysis of the *status quo* of helminth infestation of their animals should precede the design of appropriate and accessible means to effectively counteract eventual problems. This was the objective of the present study.

3.2. Material and methods

3.2.1. Study area

The Cholistan desert covers an area of 2.6 Mio. hectares and is located in the southern Punjab between latitudes 27°42′ and 29°45′ N and longitudes 69°52′ and 75°24′ E (FAO, 1993). Average annual rainfall is only 128 - 175 mm, therefore crop cultivation is only possible under irrigation near streams or wells. The region’s total livestock population has been estimated at 1.29 Mio. heads (Livestock Census of Pakistan, 2006), which is almost twice that of the human population in this area.

The people of Cholistan lead a semi-nomadic life, moving from one place to another in search of water and fodder for their animals. For their livestock and for themselves, the local tribes store rainwater in man-made ponds in the ground or between sand-hills – these ponds are called ‘*tobas*’. Habitations are small and scattered around the *tobas*. For this study five localities that were at least 30 km distant from each other were selected, each locality comprising several villages and *tobas*, respectively (Figure 3.1).

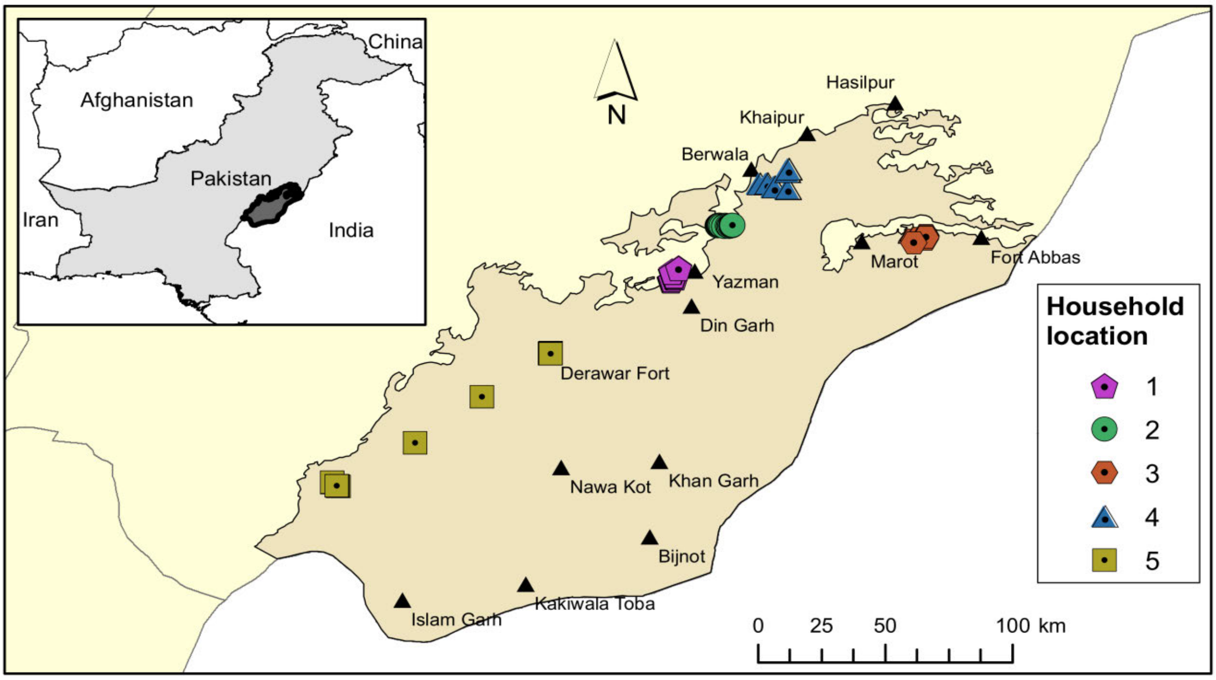

Figure 3.1: Map of the study area in the Cholistan desert, Pakistan, with the homesteads of the sampled sheep and goat herds and position of the five locations.

3.2.2. Sample collection

Five hundred sheep and 500 goats, from a total of 100 pastoral flocks (comprising each between 5 – 250 animals) across the 5 localities were randomly selected. A ratio of 20 : 40 : 40 of suckling, young, and adult animals was sampled in each species, and the male to female ratio was 30 : 70. From January to May 2011, one sample of fresh faeces (approx. 10 - 15 g) was collected from the rectum of each animal by using plastic gloves. The sample was put in a sterile zipper polythene bag coded with the locality, household ID, species, age and sex of the animal. Samples were placed into an air tight cool box until arrival in the laboratory and were refrigerated at 4°C until analysis (Hayat and Akhtar, 2000). After faecal examination, samples were preserved in 10% formalin for backup purpose.

3.2.3. Faecal examination

Examination of faecal samples was performed by using standard direct and indirect parasitological techniques (flotation and sedimentation) as suggested by Hayat and Akhtar (2000) and Soulsby (1987). Eggs of the different helminths were identified on the basis of morphological appearance and size with the help of keys (MAFF, 1979; Soulsby, 1987; Urquhart *et al*., 1996). For the identification of certain nematodes, coproculture was performed to obtain larval stages as described by MAFF (1979). Faecal samples containing parasitic eggs that could not exactly be identified were finely crushed with a pestle and mortar or a spatula, and were placed in a glass jar or petri-dish which was closed and incubated at 26°C for 7 days.

After incubation, samples were examined for the presence of larvae, which were identified with the help of keys (MAFF, 1979).

3.2.4. Data analysis

Differences between independent variables (location, host species, sex and age group) with respect to prevalence of individual parasite species, or helminth groups, respectively, were explored using Chi-square test (categorical variables) or Kruskal-Wallis test (continuous variables), whereby continuous variables had first been tested for normality (Kolmogorov-Smirnov test). Data was analyzed using SPSS 17.0 software (SPSS Inc., Chicago, USA). Relative prevalence of different helminth species or groups, respectively, was calculated as follows:

Prevalence (%) = [Number of positive samples / Total number of samples examined] x 100

3.3. Results

The overall prevalence of gastrointestinal (GI) parasites in the 1000 sheep and goats was 78.1%, with 78.2% (n=391) in goats and 78.0% (n=390) in sheep (Figure 3.2). In goats, 81.1% (116 of 143) male and 77.3% (275 of 357) female animals harbored GI parasites, whereas 73.6% (95 of 129) male and 79.5% (295 of 371) female animals were infected in sheep (Figure 3.3). As far as different age groups were concerned, 85.2% (75 of 88) suckling, 80.6% (166 of 206) young and 72.8% (150 of 206) adult goats were parasite-infested, whereas in sheep infection rates amounted to 88.5% (69 of 78) in suckling, 79.3% (138 of 174) in young and 73.8% (183 of 248) in adult animals (Figure 3.4).

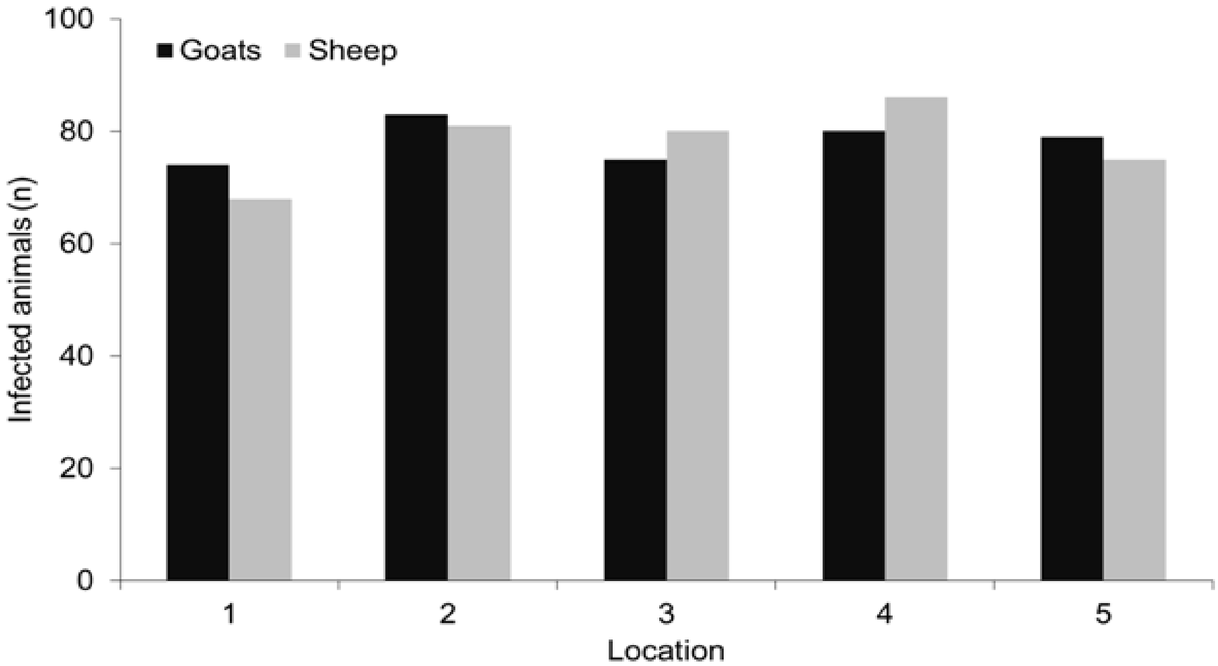

Figure 3.2: Prevalence of gastrointestinal parasites in goats and sheep (100 each per location) of pastoralists distributed across five locations in the Cholistan desert, Pakistan.

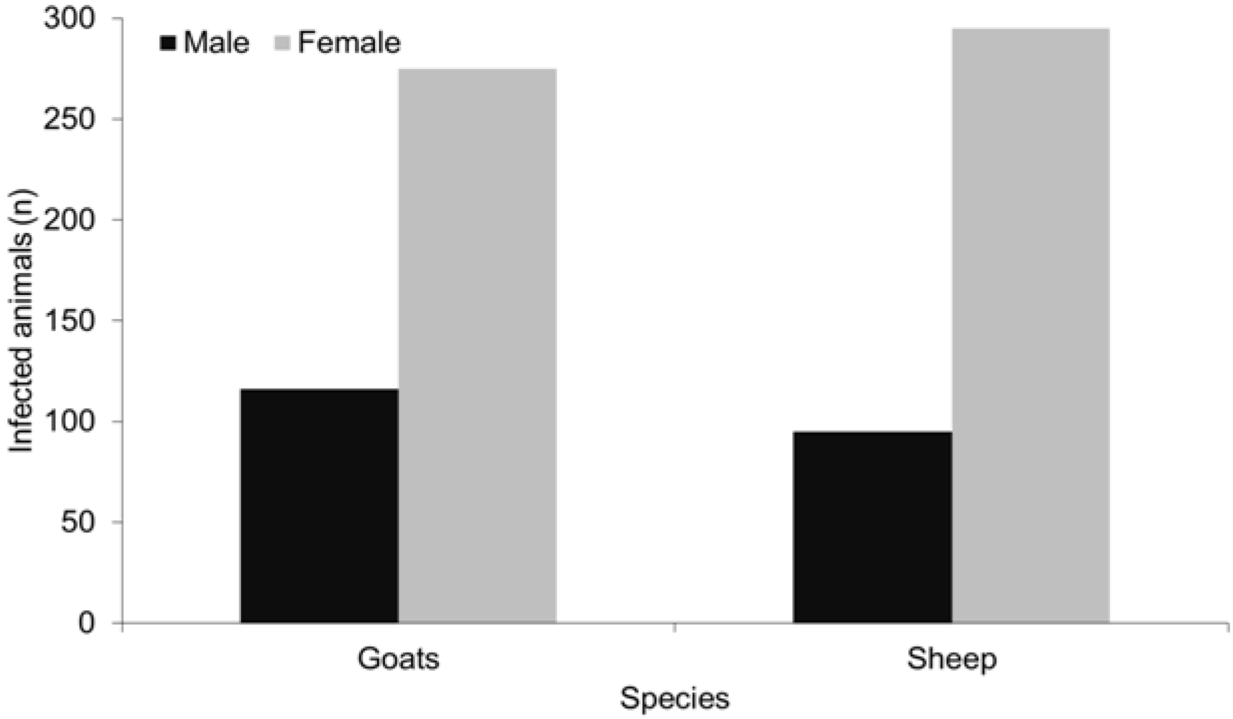

Figure 3.3: Prevalence of gastrointestinal parasites in goats (male=143, female=357) and sheep (male=129, female=371) of pastoralists in the Cholistan desert, Pakistan.

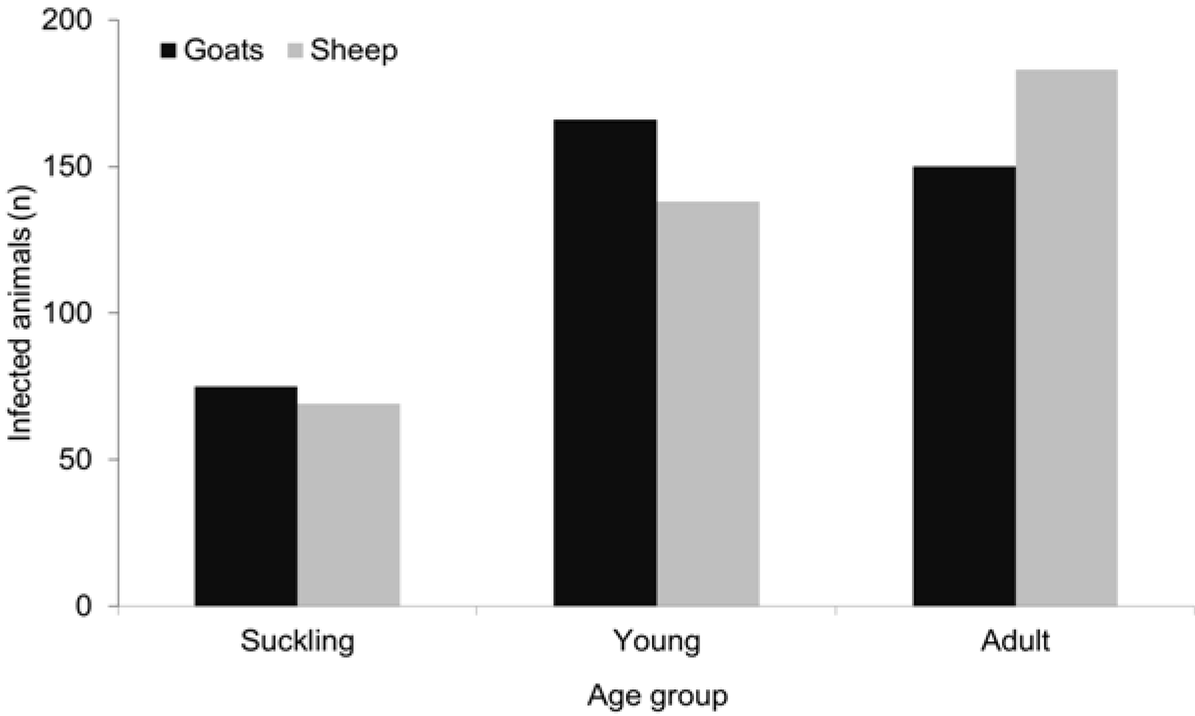

Figure 3.4: Prevalence of gastrointestinal parasites in goats (g) and sheep (s) of different age groups (suckling: g=88, s=78; young: g=206, s=174; adult: g=206, s=248) of pastoralists in the Cholistan desert, Pakistan.

As far as the identified 24 parasite species are concerned, 15 were nematodes, 4 were trematodes, 4 were cestodes and one was a protozoa. The protozoa species was mostly found in combination with helminths, its exclusive prevalence was 0.8%. While suckling animals hosted on average 1.5 – 7.6 different parasite species, this range reduced to 1.8 – 6.0 in young and 1.4 – 5.6 in adult animals, with significant differences between locations, sex and age groups but not between species (Table 3.1). The prevalence of 18, 17, 13 and 11 individual parasites varied significantly between locations, animal species, sex and age groups, respectively (Table 3.2). *Haemonchus contortus* was the most dominant parasite (13.3%), followed by *Trichuris ovis* (5.6%) and *Trichostrongylus axei* (4.7%).

Location significantly ($P \leq 0.01$) affected the overall prevalence (n=1000) of only nematodes (37.5%) and only trematodes (7.9%) in individual animals, with locations 2 and 5 having the highest nematode (9.6%) and trematode infestation (2.8%), respectively, and at the same time the lowest prevalence of trematodes (0.5%) and nematodes (5.6%). Sex had a significant effect ($P \leq 0.05$) on the prevalence of only cestodes (0.2% in males versus 2.4% in females). Mixed infections (Figure 3.5) were subdivided into (i) simple poly-parasitism, that is infection with species belonging to two different parasite groups (protozoa or helminth species) and (ii) multiple poly-parasitism, namely infection with parasites belonging to more than two different groups. Location and animal species had a significant effect ($P \leq 0.01$) on the prevalence of simple poly-parasitism, whereas occurrence of multiple poly-parasitism was significantly affected by location ($P \leq 0.01$), sex ($P \leq 0.05$) and age group ($P \leq 0.05$). Location and species together had a significant effect on the prevalence of only nematodes and only trematodes, and on the occurrence of simple and multiple poly-parasitism ($P \leq 0.01$ in all cases). In female animals, interactions between animal species and age group significantly affected poly-parasitism of nematodes plus trematodes ($P \leq 0.01$) and nematodes plus cestodes ($P \leq 0.05$). In young animals, interactions between animal species and sex significantly ($P \leq 0.01$) affected the infestation with only nematodes (female sheep 15.6%, male sheep 3.8%, female goats 13.0%, male goats 5.1%) as well as poly-parasitism of nematodes plus trematodes ($P \leq 0.05$), while in adult animals the interactions between animal species and sex affected simple poly-parasitism (nematodes plus trematodes as well as nematodes plus cestodes; $P \leq 0.01$) and multiple poly-parasitism ($P \leq 0.05$). Only in sheep, but not in goats, the interaction between sex and age group significantly affected the prevalence of cestodes ($P \leq 0.01$) and multiple poly-parasitism ($P \leq 0.05$). No case of simple poly-parasitism with trematodes and cestodes was found (Figure 3.5).

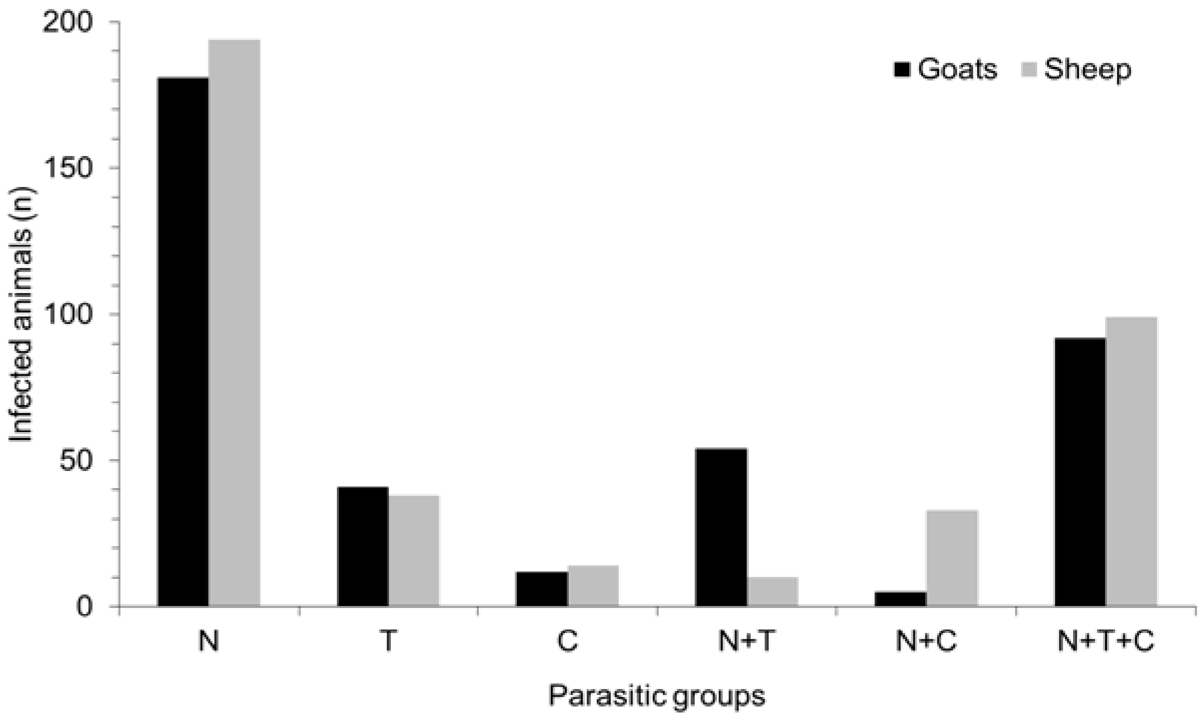

Figure 3.5: Prevalence of single or mixed infestations with gastrointestinal nematodes (N), trematodes (T) and cestodes (C) in 500 goats and 500 sheep of pastoralists in the Cholistan desert, Pakistan.

Table 3.1: Total mean number of different species of gastrointestinal parasites (±SD) found in 1000 goats and sheep* of pastoralists in the Cholistan desert, Pakistan, and the respective statistical effects of location**, age group#, livestock species and sex.

	Male			Female		
Location	**Suckling**	**Young**	**Adult**	**Suckling**	**Young**	**Adult**
1	7.6 ±6.32	3.0 ±5.45	2.1 ±3.81	1.5 ±2.04	2.7 ±3.10	1.4 ±2.70
2	7.0 ±5.48	3.1 ±4.80	2.2 ±3.49	5.0 ±4.63	3.6 ±4.34	2.6 ±3.94
3	3.6 ±3.86	3.9 ±4.22	2.5 ±3.02	3.1 ±4.21	1.8 ±2.85	1.7 ±2.79
4	4.0 ±4.17	6.0 ±5.25	5.6 ±4.84	4.9 ±5.14	3.9 ±4.37	3.6 ±4.05
5	5.1 ±5.29	4.3 ±5.26	3.8 ±4.80	2.7 ±3.53	3.5 ±4.58	3.4 ±3.99

Variable	**Df**	**Mean**	**SD**	**χ^2**	***P***
Location	4	3.00	1.414	30.15	<0.001
Age group	2	2.29	0.733	19.41	<0.001
Species	1	1.50	0.500	0.59	0.444
Sex	1	1.73	0.445	4.46	0.035

* Kruskal Wallis test; as livestock species had no effect on number of species of gastrointestinal parasites per animal, average values are presented across goats and sheep.

** For emplacement of locations see Figure 3.1.

Age groups: suckling (≤3 months), young (4-18 months), adult (>18 months).

Table 3.2: Prevalence of different species of gastrointestinal parasites in goats and sheep of pastoralists in the Cholistan desert, Pakistan, and the respective statistical effects of livestock species, location, age and sex.

Parasite	Prevalence (%)			Species	Location*	Age**	Sex
	Goats (n=500)	Sheep (n=500)	Overall (n=1000)	$P \leq$ [#]			
Bunostomum phlebotomum	1.4	1.4	1.4	n.s.	0.01	0.05	0.05
Chabertia ovina	0.6	0.4	0.5	0.05	0.05	n.s.	n.s.
Cooperia spp.	0.0	0.2	0.1	0.01	n.s.	0.05	0.05
Cotylophora cotylophorum	0.4	0.6	0.5	0.01	0.01	0.01	n.s.
Dicrocoelium dendriticum	1.4	0.4	0.9	0.05	0.01	n.s.	0.05
Echinococcos granulosus	0.8	0.6	0.7	0.05	n.s.	n.s.	0.01
Eimeria spp.	1.2	0.4	0.8	n.s.	0.01	0.05	0.01
Fasciola gigantica	0.6	0.8	0.7	0.01	0.01	0.05	n.s.
Fasciola hepatica	3.2	2.2	2.7	0.01	n.s.	n.s.	0.05
Haemonchus contortus	12.8	13.8	13.3	n.s.	n.s.	0.05	0.01
Haemonchus placei	0.0	0.2	0.1	n.s.	0.01	n.s.	0.01
Moniezia benedeni	0.2	0.2	0.2	0.01	0.05	0.01	0.01
Moniezia expansa	1.0	1.2	1.1	0.01	0.01	n.s.	n.s.
Nematodirus spp.	1.4	0.8	1.1	n.s.	n.s.	0.05	n.s.
Oesophagostomum columbianum	1.0	2.0	1.5	0.01	0.01	0.05	n.s.
Oesophagostomum radiatum	1.8	1.4	1.6	0.01	0.01	n.s.	n.s.
Ostertagia circumcincta	0.8	1.2	1.0	0.01	n.s.	n.s.	0.01
Ostertagia oestertagi	1.6	3.0	2.3	0.01	0.05	0.05	0.05
Paramphistomum cervi	3.0	4.2	3.6	0.01	0.01	n.s.	n.s.
Strongyloides papillosus	1.4	1.2	1.3	0.01	0.01	n.s.	n.s.
Trichostrongylus colubriformis	0.2	1.2	0.7	n.s.	0.01	0.01	0.01
Trichostrongylus axei	3.8	4.6	4.2	n.s.	0.05	n.s.	n.s.
Trichostrongylus spp.	2.2	3.4	2.8	0.01	0.01	n.s.	0.05
Trichuris ovis	7.2	4.0	5.6	n.s.	0.01	n.s.	n.s.

*For locations see Figure 1; **Age groups: suckling (≤3 months), young (4-18 months), adult (>18 months);
[#]Chi square test; n.s. = non significant.

3.4. Discussion

Epidemiology is the foundation on which control of parasitic diseases has to be based. Therefore the findings of this study are indicative for other small ruminant herds under similar management in this environment. The high prevalence of parasites in the screened pastoral sheep and goat flocks supports the notion that parasitic infestations are among the main health problems in small ruminants globally (Wang *et al*., 2006; Mbuh *et al*., 2008; Lone *et al*., 2012; Badran *et al*., 2012; Kantzoura, 2012).

The helminth species and groups recorded in the study area have also been reported previously by Raza *et al*. (2007), Gadahi *et al*. (2009), Khan *et al*. (2010) and Ayaz *et al*. (2013) from different areas of Pakistan and other parts of the world (Biu *et al*., 2009: Nigeria; Mohanta *et al*., 2007: Bangladesh; Kanyari *et al*., 2009: Kenya; Dagnachew *et al*., 2011: Ethiopia). Yet, the mentioned studies also reported some other helminth species in addition to those recorded in Cholistan, and variation also exists in the prevalence of different helminth species in different regions, which may be attributed to different host factors and climatic conditions required for the development of the free-living stages of different parasites (Tariq *et al.,* 2010). Farooq *et al*. (2012) assessed the prevalence of gastrointestinal helminths infestation among wild and domestic ruminants in Cholistan desert and recorded higher prevalence in sheep (44%) than goats (39%); with *Haemonchus* and *Trichostrongylus* being the most frequently recorded genera.

The Cholistan sheep and goats were infected with the same principal parasitic species, and one reason for this could be that samples were collected from animals in mixed flocks. Grazing patterns and managerial practices of the pastoralists are almost the same for both species, and sheds, pastures and watering places are shared between sheep and goats. The year-round movements of sheep and goat flocks over a large area may greatly facilitate the spread of parasites. Wild ruminants occurring in the region, such as chinkara (*Gazella bennettii)* and blackbuck (*Antilope cervicapra*) constitute a reservoir of helminths for their domestic relatives (Farooq *et al*., 2012).

The age of the host animal was an important factor influencing the prevalence of GI parasites. The higher infection of suckling than young and adult animals may be attributed to a weaker immunological response of young animals. Several authors (Urquhart *et al*., 1996; Tariq *et al.,* 2010; Zeryehun, 2012) reported that older animals recover from parasitic infection more quickly as the immunity of the host increases with age; animals may hence become immune, especially as they undergo repeated exposure (Dagnachew *et al*., 2011).

It is interesting to note that in goats the prevalence of helminths was higher in males compared to females, while in sheep the opposite was found. Normally females are assumed to be more heavily infested due to hormonal differences and stress during pregnancy. In goats the present results may be due to the stall feeding of female animals during pregnancy, which reduces exposure to pasture contamination (Pal and Qayyum, 1992; Maqsood *et al*., 1996; Ayaz *et al*., 2013).

In the present study nematode infections were highly prevalent, followed by trematodes and cestodes. Similar results have been reported for Owerri, southeastern Nigeria (Opara *et al*., 2005); Muzaffargarh, Pakistan (Raza *et al*., 2007), Central Oromia, Ethiopia (Kumsa *et al*., 2011) and Haramaya, southeastern Ethiopia (Zeryehun, 2012). Similarly, the occurrence of simple and multiple poly-parasitism in small ruminants agrees with findings from several locations in Pakistan (Raza *et al*., 2007: Muzaffargarh; Gadahi *et al*., 2009: Rawalpindi and Islamabad) and Ethiopia (Kumsa *et al*., 2011; Tefere *et al*., 2012; Zeryehun, 2012). Poly-parasitism is an important cause of morbidity and loss of production in sheep and goats (Kumsa *et al*., 2011). The impairment of the host's immune system by poly-parasitism increases the animal's susceptibility to other diseases or parasites (Wang *et al*., 2006).

Many nematode species do not require an intermediate host for the completion of their life cycle (Gulland and Fox, 1992), and for egg hatching and larval development they find suitable conditions around the water reservoirs of the *tobas*. The most prevalent nematode determined in this study was *Haemonchus contortus,* which agrees with findings from Himachal Pradesh, India (Katoch, 1999), Bokova, Cameroon (Mbuh *et al*., 2008) and Jatoi, Pakistan (Raza *et al.,* 2012). The high prevalence could be related to the fact that this nematode has a relatively short generation interval and lays up to 10,000 eggs per day for several months. Additionally, this parasite is able to develop resistance against anthelmintics faster than other helminth species (Radostits *et al*., 1994; Katoch, 1999).

3.5. Conclusions

In Cholistan pastoralist flocks of small ruminants are heavily infested with a variety of GI parasites including some that potentially entail substantial economic losses. Especially suckling animals carry a severe helminth burden, which indicates that particular attention should be paid to their management. In view of high prices, unavailability or inaccessibility of drugs and veterinary services, systematic deworming of animals with a broad-spectrum anthelminthic cannot be recommended to pastoralists unresentedly. Therefore inexpensive locally applied measures such as the use of plant-based remedies against GI parasites should be systematically evaluated for their effectiveness against the most prevalent helminth species, so as to devise cheap but effective remedies that pastoralists can synthesize themselves and use regularly.

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CHAPTER 4

COMPARATIVE *IN VITRO* EFFICACY OF SELECTED MEDICINAL PLANTS FROM CHOLISTAN AGAINST GASTROINTESTINAL HELMINTHS OF SHEEP AND GOATS

Comparative *in vitro* efficacy of selected medicinal plants from Cholistan against gastrointestinal helminths of sheep and goats

Abstract

Gastrointestinal helminths are a prime risk to livestock, especially small ruminants, affecting the animals' well-being and productive potential. Synthetic anthelmintics can control the problem but high prices, unavailability, side effects, and development of parasite resistance limit drug use in many pastoral systems. The strategic use of traditional medicinal plants might therefore be an effective low-cost alternative. To this end, the *in vitro* anthelmintic activity of leaf extracts of five ligneous plants (*Capparis decidua*, *Salsola foetida, Suaeda fruticosa, Haloxylon salicornicum, Haloxylon recurvum)* from the Cholistan desert, Pakistan, was investigated against adult worms of *Haemonchus contortus*, *Trichuris ovis* and *Paramphistomum cervi.* Various concentrations (range 7.8 to 500 mg dry matter/ml) of three types of extracts, i.e. aqueous, methanol, and aqueous-methanol (30:70), of each plant were used at different time intervals to access their anthelmintic activity. Levamisol (0.55 mg/ml) and oxyclozanide (30 mg/ml) served as positive, and phosphate buffered saline as negative control.

All extracts exhibited minimum and maximum activity at 2 h and 12 h after parasite exposure; the 500 mg/ml extract concentrations were most effective. Results of the Kruskal-Wallis test showed that plant species ($P<0.05$), extract type ($P<0.01$), parasite species ($P<0.01$), extract concentration ($P<0.01$), time of exposure ($P<0.01$) and their interactions ($P<0.01$) had significant effects on the number of immobile/dead helminths. From the comparison of LC_{50} values it appeared that the aqueous extract of *C. decidua* was more potent against *H. contortus* and *T. ovis,* while the aqueous extract of *S. foetida* was effective against *P. cervi.* The methanol extract of *H. recurvum* was most potent against all three types of parasites, and its aqueous-methanol extract was also very effective against *T. ovis* and *P. cervi*. Further studies are now needed to investigate the *in vivo* anthelmintic activity of these plants and plant extracts, respectively, in order to develop effective, cheap and locally available anthelmintics for pastoralists in Cholistan and neighboring desert regions.

Keywords: Anthelmintic activity; *Haemonchus contortus*; LC_{50}; Small ruminants; *Trichuris ovis*; *Paramphistomum cervi*.

4.1. Introduction

Pakistan's livestock sector is contributing approximately 55% of the agricultural value added and 11.6% of the national gross domestic production (Economic Survey of Pakistan, 2012). Sheep and goat keeping is an important livestock sub-sector, given the relatively low costs of inputs including life animals, housing structures and feedstuffs, especially when compared to dairy cattle or buffaloes (Terefe et al., 2012).

Helminthiasis is a major constraint to small ruminant keeping in extensive rural holdings (Raza et al., 2007), reducing feed consumption and/or feed conversion efficiency, delaying growth or inducing weight losses, decreasing milk production, fertility, morbidity and even mortality at heavy infestation (Terefe et al., 2012). In addition to these threats, helminth infestations also deteriorate the animal's immune status and the host becomes susceptible to other (pathogenic) infections, eventually resulting in substantial economic losses (Garedaghi et al., 2011).

Synthetic anthelmintics have long been considered the only effective way of controlling this problem but high prices, unavailability and scarcity in remote areas, side effects, chemical residues in products and environmental toxicity problems, as well as development of resistance of the targeted parasites (Jabbar et al., 2006a; Saeed et al., 2007; Ji et al., 2012) contribute to their very limited use in many pastoral systems (Gilleard, 2006). Vaccination may be an alternative way to control parasitic infestations, but the antigenic complexity and variation at various developmental stages of the parasites has slowed the process of vaccine development (Maizels et al., 1999).

The search for new and more sustainable ways of controlling parasitic and other diseases of livestock has given rise to the study of ethno-botanicals (Mathias, 2004). The use of medicinal plants or other ethno-botanical remedies is economical (Ghotge et al., 2002), safe, and generally has no problem of drug resistance; such practices may therefore be a valid substitute of allopathic anthelmintics. Furthermore, these remedies are easily available, simple to prepare and administer, at minute or free of cost to the farmer (Jabbar et al., 2005) and are considered powerful healing agents for the treatment of different parasitic diseases (Kone et al., 2012).

Plants have served through centuries as source of medication for a variety of diseases. The history of ethno-botany is almost as old as human civilization (Sarojini et al., 2012) and most of the population of the Indo-Pakistan subcontinent, as well as of many other regions, has for centennials relied on plants for curing animals and humans (McCorkle et al., 1995; Jabbar et al., 2006b). This has also been acknowledged by the World Health Organisation's estimate that 80% of the people in developing countries or 60% of the global human population largely depend on plant-based remedies for the control and treatment of various human and animal diseases (World Health Organization, 2010). Research on fields of applications and use practices as well as doses and administration routes associated with plant resources are important for the discovery of new medicines (Kone et al., 2012). The search for medicinal plants to treat diseases of bacterial, viral, fungal or parasitic origin is even more urgent in the context of countries like Pakistan that have an agriculture-based economy, a large proportion of (poor) rural dwellers, and are bestowed with a unique biodiversity. About 600 of the country's 6000 known plant species are considered of therapeutic value (Hamayun et al., 2003; Khan et al., 2012).

Previous studies, albeit few, attempted to determine the anthelmintic activity of some plants indigenous to Pakistan (Lateef et al., 2003; Iqbal et al., 2005; Jabbar et al., 2006b; Ibrar et al., 2007). However, given the localized availability of the plants and their still lacking application on a country-wide level through (community-based) distribution programs, we focused on the very remote and poor desert region of Cholistan, where the majority of the rural livelihoods rely on sheep and goat keeping. Communal ceremonies like weddings, funerals and tribal celebrations include slaughtering and exchange of animals, and traditionally wealth has been assessed from the size of the livestock herd of an individual (Farooq et al., 2008). Based on interviews with 120 pastoralists, we identified five medicinal plants that were said to be effectively used against gastrointestinal parasites in sheep and goats. These were tested *in vitro* against three (*Haemonchus contortus, Trichuris ovis* and *Paramphistomum cervi*) of the four most prevalent helminths (*Haemonchus contortus, Trichuris ovis, Trichostrongylus axe*i and *Paramphistomum cervi*) in the region that had been identified through a large-scale screening (Chapter 3). The comparison of the anthelmintic efficacy of the plants and of various extract types at different time intervals and concentration levels should help to identify the most appropriate remedy against the specific parasite.

4.2. Material and methods

4.2.1. Collection of plant material

On the basis of the number of mentions and stated efficacy in a baseline survey, the following five ligneous plants (shrubs) were selected for the evaluation of their anthelmintic activity: *Capparis decidua* L., *Salsola foetida* L., *Suaeda fruticosa* Forssk., *Haloxylon salicornicum* (Moq.) Bunge and *Haloxylon recurvum* Bunge ex. Boiss. A total of 10 kg of fresh leaves and adhering soft branches of each plant was collected as a pool from different areas of the Cholistan desert (situated between 27°42′ - 29°45′ N, and 69°52′ - 75°24′ E) during June - July 2011. The plant material was put into big cotton bags and labelled.

4.2.2. Processing of plant material

The sampled plant material was air-dried in a ventilated room and afterwards was cleaned of adulterants (weeds, soil particles). Then it was ground into a fine powder by using a stainless steel electrical blender; the powders were stored in sealed cellophane bags at 4°C until use (maximum storage time 10 months). A sample of 100 g dried plant material was kept for the analysis of the proximate composition and phenolic compounds. Dry matter (DM) and organic matter (OM) were determined according to Naumann and Bassler (1997), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by a modification of the method of Van Soest et al. (1991) using a semi-automated Ankom 220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) without using decalin or sodium sulphite. Total phenols and total tannins were determined by the modified Folin–Ciocalteu method (Makkar, 2003) whereby polyvinyl-polypyrrolidone was used to distinguish non-tannin phenols from tannin phenols. Condensed tannins were extracted according to Porter et al. (1986).

Table 4.1: Average proximate composition and phenolic compounds of five important medicinal plants from the Cholistan desert, Pakistan. All values are replicates of 5 analyses per plant, given in % of dry matter.

Plant name	Organic matter	Neutral detergent fiber	Acid detergent fiber	Total phenoles	Non-tannin phenoles	Total tannins	Condensed tannins
Capparis decidua	89.8	57.9	41.7	0.73	0.66	0.07	0.02
Salsola foetida	60.3	26.3	21.2	1.16	0.88	0.28	0.04
Suaeda fruticosa	85.1	48.1	39.2	1.48	1.34	0.14	0.02
Haloxylon salicornicum	59.3	22.3	15.2	1.48	1.09	0.39	0.04
Haloxylon recurvum	72.5	29.9	15.9	5.90	4.31	1.59	0.05

4.2.3. Extract preparation

At the Pathobiology Laboratory of Bahauddin Zakariya University, Multan, three different types of extract were prepared of each plant: aqueous, methanol and aqueous-methanol extract. The preparation of the aqueous extract followed the procedures described by Onyeyili et al. (2001). The powdered plant material (250 g dry matter) was soaked in 1 l of water over night and then boiled for 1.5 hours. The mixture was allowed to cool down and was then filtered using muslin cloth and Whitman No.1 filter paper. The residual plant material on the filter was diluted again in 1 l of water, and the described process was repeated a second and third time. The three filtrates were pooled and from the 3 l water was evaporated in a force draft oven at 50°C until a volume of 50 - 90 ml was reached; this took 5 to 7 days.

Methanol and aqueous-methanol (30:70) extracts were prepared by cold maceration technique, modifying the method of Tabassam et al. (2008). Powdered plant material (250 g dry matter; see Figure 4.1) was soaked at a ratio of 1:4 in each solvent for three days. The filtrate was collected through a piece of muslin cloth and Whitman No.1 filter paper. The residual plant material on the filter was soaked again in 1 l of solvent for a second and third time. The three filtrates (3 l) were pooled and condensed to 50 - 70 ml volume in a forced draft oven at 40 - 45°C for 3 to 5 days.

The concentration of each extract was calculated on the basis of dried powdered plant matter soaked in the solvents. The concentration of the extracts was maintained at a ratio of 1 g extracted plant material per 1 ml of solvent by adding distilled water to the concentrated extract until a volume of 250 ml was reached; the thus prepared crude extracts were stored at 4°C for a maximum of three months (duration of one experimental run).

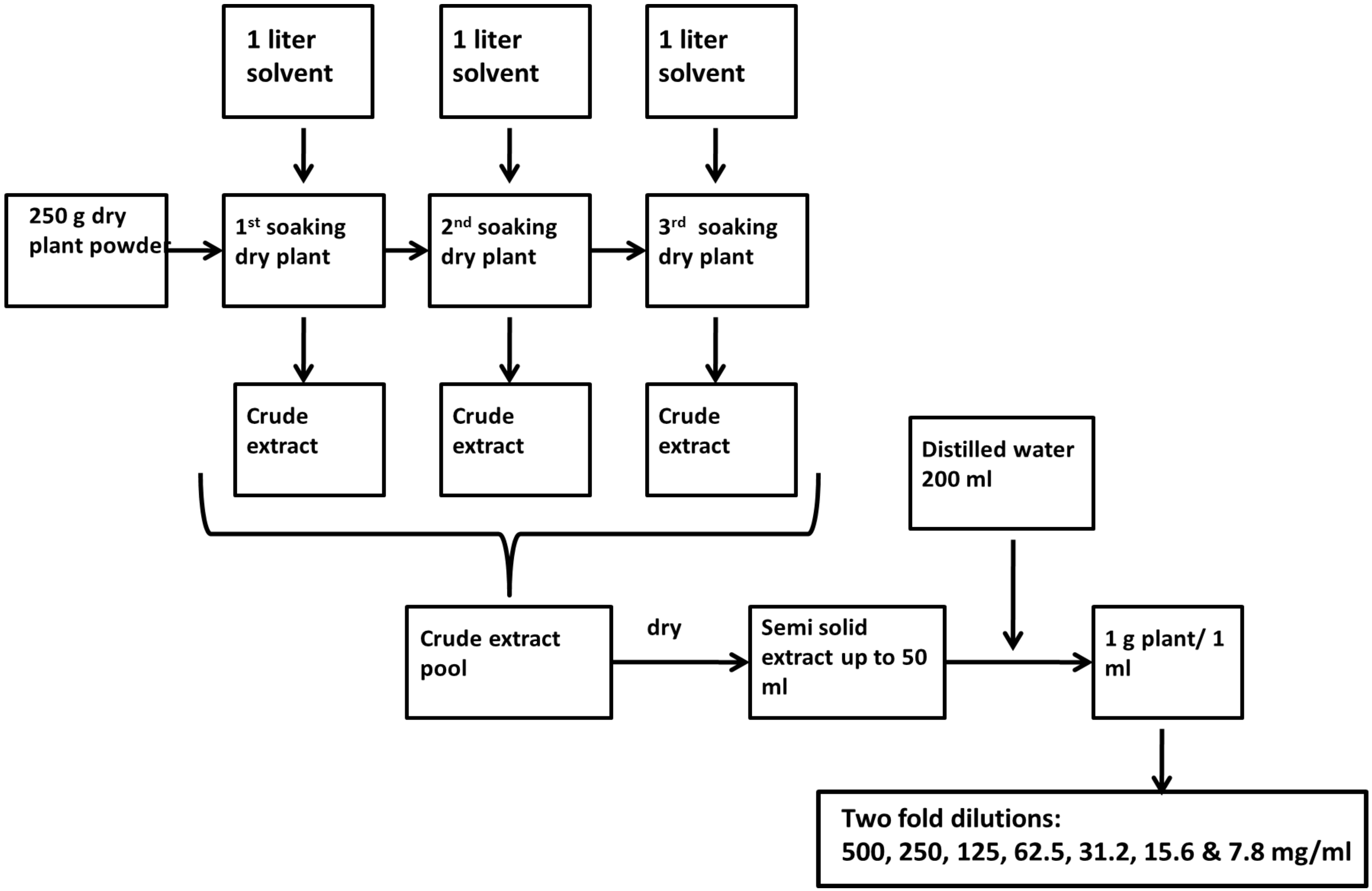

Figure 4.1: Schematic presentation of the preparation of plant extracts with methanol and aqueous-methanol solvent.

4.2.4. Determination of anthelmintic activity

For each test running 24 hours, adult and motile helminths were freshly collected from 20 - 30 sheep and/or goats slaughtered at the abattoir of Multan. *Haemonchus contortus* was collected from abomasal contents, *Trichuris ovis* from contents of the large intestine and *Paramphistomum cervi* from rumen contents. These three parasite species had been identified as the 1st, 2nd and 4th most prevalent one in pastoral sheep and goat flocks in the Cholistan desert (Chapter 3). *Paramphistomum cervi* (4th) was selected instead of *Trichostrongylus axe*i (3rd) because this helminth was frequently observed in mixed infestations. Collected parasites were washed in phosphate buffered saline (PBS) solution (Alawa et al., 2003) and were directly used for the evaluation of anthelmintic activity via adult motility assay (Singh et al., 1985).

For this assay, 10 individual helminths per species were counted into a Petri dish and, at an ambient temperature of 25 - 30°C, were exposed to seven different concentrations (see below) of the three types of extracts (aqueous, methanol and aqueous-methanol), whereby each treatment was replicated three times. For each combination of plant and extract type, the tested dilution series was as follows: 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 mg extracted plant material per ml solvent (Figure 4.1). Levamisole (0.55 mg/ml) and oxyclozanide (34 mg/ml) served as positive control, and pure PBS served as negative control in each dilution series.

The inhibition of motility or the dead of individual helminths were indiscriminately used as criterion to evaluate the anthelmintic activity. Motility loss / mortality were observed at 2, 4, 6, 8, 10, 12 and 24 hours after adding the extract to the Petri dishes, using a convex lens magnifying glass (5X). At the end of each observation interval, the treated worms were kept for five minutes in lukewarm fresh PBS to test recapture of motility. The number of immobile/dead individuals was recorded for each combination of plant species and extract type at the specific concentration and time interval.

4.2.5. Statistical analyses

Data were analyzed using Microsoft Excel 2007 and SPSS 17.0 software (SPSS Inc., Chicago, USA). The effect of plant species (n=5), extract type (n=3), concentration of extract (n=7) and time of exposure (n=7) on motility/mortality of the three helminth species was determined using the Kruskal-Wallis test for not normally distributed data, whereby the dependent variable (number of dead parasites) had first been tested for normality (Kolmogorov-Smirnov test). The 50% lethal concentration (LC_{50}), that is the concentration of plant extract required to kill 50% of the adult parasites, was calculated for each combination of plant, parasite and extract type using simple linear regression analysis across concentrations and time intervals (n=42 observations in each case) as follows:

$$y = ax + b$$

where y is the number of dead parasites and x is extract concentration.

4.3. Results

In all tests run, 100% mortality of *H. contortus* and *T. ovis* was obtained by the positive control with levamisole within 2 hours of exposure, while 100% mortality of *P. cervi* was observed with oxyclozanide within 4 hours of exposure. No mortality of helminths was observed in PBS (negative control). All five test plants exhibited anthelmintic activity; however, a wide variation was observed in the effectiveness of plant species, extract types and dilutions against the three helminth species. Across all treatments however, the highest and lowest anthelmintic efficacy was obtained at a concentration of 500 and 7.8 mg/ml, respectively, and at 12 and 2 hours of exposure (Tables 4.2 - 4.7). The aqueous and aqueous-methanol extracts of *C. decidua* and the methanol extract of *H. salicornicum* killed a maximum of *H. contortus* (7.1, 6.6, and 7.7, respectively) at a concentration of 500 mg/ml (Table 4.2). In case of *T. ovis,* the aqueous extract of *C. decidua* and the methanol and aqueous-methanol extracts of *H. recurvum* were more potent at a concentration of 500 mg/ml (Table 4.3), whereas the aqueous extract of *S. foetida* as well as the methanol and aqueous-methanol extracts of *H. recurvum* killed the highest mean number of *P. cervi* (Table 4.4).

Table 4.2: Mean dead *Haemonchus contortus* at various concentrations of aqueous, methanol and aqueous-methanol extracts of 5 selected plants. Values are means of three replicates per treatment with 10 adult parasites per replicate.

		Concentrations (mg extracted plant/ml)							
Extract	**Plant**	**500.0**	**250.0**	**125.0**	**62.5**	**31.3**	**15.6**	**7.8**	**SEM**
Aqueous extract	*C. decidua*	7.11	6.44	5.33	4.00	2.44	1.39	0.72	0.558
	S. foetida	6.61	5.89	4.72	3.67	2.33	0.72	0.17	0.524
	S. fruticosa	6.22	5.67	4.72	3.89	2.17	1.11	0.44	0.486
	H. salicornicum	3.39	2.39	1.83	1.28	0.72	0.39	0.22	0.301
	H. recurvum	3.39	2.67	2.39	1.44	0.67	0.33	0.17	0.308
	SEM	0.575	0.592	0.544	0.532	0.399	0.231	0.138	
Methanol extract	*C. decidua*	6.33	5.78	5.28	3.72	2.50	1.28	0.67	0.533
	S. foetida	6.28	5.78	5.28	3.83	2.72	1.50	0.89	0.546
	S. fruticosa	7.06	6.39	5.89	5.44	4.00	2.94	2.22	0.502
	H. salicornicum	7.67	7.33	6.28	5.39	4.28	3.33	2.33	0.461
	H. recurvum	7.39	7.06	6.50	5.61	4.94	3.72	2.89	0.483
	SEM	0.528	0.565	0.593	0.559	0.518	0.442	0.371	
Aqueous methanol extract	*C. decidua*	6.61	6.00	4.83	3.83	2.22	1.44	0.78	0.461
	S. foetida	6.22	5.44	4.56	3.94	1.94	0.94	0.11	0.455
	S. fruticosa	5.61	4.61	3.67	2.61	1.72	0.72	0.33	0.417
	H. salicornicum	5.67	4.33	3.56	2.17	1.06	0.61	0.22	0.441
	H. recurvum	5.44	4.89	3.94	2.72	1.44	0.78	0.33	0.485
	SEM	0.498	0.515	0.486	0.451	0.323	0.239	0.130	

For full plant names see Table 4.1.
Negative control (Phosphate buffered saline): no dead parasite; Positive control (Levamisole, 0.55 mg/ml): all parasites dead at 2 h after exposure.

Table 4.3: Mean dead *Trichuris ovis* at various concentrations of aqueous, methanol and aqueous-methanol extracts of 5 selected plants. Values are means of three replicates per treatment with 10 adult parasites per replicate.

		Concentrations (mg extracted plant /ml)							
Extract	**Plant**	**500.0**	**250.0**	**125.0**	**62.5**	**31.3**	**15.6**	**7.8**	**SEM**
Aqueous extract	*C. decidua*	6.28	5.22	4.50	3.33	2.39	1.39	0.61	0.499
	S. foetida	5.72	5.06	4.11	3.28	1.78	0.94	0.22	0.454
	S. fruticosa	6.11	5.56	4.22	3.22	2.11	0.94	0.39	0.478
	H. salicornicum	3.06	2.22	1.61	1.06	0.72	0.22	0.17	0.271
	H. recurvum	3.50	2.61	2.17	1.39	0.611	0.28	0.11	0.289
	SEM	0.524	0.544	0.513	0.459	0.349	0.228	0.121	
Methanol extract	*C. decidua*	5.67	5.33	4.61	3.22	2.22	1.28	0.72	0.580
	S. foetida	6.61	5.78	4.78	3.67	2.50	1.33	0.56	0.493
	S. fruticosa	6.33	5.72	4.78	4.00	2.72	1.39	0.56	0.498
	H. salicornicum	6.28	5.44	4.72	3.94	2.72	1.94	0.89	0.548
	H. recurvum	6.67	5.83	5.56	5.17	3.61	2.28	1.17	0.601
	SEM	0.586	0.634	0.669	0.629	0.517	0.411	0.258	
Aqueous methanol extract	*C. decidua*	6.17	5.11	4.11	3.11	2.28	1.11	0.50	0.437
	S. foetida	5.89	4.67	3.50	2.83	1.72	0.89	0.22	0.438
	S. fruticosa	5.72	5.06	4.17	3.17	2.22	1.44	0.78	0.445
	H. salicornicum	6.94	6.22	5.39	4.17	3.22	1.83	0.89	0.542
	H. recurvum	7.33	6.06	5.67	4.61	3.67	2.50	1.17	0.541
	SEM	0.500	0.533	0.553	0.528	0.475	0.355	0.214	

For full plant names see Table 4.1.
Negative control (Phosphate buffered saline): no dead parasite; Positive control (Levamisole, 0.55 mg/ml): all parasites dead at 2 h after exposure.

Table 4.4: Mean dead *Paramphistomum cervi* at various concentrations of aqueous, methanol and aqueous-methanol extracts of 5 selected plants. Values are means of three replicates per treatment with 10 adult parasites per replicate.

		Concentrations (mg extracted plant/ml)							
Extract	**Plant**	**500.0**	**250.0**	**125.0**	**62.5**	**31.3**	**15.6**	**7.8**	**SEM**
Aqueous extract	*C. decidua*	5.44	4.67	4.06	2.94	1.94	1.00	0.44	0.457
	S. foetida	5.56	5.22	4.33	3.17	2.06	0.61	0.33	0.468
	S. fruticosa	4.50	3.72	2.83	2.17	1.39	0.67	0.28	0.423
	H. salicornicum	3.06	1.94	1.67	1.22	0.72	0.39	0.17	0.284
	H. recurvum	3.06	2.28	1.83	1.00	0.72	0.44	0.11	0.270
	SEM	0.532	0.551	0.500	0.417	0.324	0.187	0.105	
Methanol extract	*C. decidua*	5.28	4.22	3.06	2.06	1.17	0.61	0.44	0.418
	S. foetida	3.28	2.50	1.33	0.78	0.50	0.28	0.06	0.259
	S. fruticosa	3.72	3.11	2.06	1.33	0.78	0.33	0.11	0.295
	H. salicornicum	6.11	5.28	4.22	2.83	2.00	1.00	0.22	0.472
	H. recurvum	7.39	6.67	6.06	4.67	2.83	2.11	1.39	0.561
	SEM	0.528	0.548	0.549	0.479	0.372	0.279	0.198	
Aqueous methanol extract	*C. decidua*	4.83	4.28	3.44	2.61	1.78	1.06	0.33	0.359
	S. foetida	4.22	3.83	3.33	2.33	1.67	0.83	0.28	0.334
	S. fruticosa	2.89	1.61	0.83	0.56	0.22	0.11	0.00	0.195
	H. salicornicum	5.28	4.44	3.78	2.50	1.72	0.89	0.44	0.434
	H. recurvum	6.67	6.06	5.33	4.11	1.39	0.89	0.50	0.554
	SEM	0.488	0.515	0.498	0.435	0.325	0.227	0.130	

For full plant names see Table 4.1.
Negative control (Phosphate buffered saline): no dead parasite; Positive control (Oxyclozanide, 34 mg/ml): all parasites dead at 4 h after exposure.

At 500 mg/ml concentration, aqueous extracts of *C. decidua* and *S. foetida*, methanol extracts of all plants and aqueous-methanol extracts of *C. decidua, H. salicornicum* and *H. recurvum* killed all *H. contortus* after 12 hours of exposure (Table 4.5). Aqueous extracts of *C. decidua* and *S. fruticosa,* methanol extracts of all plants and aqueous-methanol extracts of *S. foetida, H. salicornicum* and *H. recurvum* killed all *T. ovis* after 12 hour of exposure (Table 4.6). In case of *P. cervi*, no aqueous plant extract killed all parasites (*C. decidua* and *S. foetida aqueous extracts* killed the maximum of 9.67 *P. cervi*), whereas the methanol extracts of *H. salicornicum* and *H. recurvum* and the aqueous-methanol extract of *H. recurvum* killed all 10 helminths after 12 hours of exposure (Table 4.7). Across the whole series of tests carried out, plant species ($P<0.05$), extract type ($P<0.01$), parasite species ($P<0.01$), extract concentration ($P<0.01$), time of exposure ($P<0.01$) and their interactions ($P<0.01$) had statistically significant effects on the number of immobile/dead helminths (Table 4.8).

Table 4.5: Effect of time (h) on mean dead *Haemonchus contortus* in aqueous, methanol and aqueous-methanol extracts of five selected medicinal plants at a concentration of 500 mg/ml. Values are means of three replicates per treatment with 10 adult parasites per replicate.

		Time (h of exposure)						
Extract	**Plant**	**2**	**4**	**6**	**8**	**10**	**12**	**SEM**
Aqueous extract	*C. decidua*	2.00	4.67	7.00	9.00	10.00	10.00	1.321
	S. foetida	2.00	4.33	6.00	8.00	9.33	10.00	1.260
	S. fruticosa	2.00	4.00	6.00	7.00	8.67	9.67	1.173
	H. salicornicum	0.00	1.00	3.00	4.00	5.00	7.33	1.093
	H. recurvum	0.33	1.00	3.00	4.00	5.00	7.00	1.020
	SEM	0.452	0.823	0.837	1.030	1.082	0.672	
Methanol extract	*C. decidua*	2.00	3.00	5.00	8.00	10.00	10.00	1.430
	S. foetida	1.67	2.67	5.00	8.33	10.00	10.00	1.504
	S. fruticosa	3.00	4.67	6.67	8.00	10.00	10.00	1.162
	H. salicornicum	4.00	5.67	7.33	9.00	10.00	10.00	1.004
	H. recurvum	4.00	5.00	6.67	8.67	10.00	10.00	1.048
	SEM	0.488	0.583	0.478	0.194	0.000	0.000	
Aqueous methanol extract	*C. decidua*	3.00	4.00	6.00	7.67	9.00	10.00	1.133
	S. foetida	2.67	4.00	6.00	7.00	8.33	9.33	1.039
	S. fruticosa	1.67	3.33	5.00	6.00	8.00	9.67	1.203
	H. salicornicum	2.00	3.00	5.00	6.33	7.67	10.00	1.214
	H. recurvum	1.33	3.00	4.33	6.00	8.00	10.00	1.312
	SEM	0.309	0.226	0.323	0.324	0.226	0.133	

For full plant names see Table 4.1.
Negative control (Phosphate buffered saline): no dead parasites;
Positive control (Levamisole, 0.55 mg/ml): all parasites dead at 2 h after exposure.

Table 4.6: Effect of time (h) on mean dead *Trichuris ovis* in aqueous, methanol and aqueous-methanol extracts of 5 selected plants at a concentration of 500 mg/ml. Values are means of three replicates per treatment with 10 adult parasites per replicate.

		Time (h of exposure)						
Extract	**Plant**	**2**	**4**	**6**	**8**	**10**	**12**	**SEM**
Aqueous extract	*C. decidua*	2.00	4.00	5.33	7.67	8.67	10.00	1.237
	S. foetida	1.67	3.33	5.33	6.33	8.00	9.67	1.203
	S. fruticosa	2.67	3.67	4.67	6.67	9.00	10.00	1.207
	H. salicornicum	0.00	1.00	2.67	3.67	5.00	6.00	0.940
	H. recurvum	0.67	1.67	3.00	4.00	5.33	6.33	0.881
	SEM	0.476	0.591	0.534	0.782	0.847	0.915	
Methanol extract	*C. decidua*	0.00	2.00	4.00	8.00	10.00	10.00	1.745
	S. foetida	3.00	4.33	5.33	7.33	9.67	10.00	1.172
	S. fruticosa	2.67	3.67	4.67	7.67	9.33	10.00	1.259
	H. salicornicum	2.00	3.33	4.33	8.00	10.00	10.00	1.431
	H. recurvum	2.67	3.67	5.00	8.67	10.00	10.00	1.342
	SEM	0.542	0.386	0.236	0.221	0.133	0.000	
Aqueous methanol extract	*C. decidua*	2.67	3.67	5.33	7.00	8.67	9.67	1.131
	S. foetida	2.00	3.33	5.33	6.67	8.00	10.00	1.210
	S. fruticosa	2.00	3.33	4.33	6.67	8.33	9.67	1.221
	H. salicornicum	2.67	4.67	6.00	8.33	10.00	10.00	1.224
	H. recurvum	4.00	5.00	6.33	8.67	10.00	10.00	1.058
	SEM	0.365	0.350	0.343	0.429	0.422	0.817	

For full plant names see Table 4.1.
Negative control (Phosphate buffered saline): no dead parasite;
Positive control (Levamisole, 0.55 mg/ml): all parasites dead at 2 h after exposure.

Table 4.7: Effect of time (h) on mean dead *Paramphistomum cervi* in aqueous, methanol and aqueous-methanol extracts of 5 selected plants at a concentration of 500 mg/ml. Values are means of three replicates per treatment with 10 adult parasites per replicate.

		Time (h of exposure)						
Extract	**Plant**	**2**	**4**	**6**	**8**	**10**	**12**	**SEM**
Aqueous extract	*C. decidua*	1.00	3.00	5.00	6.33	7.67	9.67	1.284
	S. foetida	1.67	3.33	4.67	6.00	8.00	9.67	1.210
	S. fruticosa	0.67	1.67	3.33	5.00	7.00	9.33	1.341
	H. salicornicum	0.00	1.00	2.00	3.33	4.67	7.33	1.090
	H. recurvum	0.00	1.00	2.67	3.67	4.67	6.33	0.956
	SEM	0.316	0.494	0.573	0.602	0.726	0.688	
Methanol extract	*C. decidua*	1.67	3.00	4.33	6.00	7.67	9.00	1.143
	S. foetida	0.67	1.67	2.33	3.33	5.33	6.33	0.892
	S. fruticosa	1.00	2.00	3.00	4.33	5.33	6.67	0.867
	H. salicornicum	2.00	3.33	5.33	7.33	8.67	10.00	1.270
	H. recurvum	3.00	5.33	7.00	9.00	10.00	10.00	1.153
	SEM	0.408	0.645	0.833	1.017	0.921	0.799	
Aqueous methanol extract	*C. decidua*	2.00	3.00	4.33	5.00	6.33	8.33	0.934
	S. foetida	1.00	2.67	3.67	5.00	6.00	7.00	0.905
	S. fruticosa	1.00	1.67	2.33	3.00	4.00	5.33	0.648
	H. salicornicum	2.00	3.33	4.00	5.00	7.67	9.67	1.172
	H. recurvum	1.67	3.67	6.67	8.33	9.67	10.00	1.377
	SEM	0.226	0.343	0.704	0.859	0.939	0.865	

For full plant names see Table 4.1.
Negative control (Phosphate buffered saline): no dead parasite;
Positive control (Oxyclozanide, 34 mg/ml): all parasites dead at 4 h after exposure.

Table 4.8: Effect* of plant, treatment**, extract type, parasite species, concentration of extract and time of exposure on the number of dead parasites.

Independent variable	**df**	**Mean**	**SD**	**χ^2**	**P**
Plant species	4	3.00	1.414	15.193	0.004
Treatment	6	3.87	1.553	330.37	<0.001
Extract type	2	2.00	0.816	116.31	<0.001
Parasite species	2	2.00	0.817	119.67	<0.001
Concentration of extract	6	4.00	2.001	1979.91	<0.001
Time of exposure	5	3.50	1.708	2346.83	<0.001
Concentration x plant	21	12.00	8.718	1517.77	<0.001
Concentration x extract type x plant	38	24.00	21.231	1279.75	<0.001
Concentration x extract type x parasite	25	16.00	13.401	1514.31	<0.001

* Kruskal Wallis test: data were non-normally distributed
** Treatment: the five plants plus the positive and negative control (see footnotes of Tables 4.2-4.7)

The R^2 values of the simple linear regressions used to calculate the LC_{50} values ranged from a minimum of 0.14 (*T. ovis*, *H. recurvum,* methanol extract) to a maximum of 0.58 (*P. cervi*, *S. foetida,* aqueous-methanol extract). LC_{50} values strongly depended on the parasite species ($P<0.01$) and the extract type ($P\leq0.01$). From their comparison (Table 4.9) it appeared that among the aqueous extracts the ones of *C. decidua* (at 251.8 and 304.9 mg/ml, respectively) showed a clear *in vitro* anthelmintic activity against adult worms of *H. contortus* and *T. ovis,* while the aqueous extract of *S. foetida* (at 371.8 mg/ml) was very effective against *P. cervi*. Among the methanol extracts those of *H. recurvum* (at 89.4, 238.4 and 193.2 mg/ml, respectively) were most potent against all three helminth species as compared to the methanol extracts of the other plants. Among the aqueous-methanol extracts, the one of *C. decidua* (at 287.2 mg/ml) was most effective against *H. contortus,* whereas the aqueous-methanol extract of *H. recurvum* (at 200.3 and 284.2 mg/ml) was most effective against *T. ovis* and *P. cervi* (Table 4.9).

When ranking the plant species for their effectiveness against a certain parasite species at a given solvent use, against *H. contortus* the aqueous extract of *C. decidua* (251.8 mg/ml) was most potent, flowed by *S. foetida* (292.9 mg/ml) and *S. fruticosa* (302.3 mg/ml); when considering the methanol extracts *H. recurvum* (89.4 mg) was more effective than *H. salicornicum* (121.9 mg/ml) and *S. fruticosa* (164.0 mg/ml), and in the case of aqueous-methanol extracts *C. decidua* (287.2 mg/ml) killed most *H. contortus* followed by *S. foetida* (322.2 mg/ml) and *H. salicornicum* (398.9 mg/ml).

To affect *T. ovis* with an aqueous plant extract, *C. decidua* (304.9 mg/ml) was most potent, followed by *S. fruticosa* (329.4 mg/ml) and *S. foetida* (340.0 mg/ml); in the case of methanol extract *H. recurvum* (238.4 mg/ml) was more potent than *S. fruticosa* (278.6 mg/ml) and *H. salicornicum* (289.4 mg/ml). With an aqueous-methanol extract *H. recurvum* (200.3 mg/ml) gave best results, followed by *H. salicornicum* (240.2 mg/ml) and *C. decidua* (326.5 mg/ml).

To control *P. cervi* with an aqueous extract, *S. foetida* (371.8 mg/ml) was most effective, followed by *C. decidua* (373.6 mg7ml) and *S. fruticosa* (556.7 mg/ml); with a methanol and aqueous-methanol extract *H. recurvum* (193.2 and 284.2 mg/ml, respectively) was most potent, followed by *H. salicornicum* (344.2 and 396.1 mg/ml, respectively) and *C. decidua* (441.1 and 440.8 mg/ml, respectively).

Table 4.9: LC_{50} values of different plant extracts and statistical effect* of plant, parasite and extract type on LC_{50} values. The gray highlights indicate the most effective concentration for each plant species x extract type combination against the specific parasite.

Plant species	**Extract Type**	**LC_{50} (mg/ml)**			
		Haemonchus contortus	***Trichuris ovis***	***Paramphistomum cervi***	
Capparis decidua	A	251.8	304.9	373.6	
	M	281.0	334.4	441.1	
	AM	287.2	326.5	440.8	
Salsola foetida	A	292.9	340.0	371.8	
	M	291.7	294.6	780.7	
	AM	322.2	365.4	520.6	
Suaeda fruticosa	A	302.3	329.4	556.7	
	M	164.0	278.6	626.6	
	AM	406.1	340.2	986.0	
Haloxylon salicornicum	A	735.0	902.4	889.6	
	M	121.9	289.4	344.2	
	AM	398.9	240.2	396.1	
Haloxylon recurvum	A	718.5	735.5	889.2	
	M	89.4	238.4	193.2	
	AM	400.1	200.3	284.2	
Independent variable	**Df**	**Mean**	**SD**	**χ^2**	**P**
Plant species	4	3.00	1.430	1.49	0.828
Parasite species	2	2.00	0.826	9.16	0.010
Extract type	2	2.00	0.826	7.99	0.018
Plant x extract type	3	6.00	3.961	20.01	0.029
Plant x parasite	10	6.00	3.960	11.35	0.331
Extract type x parasite	5	4.00	2.430	9.02	0.108

*Kruskal Wallis test: data were non-normally distributed;
Extract type: A: aqueous, M: methanol, AM: aqueous-methanol.

4.4. Discussion

Parasitic infestations and related diseases are considered as major health threat to extensively kept sheep and goat flocks (Iqbal et al., 2005). Among different parasitic diseases, helminth infections are not only rampant (Saeed et al., 2007) but the worms are also developing resistance against the commonly used synthetic drugs throughout the globe (Jabbar et al., 2006a). Considering these serious problems along with the high costs of synthetic anthelmintics and their various side effects, the exploration of alternate medication based on plants used in traditional healthcare systems is indicated (John et al., 2006).

All five plants tested in the current study expressed anthelmintic activity against the three species of helminths most prevalent in pastoral sheep and goat flocks in the Cholistan desert (Chapter 3); yet, their efficacy varied with respect to the targeted parasite species as well as with regard to the solvent used for extract preparation. As a matter of principle, the anthelmintic ability of a plant (part) is due to the presence of one or a variety of secondary metabolites.

The observed variation in the anthelminthic efficacy of the tested plants is affected by the solvent used (Malu et al., 2009), but in the first instance determined by differences in the nature and concentration of their secondary metabolites responsible for killing the parasites (Eguale et al., 2007). Increasing the concentration of the plant extracts and the time interval of exposure resulted in increased parasite mortality, indicating dose- and time-dependent activity. Several studies evaluating the *in vitro* and *in vivo* activity of different plant-based anthelmintics also reported their dose- and time-dependent effect (Costa et al., 2011; Ahmed et al., 2012; Cala et al., 2012; Ji et al., 2012; Moreno et al., 2012; Nalule et al., 2013).

Although only of limited explanatory power, the chemical analysis revealed the presence of condensed tannins and other phenolic compounds in all five plants. Especially tannins exhibit anthelmintic activity by two mechanisms: firstly, through irreversible binding they can change the chemical and physical properties of protein surfaces of the parasite, such as cuticle, oral cavity, esophagus, cloaca and vulva; in consequence, helminths lose their grip onto the host's gastrointestinal epithelium and are expelled from the body (Athanasiadou et al., 2001; Cenci et al., 2007). Secondly, the interaction of tannins with free dietary proteins may reduce the availability of nutrients to the parasite, affecting its live cycle and in the last resulting in death by starvation (Athanasiadou et al., 2001; Hoste et al., 2006).

The LC_{50} values of the five plants varied for different parasites, but they were also different for the three solvents used to prepare extracts. This might be due to different chemical components extracted by the different solvents and their biological effects on the parasites (Eloff, 1998). Of *C. decidua*, the aqueous extract was most potent against all three helminths, indicating that the active components responsible for the anthelmintic activity of this plant were hydrophilic in nature. Of *S. foetida* and *S. fruticosa* the methanol extract was most effective against *H. contortus* and *T. ovis*, while their aqueous extract was most effective against *P. cervi*. This points to lipophilic secondary metabolites being responsible for the activity against *H. contortus* and *T. ovis*, and hydrophilic compounds killing *T. ovis*. In case of *H. salicornicum and H. recurvum*, lipophillic compounds were active against *H. contortus* and *P. cervi,* while a combination of lipophillic and hydrophilic compounds were jointly effective against *T. ovis*. Although detailed profiles of the secondary metabolites of the plants used in this study are still to be determined, in general, plants may contain alkaloids (fat soluble but poorly soluble in water), tannins (water and fat soluble), phenols (water soluble), saponins (water soluble), carotenoids (fat soluble), glucosinolates (water soluble) and phytosterols (fat soluble) that are responsible for their anthelmintic activity. These plant metabolites may have worked individually or caused synergistic effect against the helminths (Briskin, 2000).

Overall, the methanol and aqueous-methanol extracts used in this study exhibited a better anthelmintic activity than pure aqueous extracts. Anthelmintic drugs can reach target sites in helminths either by oral route or by diffusion through their cuticle. Several studies showed that the trans-cuticle diffusion is the common pathway of entry for non-nutrient and non-electrolyte substances and most of the broad-spectrum anthelmintics affect and enter helminths by this route (Debella, 2002; Eguale et al., 2007). Lipophilic anthelmintics such as albendazole have a greater capability to cross the external surface of the helminths than hydrophilic compounds (Geary et al., 1999). The higher effectiveness of the methanol and aqueous-methanol plant extracts as compared to the aqueous extract could therefore be due to their better trans-cuticle absorption (Eloff, 1998).

4.5. Conclusions

The current *in vitro* study revealed that the aqueous extract of *C. decidua*, as well as the methanol and aqueous-methanol extract of *H. recurvum* have the potential to be developed into plant-based drugs for treatment against *H. contortus*, *T. ovis* and *P. cervi* infestations. However, as a next step, *in vitro* studies are needed to examine the activity of the extracts under first on-station experimental conditions and then in field tests with pastoralists. Although research is required to isolate and identify the active ingredients responsible for the anthelmintic activity, the present results indicate that some of the locally used medicinal plants have the potential to be developed into effective low cost anthelmintics for the systematic curative treatment of small ruminant herds of poor pastoralists in the Cholistan and neighboring desert regions of Sindh province, Pakistan, and probably even Rajasthan, India.

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4.6. References

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CHAPTER 5

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General discussion

5.1.1. Socioeconomic conditions and livestock production in Cholistan desert

The insights into pastoralists' socio-economic characteristics across the five locations (Chapter 2) underlined how important livestock production is for the livelihoods of these groups, pointing especially to risk aversion strategies (Akhter and Arshad, 2006) and also wealth assessment based on the number of animals owned (Farooq et al., 2008). The illiteracy rate among pastoralists was very high and water points (*tobas*, wells) located in desert are considered boundaries of the land belonging to different clans. Since different species of animals and herds share the same water sources and grazing areas, free mixing of diseased animals with healthy ones expose livestock to various types of diseases. This was illustrated by the numerous diseases and EVM treatments reported by pastoralists (Chapter 2), and the high helminth infestation (Chapter 3) and overall disease prevalence in the region (Farooq et al., 2008) as well as high but fluctuating mortality rates (5-60%), especially among sheep and goats herds (Food and Agriculture Organization, 1993; Akhter and Arshad, 2006).

5.1.2. Helminth infestations: a real threat for pastoralists

On average every pastoralist recommended at least one traditional remedy for the medication of helminth infestations and overall the highest number of remedies (18.6%) was recorded for the treatment of gastrointestinal problems (Chapter 2). This view is also supported by data provided by the Food and Agriculture Organization (1993) and Akhter and Arshad (2006). The results of the prevalence study (Chapter 3) also confirmed this assumption; overall helminth infestation was very high (78%) in both sheep and goat herds. Especially suckling animals carry a severe helminth burden as this age group has a weaker immunological response against parasites and has maximum chance of mortality due to dehydration and undernutrition consequences of parasitic infestation. Losses caused by helminths depend on the nature and intensity of the infestation and the herd and animal management practices. Overall, they retard growth (Kochapakdee et al., 1995), lower productivity (Perry and Randolph, 1999), increase mortality (Sykes, 1994) and lead to high economic losses (Garedaghi et al., 2011).

5.1.3. Livestock health management practices in Cholistan

For the medication of important diseases, veterinary health care facilities are not available in the desert or limited to very few poorly equipped small units in peripheral towns. Livestock owners often become distressed and helpless when their livestock seriously gets ill (Akhter and Arshad, 2006). Due to their poverty and remoteness, livestock keepers cannot easily contact veterinary personnel, therefore, a large number of the animals infected with diseases were either not provided any medication or were given traditional plant based remedies (Chapter 2). Local healers at toba or village level have reasonable knowhow to diagnose diseases but their scope of medication is limited (Arshad et al., 2003) as they are relying on traditional plant-based remedies without any scientific validation. In the region of study pastoralists were well aware of the hazards caused by helminthiasis and considering it a major constraint for their livestock production.

5.1.4. Scientific validation of the effectiveness of traditional plant-based remedies

The five plants that were tested for their *in vitro* efficacy in this study, namely *Capparis decidua*, *Salsola foetida, Suaeda fruticosa, Haloxylon salicornicum* and *Haloxylon recurvum* appeared to be the ones most frequently used against infestations with gastrointestinal parasites (Chapter 2). Since many of these remedies have a prolonged and uneventful use, this may serve as an indirect testimony of their efficacy. However, in the absence of an objective proof and scientific evaluation of their efficacy (Chapter 4), the validity of these remedies is questionable and their use remains locally restricted. Screening of plants for their anthelmintic activity (Chapter 4) had the objective of validating the claims of pastoralists, using different plants as curing agent (Chapter 2) in general, and exploring the possibilities of discovering new plants based anthelmintic remedies in particular.

The study area, from an ethno-botany point of view, is one of the least investigated areas of Pakistan with the exception of a few studies (Arshad et al., 2003; Farooq et al., 2008; Khan, 2009). Since the use of traditional plant-based remedies is economically sound (Ghotge et al., 2002), environmentally safe, and generally does not lead to drug resistance of the targeted disease agents, these remedies may be a valuable substitute of allopathic anthelmintics. Furthermore, these remedies are easily available, simple to prepare and administer, at minute or no cost to the farmer (Jabbar et al., 2006) and considered to be effective against different parasitic diseases (Kone et al., 2012).

All five plants scientifically validated in the present study showed *in vitro* anthelmintic activity (Chapter 4) against the three helminth species most prevalent in pastoral sheep and goat flocks (Chapter 3); yet, their efficacy varied with respect to the targeted parasite species as well as with regard to the solvent used for extract preparation. Based on their LD_{50}-values, the aqueous extract of *Capparis decidua,* the methanol extract of *Haloxylon recurvum* and the aqueous-methanol extract of *Haloxylon recurvum* show great potential to be developed into plant-based drugs for treatment against *Haemonchus contortus*, *Trichuris ovis* and *Paramphistomum cervi* infestations, respectively. However, results of an *in vitro* screening of the anthelmintic activity of plant extracts do not allow a firm conclusion on their *in vivo* bioactivity. This is due to considerable variation in the conditions encountered *in vivo* (interaction of the remedy with feed, absorption of the remedy along the various gastrointestinal compartments and host and parasite metabolism). Therefore, further studies are now needed to investigate the *in vivo* anthelmintic activity of these plant extracts in order to develop effective, cheap and locally available anthelmintics for the systematic curative treatment of small ruminant herds of poor pastoralists in Cholistan and neighboring desert regions of Sindh province, Pakistan, and probably even Rajasthan, India.

5.2. Conclusions

1. The pastoralists in Cholistan desert region are using a wide array of plant-based traditional remedies for the medication of different livestock diseases, but scientific validation of the remedies' efficacy is lacking.

2. Pastoralist flocks of small ruminants are heavily infested with a variety of gastrointestinal parasites including some that potentially entail substantial economic losses. Especially suckling animals carry a severe helminth burden, which indicates that particular attention should be paid to their management.
3. The aqueous extract of *Capparis decidua*, as well as the methanol extract of *Haloxylon recurvum* and the aqueous-methanol extract of *Haloxylon recurvum* has the potential to be developed into plant-based drugs for treatment against *Haemonchus contortus*, *Trichuris ovis s*and *Paramphistomum cervi*, respectively.

4. To further corroborate and validate the present findings, *in vivo* studies on station and in pastoral herds are needed before these locally used plant-based remedies can be developed into effective low-cost anthelmintics for the systematic curative treatment of small ruminants in Cholistan and neighboring desert regions in Pakistan, and probably even India.

5.3. Recommendations

Locally adapted, low-cost and effective treatments of economically important animal diseases are an important contribution to the sustainable development of livestock-based pastoral societies in remote areas of Pakistan and neighboring countries. From the present study, the following specific recommendations for policy makers, research institutions and farmers are emerging:

1. Documentation of ethno-botanical knowledge should be expanded to other areas of Pakistan that also have rich botanical and cultural heritage and vast indigenous knowledge, which is however threatened to vanish due to regional and global change phenomena. In this context, it might also be worthwhile to consider the integration of such traditional knowledge into the curriculum of biological sciences graduates.

2. The most promising plants indicated by local communities should be screened *in vitro* for their efficacy through scientific approaches; these must also include the isolation and identification of the active ingredients (secondary plant compounds) responsible for a remedy's effectiveness.

3. In a further step, *in vivo* on-station experiments are needed to corroborate the *in vitro* results; subsequently, dose standardization and toxicity studies for drug development as well as field testing of the remedies with pastoralists are indispensable.

4. *In situ* conservation strategies are needed to preserve the floristic (and overall) biodiversity in remote areas of Pakistan, where poor living conditions and high environmental variability easily entail anthropogenic overexploitation of natural resources. By identifying appropriate propagation, harvesting, conservation and marketing strategies, valuable ethno-veterinary plants might not only be preserved but might also add to the income of local populations.

5. To achieve the above aims, on-site training of pastoralists must be envisaged to enhance pastoralists' skills for livestock management and health care, remedy preparation and application as well as plant propagation, harvest and remedy sale.

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14. 2nd International Conference of Plant Scientists & 11th National Meeting of Plant Scientists. Organized by Department of Botany, GC University, Lahore, Pakistan. February 22-24, 2011.
15. National Conference on the Strategies to improve Red Meat Production in Pakistan. Organized by Faculty of Veterinary Sciences, Bahauddin Zakariya Univesity, Multan, Pakistan. October 13-14, 2010.

Scientific publications

Books

1. **Raza, M.A.,** Murtaza, S., Bachaya, H.A., 2012. Helminthiasis. ISBN 978-3-659-21184-3, Lambert Academic Publishing (LAP) Germany.
2. **Raza, M.A.**, Fatima, G., Rasool, Q.A., 2012. Ethnobotany. ISBN 978-3-659-19074-2, Lambert Academic Publishing (LAP) Germany.

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1. Ayaz, M.M., **Raza, M.A.,** Murtaza, S., Akhtar, S., 2013. Epidemiological survey of helminths of goats in southern Punjab, Pakistan. Tropical Biomedicine, 30: 62-70.
2. Bachaya, H.A., **Raza, M.A.,** Khan, M.N., Iqbal, Z., Abbas, R.Z., Murtaza, S., Badar, N., 2012. Predominance and detection of different Eimeria species causing coccidiosis in layer chickens. The Journal of Animal and Plant Sciences, 22: 597-600.
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9. **Raza, M.A.**, Murtaza, S., Bachaya, H.A., Hussain, A., 2009. Prevalence of *Paramphistmum cervi* in ruminants slaughtered in district Muzaffar garh. Pakistan Veterinary Journal, 29: 214-215.
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11. **Raza, M.A.**, Iqbal, Z., Jabbar, A., Yaseen, M., 2007. Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakiatan. Journal of Helminthology, 81: 323-328.
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Abstracts / Proceedings / Presentations

1. **Raza, M.A.,** E. Schlecht and M. Younas, 2012. Comparative *in vitro* efficacy of selected medicinal plants from Cholistan against gastrointestinal helminthes of sheep and goat. Tropentag, (Resilience of agricultural systems against crises) September 19-21, 2012, Georg-August University of Göttingen, Göttingen, Germany.
2. Fatima, G., I.A. Khan, M.J. Jaskani, M. Hameed and **M.A. Raza,** 2012. Comparative studies of root anatomy in some Date palm (*Phoenix dactylifera* L.) cultivars from diverse origin. Tropentag, (Resilience of agricultural systems

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10. Bachaya, H.A., **M.A. Raza,** S. Murtaza and I.R. Akbar, 2010. Sub-clinical mastitis in buffalo in Muzaffar Garh district of Punjab (Pakistan). Presented in National Conference on the Strategies to improve Red Meat Production in Pakistan; October 13-14, 2010. Faculty of Veterinary Sciences, Bahauddin Zakariya Univesity, Multan (Pakistan).
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Erklärung

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt und keine anderen als die in der Dissertation angegebenen Hilfsmittel benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren verwendet worden.

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