

**Sero-prevalence and Impact of Selected Diseases Affecting
Small Ruminants Production in Krodofan, Blue Nile and
Gadarif States/ Sudan**

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Dedication

This thesis is dedicated with love and respect to:

My mother and father.

My wife and my beloved children Waad and
Mohammed.

All participating pastoralists for being cooperative

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Abbreviations

°C	Degree Centigrade
μ	Microlitre
ABTS	2,2-Azinodi 3-ethybenzothiazoline-6-sulfonic acid
AVIS	Advance Veterinary Information System
CCFR	Crude Case Fatality Rate
CCPP	Contagious Caprine Pleuro Pneumonia
c-ELIZA	Competitive Enzyme Linked Immunosorbent Assay
CVRL	Central Veterinary Research Laboratory
DNA	Deoxyribonucleic acid
ELIZA	Enzyme Linked Immunosorbent Assay
EUR	Euro
FAO	Food and Agriculture Office
GDP	Gross Domestic Product
H.S.	Hemorrhagic Septicemia
ICARDA	International Centre for Agricultural Research in Dry Areas
IFAD	International Fund for Agricultural Development
ILRI	International Livestock Research Institute
MAP-1B	Major Antigenic Protine fragment B
MOAR&F	Ministry of Animal Resources and Fisheries
NENA	Near East and North Africa
PBST	Phosphate Buffer Saline supplemented with Tween 20
PBSTM	Phosphate Buffer Saline supplemented with Tween 20 and skimmed Milk
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PPR	Peste des petits Ruminants
PPRV	Peste des petits Ruminants Virus
RP	Rinder pest
SD	Sudanese Dinnar
SPC	Stomatitis Pneumoenteritis Complex
SPSS	Statistical Products and Services Solution
TBD	Tick borne Disease
TCRV	Tissue Culture Rinderpest Vaccine
TTBD	Tick and Tick Borne Disease

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Abstract

Although certain livestock diseases have been known to play an important role in lower productivity and market access for small ruminants in the Sudan there has been few studies conducted to elucidate their exact magnitude in terms of their prevalence.

Across sectional survey was therefore conducted during the period May 2005 to September 2006 to provide an overview of the most important diseases affecting small ruminants' productivity and their economic impact. The study areas namely, Blue Nile state, Gadarif state and Elkhowie area in (W. Kordofan state) were selected according to certain criteria, namely, diversity in terms of production systems, market outlets, prevalence of poverty, dominance of sheep and goats and prevalence of major transboundary diseases. Information on small ruminants' health and market constraints was collected through a structured questionnaire, along the market chain at seven entry points. These seven entry points, which comprised of categories of stakeholders were, villages and communities, households and flock owners, markets, traders, slaughter houses, quarantine facilities and veterinary clinics. Trained data collectors then administered questionnaires to the seven categories in each of the study states (sites).

Results of interviews with small ruminants' keepers indicated that PPR, Heartwater and Sheep pox were the most important diseases in all the study areas, with minor variations in importance on individual state level. However, heartwater and PPR had a slightly higher rank of importance in Gadarif state, sheep pox ranking third. However, PPR ranked the first in

importance in Blue Nile state, heartwater showing a steady importance as the second. In Elkhwei area (W. Kordofan), respondents reported Sheep pox as the most important disease, with plant poisoning ranking second.

The questionnaire survey revealed that PPR was more prevalent when the production system was seasonal movement (transhumant) in both Gadarif and Blue Nile states. Further analysis revealed significant association between prevalence for each of heartwater and PPR, and winter season. There was a strong correlation between the number of animals affected with PPR and the number of sick animals that could not be sold in Blue Nile state (Pearson correlation coefficient, 0.819), an indication that PPR was a constraint for marketing small ruminants. Similarly, there were strong positive correlations (Pearson correlation coefficients, 0.720 and 0.820 for Blue Nile and Gadarif states respectively) between the number of goats born during the year and the number that died due to PPR during the same year. This observation may be explained in terms of high susceptibility of newborns to PPR, exacerbated by poor management.

MAP1-B ELIZA (Jongejan, Utreth, Netherland) was used to test 320 serum samples from a random sample of small ruminants drawn from the three study areas, to determine sero-prevalence of heartwater. Meanwhile, Competitive enzyme-linked immunosorbent assay (c- ELIZA) ((PDSL, 2005) was used to test 600 serum samples collected from small ruminants from all study sites to determine *peste des petits ruminants* (PPR) sero-prevalence .

The results revealed that the sero-prevalence of heartwater was 73.1%, with a range between 12.0% and 98.5% in all the study sites. However, Gadarif state had the highest sero-positivity rate (98.5%) followed by Blue Nile state (93.3%) and ElKhowei with the lowest of all (12%). On the basis of species, sheep had 63% , while goats had a record high of 90% sero- prevalence in all the three study sites.

The picture for PPR gave an overall sero-prevalence of 61.8%; while individual study states had (69.3%) in Blue Nile, (68.4%) in Elkhwei area in west Kordofan and 28.6% in Gadarif states respectively. However, the study showed higher prevalence of PPR in Elkhwei area, the pastoralists are not familiar with clinical signs of PPR and have no local name for it. Pastoralist misdiagnosis is therefore a possibility and the higher proportion of small ruminants positive for antibodies to the disease in this site would therefore imply that PPR may have been newly introduced into the area. On species basis, an overall sero-prevalence in all study sites was 62.9% for sheep and 59.7% for goats.

Using chi-square statistics, there was no significant difference in sero-positivity for heartwater between sheep and goats, males and females and among different age groups of each species, ($p > 0.05$) .

With respect to PPR, and using chi-square statistics, there was significant association in the overall sero-positivity among different age groups for both species (sheep and goats). Moreover, a significant difference in sero-positivity between female and male small ruminants in all study areas was observed, ($p < .05$).

The total losses and costs resulting from identified diseases, over a period (2003, 2004, and 2005) as reported by respondents were SD. 136,483,454. PPR accounted for 29.1% while heartwater accounted for 9.9% of the losses. Since 71.98% of the respondents were entirely dependent on livestock rearing in the study areas, these losses underline the important role these diseases play in the economic wellbeing and livelihoods of poor small ruminants' keepers.

Improvement of field diagnostic facilities and use of cELISA, which is effective in the diagnosis of PPR are recommended to improve surveillance and control efforts for PPR. In addition, extension packages for small ruminants' keepers for appropriate use of communal pastures, better recognition of diseased animals, informed usage of drugs and vaccines are highly recommended.

الخلاصة

بالرغم من أن بعض أمراض الحيوانات قد عرفت بدورها الهام في انخفاض الإنتاجية وتسويق الحيوانات المجترة الصغيرة في السودان ، إلا إن الدراسات التي أجريت لتوضيح مدى انتشارها علي وجه الدقة قليلة جدا.

أجريت هذه الدراسة في الفترة من أيار / مايو 2005 إلى أيلول / سبتمبر 2006 لتقديم لمحة عامة عن أهم الأمراض التي تؤثر على إنتاجية الحيوانات المجترة الصغيرة وأثرها الاقتصادي. وقد شملت مناطق الدراسة كل من ولاية النيل الأزرق ، ولاية القضارف ومنطقة الخوي (ولاية غرب كردفان). تم اختيار هذه المناطق وفقا لمعايير محددة ، وهي التنوع في نظم الإنتاج ، ومنافذ التسويق ، وانتشار الفقر ، كثرة انتشار الضان والماعز وتفشي الأمراض العابرة للحدود. جمعت معلومات صحة المجترات الصغيرة وقيود السوق من خلال استبيانات عبر سلسلة السوق في سبع مداخل. تتكون هذه المداخل السبعة من فئات أصحاب المصلحة وهم المجتمعات المحلية بالقرى، الأسر أو أصحاب القطعان، الأسواق، التجار بالأسواق، المسالخ ، مرافق الحجر البيطري والعيادات البيطرية. قام جامعي البيانات الذين تم تدريبهم بملء الاستبيانات على الفئات السبع في كل مناطق الدراسة).

أوضحت نتائج المقابلات مع المربين أن مرض طاعون المجترات الصغيرة ، الخدر و جذري الضان هي أكثر الأمراض أهمية في مجمل مناطق الدراسة ، مع اختلافات طفيفة في نسبة الأهمية على مستوي كل ولاية . شكل مرضي الخدر و طاعون المجترات الصغيرة اعلي درجات الأهمية في ولاية القضارف. في حين شكل مرض طاعون المجترات الصغيرة اعلي درجات الأهمية في ولاية النيل الأزرق تلاه مرض الخدر في المرتبة الثانية في الثلاث سنوات الأخيرة. أما بمنطقة الخوي (ولاية غرب كردفان) فقد شكل مرض جذري الضان اعلي درجات الأهمية وتلاه التسمم النباتي.

اظهر الاستطلاع أن مرض طاعون المجترات الصغيرة أكثر انتشارا في نظام الإنتاج الموسمي في كل من القضارف والنيل الأزرق. هنالك صلة بين انتشار مرضي الخدر و طاعون المجترات الصغيرة والموسم الشتوي. فهناك ارتباط قوي بين عدد الحيوانات المصابة بمرض طاعون المجترات الصغيرة وعدد الحيوانات المريضة التي لا يمكن بيعها في ولاية النيل الأزرق (معامل الارتباط بيرسون 0.819) مما يشير إلى أن مرض طاعون المجترات الصغيرة يشكل قيذا لتسويق المجترات الصغيرة. كما أن هنالك ارتباط ايجابي قوي بين ولادات الماعز

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تم استخدام MAP1-B ELIZA لاختبار 320 عينة من مصل المجترات الصغيرة لتحديد نسبة انتشار مرض الخدر. كما تم استخدام c-ELIZA لاختبار 600 عينة لتحديد نسبة انتشار مرض طاعون المجترات الصغيرة.

أظهرت النتائج المعملية أن نسبة انتشار مرض الخدر بمجمل مناطق الدراسة 73.1% وتتراوح بين 12% بمنطقة الخوي (ولاية غرب كردفان) و 98.5% بولاية القضارف أما بولاية النيل الأزرق فقد بلغت النسبة 93.3% . ونسبة الانتشار على أساس النوع فقد بلغت 63 % بالضان و 90 % بالماعز .

كما أظهرت النتائج المعملية أن نسبة انتشار مرض طاعون المجترات الصغيرة بمجمل مناطق الدراسة 61.8% وتتراوح بين 69.3% بولاية النيل الأزرق و 28.6% بولاية القضارف أما بمنطقة الخوي (ولاية غرب كردفان) فقد بلغت النسبة 68.6%. نسبة الانتشار على أساس النوع فقد بلغت 62.9 % بالضان و 59.7 % بالماعز. لم يذكر الرعاية مرض طاعون المجترات الصغيرة بمنطقة الخوي عند إجراء المقابلات و لا يعرفون علامات سريره له إلا أن الاختبار المعملية أكد ايجابية الأجسام المضادة للمرض في هذه المنطقة مما يعني دخول هذا المرض حديثا إلى المنطقة.

عند استخدام مربع كاي ليس هناك فرق إحصائي في الإصابة بمرض الخدر بين الضان والماعز ($p > 0.05$) وبين الذكور و الإناث و بين الفئات العمرية المختلفة من كل نوع . عند استخدام مربع كاي هناك فرق إحصائي في الإصابة بمرض طاعون المجترات الصغيرة ($p < 0.05$) بين الذكور والإناث و بين الفئات العمرية المختلفة من كل نوع. اجمالى الخسائر والتكاليف الناجمة عن الأمراض خلال الثلاث سنوات الماضية (2003 ، 2004 ، 2005) بلغت 136.483.454 دينار سوداني. 28.14 % من الخسائر بسبب مرض طاعون المجترات الصغيرة بينما 8.54 % من الخسائر بسبب مرض الخدر. 71.9% من الخاضعين للاستبيان يعتمدون اعتمادا كليا على تربية المواشي . وهذا يؤكد أهمية دور هذه الأمراض اقتصاديا بالنسبة لمربي المجترات الصغيرة.

تحسين معينات التشخيص الحقلي واستخدام c-ELIZA ، يعملان علي تحسين مراقبة ومكافحة مرض طاعون المجترات الصغيرة. هذا بالإضافة إلى بعض الحزم الإرشادية التي تعمل علي توعية الرعاة بالاستخدام الجيد للمراعي ، التعريف بالأمراض ، وضرورة استخدام الأدوية و اللقاحات .

Introduction

Sudan is one of the largest countries in the Near East and North African (NENA) region, with an area of about 2.5 million squared km. One third of the total area is arable land, with approximately 21 percent currently being utilized. Over 40 percent of the total area consists of pasture and forests. The total area of grazing land is estimated at about 100 million hectares, while forests and scattered trees cover about 19 percent of the total area of the country). Human population is about 30.3 million, with an annual growth rate of 2.7%.

The country is characterized by a wide range of climatic variations, from desert in the north to forest and rich savanna with equatorial climates in the south. The country lies in the tropical zone between latitudes 3° and 22° North and longitudes 22° and 38° east. It is bounded by nine countries including Egypt and Libya to the north, Chad and Central Africa Republic to the west, Zaire, Uganda, and Kenya to the south, Ethiopia and Eritrea to the East.

Annual rainfall in the northern part of Sudan varies from close to zero to about 200 mm. In the low rainfall savannah, it rains on the average between 300-900 mm and from 900 to 1500 mm in the high rainfall savannah belt.

Agriculture dominates the Sudanese economy. It contributes about 40 percent to the GDP. The sector employs over 70 percent of the total labor force and is the principal source of most intermediate inputs for industry. In addition, it meets almost all the country's food requirements.

There are three systems of animal production in Sudan:

1) The traditional system, which comprises of nomadic, semi-nomadic, and transhumance which are all based on range

2) Intensive system, which is based on, irrigated fodder and industrial bi-products and located near the big towns and mainly for milk and poultry production.

3) Feedlots located around livestock markets where animals are drawn from the traditional production system and subjected to concentrate feeding to support export and local consumption after fattening and reconditioning.

The total animal population is estimated at about 130 millions heads. This population includes 38 millions cattle, 47 m. sheep, 39 m. goats, and 3 m. camels. Over 90 percent of this wealth is kept under the transhumant production system. The rest are found in the urban and peri-urban commercial systems. The livestock sub-sector contributes between 20-25 percent to the GDP, provides over 20% of the country's foreign exchange earnings (second to oil). In addition, it meets almost all the domestic needs of meat.

Small ruminants play multiple roles in poverty alleviation among the rural communities, where poverty is more prevalent. During the 1990s, there was a rapid increase in the total livestock production, such that the off-take of sheep quadrupled between 1989/1990 and 1999/2000 in response to strong export market in the Gulf countries, specially Saudi Arabia. However, this trend was interrupted when the veterinary authorities in Saudi Arabia banned the importation of sheep from eight African countries including Sudan in September 2000 due to the outbreak of Rift Valley Fever in the southern part of the Saudi Arabia. Exports of sheep subsequently rebounded significantly

in 2002 following the resolution of the veterinary problem in Saudi Arabia, but potential risk to Sudan's live sheep export remains.

Small ruminants' diseases in Sudan are considered one of the most important constraints and burden for the livestock owners and other stakeholders to access national, regional and international markets. The livelihoods of such livestock owners whose only assets are their animals are therefore affected and unsecured. Not only would that but the share of livestock in the national economy decline.

The important small ruminant diseases are the viral diseases (*Peste des Petits Ruminants*, sheep pox), bacterial diseases (Pneumonia, Lymphadenitis, Closterdial infections), fungal infections (Ring worm), parasitic infections (Internal & external parasites), toxicity (Plant and Chemical poisoning), protozoan infection (Babesia), miscellaneous (Foreign bodies), rickettsia (Heart water) (CCPP) and others.

The aim of this research is to study the epidemiology of the most important small ruminants' diseases in order to make a rational decision for their prevention and/or control. The ultimate aim is to improve the livelihoods of poor small ruminants keepers in the Sudan through research targeted at the selected small ruminants' diseases resulting in increase productivity and enhanced access to markets. Therefore, research objective will focus on:

1. Determination of prevalence and economic impacts of diseases of small ruminants, with a focus on *peste des petits ruminant* (PPR), and heartwater.

2. Identification of possible feasible interventions to reduce or minimize their negative impacts on the livelihoods of small ruminants' keepers in the Sudan.

Chapter One: Review of literature

1.1. *Peste des Petits Ruminants (PPR):*

1.1.1 Definition:

Peste des petits ruminants (PPR) is an acute or sub-acute viral disease of goats and sheep. It is also known as pseudorinderpest of small ruminants, pest of small ruminants, goat plague, pest of sheep and goat, stomatitis pneumoenteritis syndrome, contagious pustular stomatitis and pneumoenteritis complex, (Alillo *et al.*, 1998). It is a highly contagious, infectious and fatal viral disease of domestic and small ruminants (Roder and Obi, 1999). The disease is characterized by fever, necrotic stomatitis, gastroenteritis and pneumonia. Defra(2005) described it as a rinderpest-like contagion of goats and sheep characterized by erosive stomatitis, enteritis, pneumonia.

Economically it is the most important animal disease in southern equatorial Africa, being a major constraint to the availability of animal protein for human consumption. It is an OIE class A disease.

1.1.2. Host range:

Until recently the known natural hosts were restricted to goats and sheep but an epidemic in the Al Ain Zoo in the United Arab Emirates extended the natural host range to include smaller species of wild ungulates, with cattle being refractory to infection (Defra, 2005).

The disease affects both sheep and goats, but goats are more susceptible to infection.. To date, it has been diagnosed only in captive wild ungulates from the families of *Gazellinae* (*dorcas gazelle*), *Caprinae* (Nubian ibex and Laristan sheep) and *Hippotraginae* (gemsbok) (OIE, 2002a).

Experimentally the American white-tailed deer (*Odocoileus virginianus*) is fully susceptible. Cattle and pigs develop inapparent infections. The disease seems to have a breed-linked predisposition in goats. (OIE, 2002), and goats are affected more severely than sheep (Roeder *et al.*, 1994, Diallo, 2000a). In 2002 antibodies were detected in 14 out of 100 camel sera in eastern Sudan and Khartoum where PPRV in camels was isolated and confirmed in CVRL and University of K in 2005. (Mahasin, 2005) In Egypt 4.2% of healthy slaughtered camels were positive for PPR antibodies. Man is not known to be at risk of PPR infection (Alillo *et al.*, 1998).

1.1.3. Occurrence:

PPR occurs in goats and less often in sheep. Outbreaks were first described in West Africa in 1942 and it is now endemic in the west and central Africa Where still lingers on, such that in 2004 there were reports in Guinea, Benin, *Côte d'Ivoire*, Mali, Senegal and Guinea Bissau. It was also reported from Chad, Eritrea, Ethiopia, Ghana, Mauritania and Nigeria (Bernald, 2005). It is possible that some of the earlier reports of rinderpest in sheep and goats in Asia might have been PPR outbreaks since the two diseases are not easily distinguishable on clinical signs only. Cattle and pigs develop serum-neutralizing antibodies but no disease following experimental infection. A natural disease may occur in wild sheep, gazelle and the deer but there are

no known reservoirs in domestic animals and wildlife. The disease is not transmissible in humans. (Radostits *et al*, 2000).

(Obil, 1990, Tamburawa, 1997) studied the seasonality of PPR and found that in all the climatic zones in Nigeria the disease was encountered all year round with peaks during the wet season. In contrast Butswat *et al*. (2005) found that the peak incidence was observed during the late rainy season.

Mohammed (2001) analyzed the Ethiopian veterinary services records, together with questionnaires outcomes and they indicated that PPR occurred in all months of the year with the peak of outbreaks between October and March.

1.1.4. Classification of causative agent:

PPR is caused by a virus that belongs to the *morbillivirus* genus of the subfamily *paramixovirinae*, family *paramixoviridae*, order *mononegavirales*. It is closely related to Rinderpest virus of cattle and buffaloes as well as the viruses of canine distemper in dogs, porcine distemper in seals, measles in human (Murphy *et al*, 1995), and equine influenza recently described in Australia (Grocock, 1992). The African and the Asian strains of PPR virus have some biochemical differences, implying that both strains may have evolved separately (Abu Elzein *et al.*, 1990), presumably from the goat – adapted rinderpest vaccine induced six decades ago. (Radostits *et al*, 2000).

Originally PPRV was considered a variant of RPV adapted to small ruminants; however, the two viruses have separate epizootiologic cycles in nature, and each exists in its own right (Özkul *et al.*, 2002)

1.1.5. Resistance to physical and chemical action:

Table 1.1: Resistant of PPR virus to physical and chemical actions:

physical and chemical action	Resistant of PPR virus status
Temperature	Some viruses may resist 60°C/60 min
pH:	Stable between pH 4.0 and 10.0
Chemicals:	Susceptible to alcohol, ether, detergents
Disinfectants:	Susceptible to most disinfectants, e.g. phenol, sodium hydroxide 2%/24 hours
Chilled and frozen tissues:	Survives for long periods in chilled and frozen tissues

Source: International Animal Health Code 2002

1.1.6. History and Geographical Distribution:

1.1.6.1. Geographical Distribution:

In West Africa a fatal disease of goats with high mortality in early 1940s was described in Ivory Coast (*Coted' de Ivoire* as PPR (Gargadenec and Lalne, 1992). Osman (2005) cited that Cathou (1944) reported a syndrome similar to PPR was described under the name of “ *Peste des especes ovine et*

caprine” in Benin in early 1940s. Mornet *et al.*, (1956) described similar disease in Senegal and Ghana in 1950. Similarly, a disease affecting goats in Nigeria during the same period was studied by Johnson *et al.* (1968) and it was given different names (Stomatitis Pneumoenteritis Complex (SPC), Pseudo Rinderpest and Kata). Later studies proved that the SPC was actually PPR (Rowland and Bourdin, 1970; Hamdy *et al.*, 1976).

Dhar P. *et al.* (2002) studied the genetic relationship between isolates of distinct geographical origin and viruses from 27 outbreaks in Asian and Middle Eastern countries, reported between 1993 and 2000, as well as two recent outbreaks from the African continent and compared them with the prototype African strain and they found that of the four known lineages of PPR virus, lineage 1 and 2 viruses were exclusively in west Africa. A virus from an outbreak in Burkina Faso in 1999 fell into the lineage 1 group. The viruses of lineage 3 were isolated in east Africa, from an outbreak in Ethiopia in 1996. The same virus type was found in Saudi Arabia and southern India. However, there have been no further isolations of lineage 3 virus from India since the one reported in 1992 from Tamil Nadu. A virus of this lineage was also found circulating in Yemen in 2001. In the past 8 years a virus exclusively of the fourth lineage has spread across the Middle East and the Asian sub-continent, reaching Far East as far as Nepal and Bangladesh. This virus lineage was also reported from Kuwait in 1999. The geographical source of the new lineage 4 virus is unknown although it is most closely related to African lineage 1. The possibility that its earlier presence in northern India was masked by the circulation of Rinderpest virus, a related virus of cattle, is considered unlikely.

Two PPRVs of lineage 4, which comprises many other PPRVs, with origins possibly, in the Middle East, the Arabian Peninsula, and southern Asia, were isolated from Turkish sheep (Özkul *et al.*, 2002).

PPR has a very high rate of morbidity and mortality, and effective control of this disease is of economic importance in Africa, Asia and the Middle East.

A study was designed in Ethiopia (in East Shewa, North Wollo and South Wollo) in 2001 to estimate the level of sero- prevalence, and he found that the sero- positivity levels were 13.4%, 4.3% and .4% respectively. Similar incidence in Djibouti and Somalia were also reported, (Mohammed, 2001).

In September 1998, Iraq reported officially for the first time an outbreak of *peste des petits ruminants* (PPR) in its northern governorates to the International Office of Epizootics (OIE) and the FAO. Likewise, In September 1999, an outbreak of PPR in goats in Turkey was reported by the Ministry of Agriculture and Rural Affairs, Ankara, to the OIE for the first time (FAO, 2005). On January 2005 a confirmed outbreak of PPR in Israel was reported to the FAO (FAO, 2005).

1.1.6.2 PPRV lineages:

Dhar (*et al*, 2002) identified four different PPRV lineages namely, Lineage (1) in West Africa including Senegalese strain, Nigeria 75/1, 75/2, 75/3, 76/1 and Burkina Faso isolates, Lineage (2) in other Western African countries, including Guinea Biseau/91 and Ivory Coast/89 isolates, Lineage (3) in East Africa including Sudan/72 and Ethiopia/96 isolates, as well as in Asia

including Oman/83, India/TN/92 and Yemen/01, isolates. Lineage (4) was identified in Asia, including Indian isolates (India/UP/94, India/MH/94), Bangladesh/93, Bangladesh/00, Nepal/95, Turkey/96 And Turkey/00, Israel/94, Pakista/94 and Pakistan/98, Saudi Arabia/94, Iran/94, Irag/00 and Irag//00 and Kuwait/99 isolates. (Figure 1.1 and Figure 1.2)

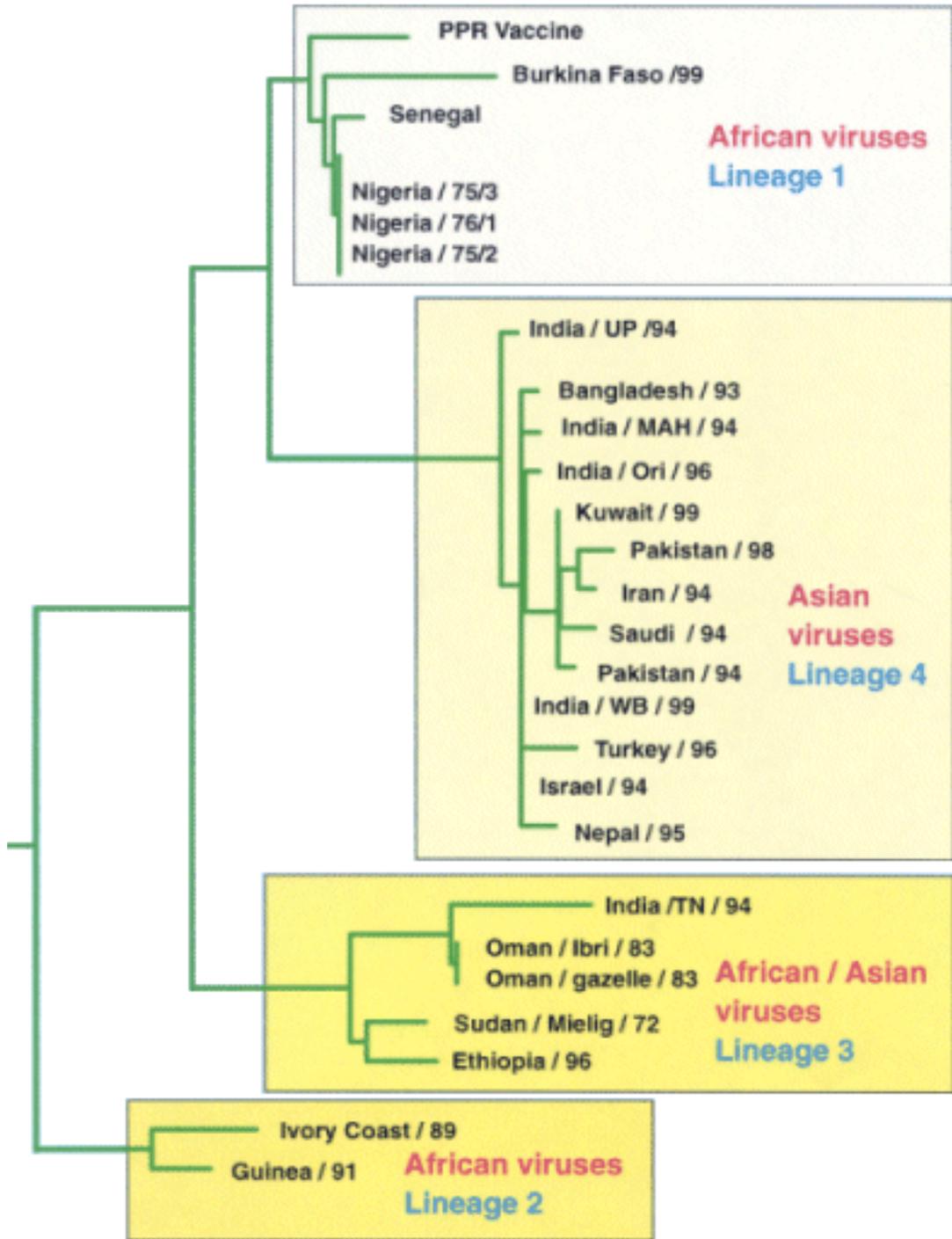


Figure 1.1: PPRV: Phylogenetic relationships Between the different isolates of PPRV

(Dhar *et al*, 2002, with permission)

Source: FAO, AVIS

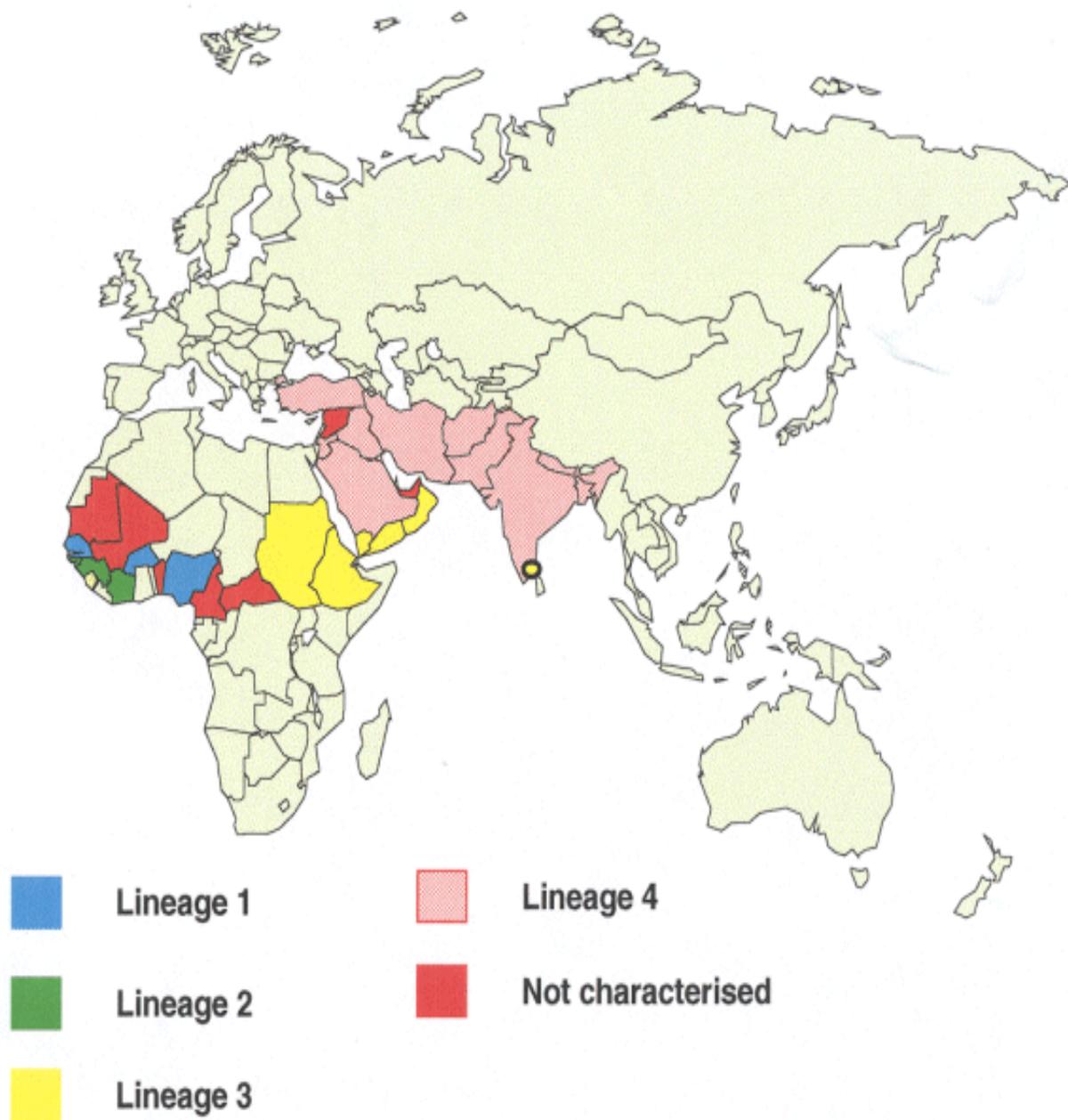


Figure No. 1.2 : Global distribution of PPR virus infection
 Source: FAO, AVIS

1.1.6.3.1 PPR in the Sudan: Historical perspective

The first report of an outbreak of a rinderpest-like disease in sheep and goats was in 1971 in the southern part of Gadarif State near Dindir River, (Elhag, 1973). The disease was diagnosed as Rinderpest (RP) on clinical signs. However, RP precipitinogens were later demonstrated by agar gel plate test (AGPT) (Elhag Ali, 1973). Subsequent isolates from an outbreak of a 1972 RP-like disease in Sennar and Meilig in Sudan were found to be closely related antigenically to the Nigerian PPRV. The isolates were considered as PPR and termed as SUD 72/ 1(Sinnar) and SUD 72/ 2 (Meilig) (Elhag & Talor, 1984).

Other outbreaks of PPR in the Sudan, include the ones from Elhilalia in Gezira state, (Awad Elkarim *et al.*, 1994) and Elfashir in North Darfur (Elsheikh (1992). Serosurveillance results demonstrated the prevalence of the disease in Khartoum, (Zeidan 1994), Darfur, Khartoum and Eastern states (Intisar (2002)and in Southern states, (Osman, 2005).

The detection of antibodies against PPR in Blue Nile, Kordofan, Darfur, Khartoum, River Nile and Southern States was considered an indication of wide spread of PPR infection in the Sudan, (Osman N. A., 2005).

1.1.6.3.2 PPR in camels:

Antibodies against PPR were detected in 14 out of 100 camel sera in eastern Sudan and Khartoum, (Haraun, *et al.*, 2002), and in Khartoum alone PPRV isolates were confirmed by the Central Veterinary Research Laboratory

(CVRL), (Mahasin, 2005). Outside the Sudan, 4.2% of healthy slaughtered camels in Egypt were positive for PPR antibodies, (Roger, *et al.*, 2001); while a 1995 outbreak of respiratory disease in Ethiopia (more than 90% and 5-76 % morbidity and mortality rates respectively) was confirmed to be PPR, (Mahasin, 2005). From suspected cases of PPR in camels in Kassala and Gedarf States, virus isolation was carried out by the CVRL and confirmed as PPR (Bernard, 2005).

1.1.6.3.3 Incidence of PPR outbreaks diagnosed at the virology department, CVRL Khartoum, 2000-2005 (Table 1.2 below).

Origin	Animal species	Date
W. Nile	Sheep	April 2000
Khartoum	Sheep	Feb. 2000
Khartoum	Sheep	Feb. 2000
Khartoum, Export animals	Sheep	June 2000
Khartoum, Abu Delaig	Sheep	June 2000
River Nile	Sheep	March 2001
Sinnar Regional lab	Sheep	March 2001
Khartoum , Wad ramly	Sheep	March 2001
Khartoum, CVRL	Goats	Dec. 2001
Gazira	Goats	Novem. 2001
Khartoum, Animal Health Admin.	Sheep	April 2001
River Nile, Atbara Regional lab.	Sheep	April 2002
Khartoum, Animal Health Admin.	Sheep	April 2002
River Nile, Bashair Farm	Sheep	Octob.2002
Gazira	Goats	Novem. 2002
W. Nile, Rabak	Sheep	Dec. 2003
Khartoum	Sheep	Janu. 2004
Blue Nile	Sheep	Janu. 2004
N. Kordofan, Obied Regional lab	Sheep	Feb. 2004
Gazira	Goats	Feb. 2004
Kassala	Goats	Feb. 2004
Different locations	Sheep/ Goats	2005

Source: Mahasin (2005):

1.1.7 Morbidity and case mortality rates

Infection rates in enzootic areas are generally high (above 50%) and can be up to 90% of the flock during outbreaks. The percentage of sheep and goats with antibodies rises with age. The disease, however, is more severe in goats than in sheep and is rapidly fatal in young animals. Case fatality rates are also much higher in goats (55 – 85%) than in sheep (less than 10%). There is no significant seasonal variation in the prevalence of the disease but since maternal antibodies are lost at about 4 months of age, the number of susceptible animals is likely to increase 3 - 4 months after peak kidding and lambing seasons. (Radostits *et al* 2000a).

Butswat *et al.* (2005) mentioned that the mortality rate due to PPR in the climatic zones of Nigeria was put at 85% and a morbidity rate of 40%, with variation of the severity of the disease between sheep and goats (ILCA, 1999). Moreover, PPR follows a cyclic pattern with significant increase in incidence rate every 3 – 4 years (Taylor *et al.*, 1990).

Females are more susceptible to PPR than their male counterparts and similarly the sheep were found to be less severely affected than goats (Obi *et al.*, 1992).

In the Sudan, morbidity and mortality rates have been shown not to exceed 66% and 37% respectively. In goats, however, kids' mortality rate may reach 50-70%, as shown in a study at the CVRL/ Khartoum, rates of 10 – 66% and 3 – 37% was recorded in different studies (Mahasin 2005). Moreover, Rates as high as 62-91% & 55% have been recorded in another

study. Meanwhile, these rates could be as high as 90 – 100% in severe outbreaks, (Mahasin 2005)

1.1.8 Virulence of PPR:

Baldock *et al.* (1999) reported that in the Middle East, the ability of PPR to gain an endemic presence within a country without causing an initial heavy mortality in indigenous small ruminant populations may be observed. Although not well studied, it is possible that the virulence of this related morbillivirus is just as variable as that of rinderpest. Unlike rinderpest, there are few instances with PPR where lengthy vaccination campaigns have been directed at the control of the virus. Even if PPR with a low level of virulence is present in a country, strains may intermittently regain a higher level of virulence and so clinical outbreaks and associated mortality will probably continue to occur. If for any reason large susceptible populations have accrued, these may be severely involved.

1.1.9 Methods of transmission:

Close contact with infected animal or contaminated fomites is usually required for transmission to take place. This is because large amounts of the virus are present in all body excretions, especially in diarrheic feces and infection is mainly by inhalation, although it could also occur through the conjunctivae and oral mucosae. (Radostits *et al.*, 2000a). The most important sources of the virus in infected animals are tears, nasal discharge, coughed secretions, and all other secretions and excretions of incubating and sick animals. (OIE, 2002).

Market goats do harbor and can transmit the virus. The first outbreaks in Saudi Arabia were associated with the importation of sheep or the return of unsold lambs from livestock markets. (Radostits *et al*, 2000a). The disease can be experimentally transmitted through close contact with an infected animal or through inoculation of infected tissues or blood. (Radostits. *et al.*, 2000a).

In the context of the African continent south of the Sahara, this disease cycles endemically in the nomadic herds and flocks under transhumant system of management where they graze in the sub-Saharan sahel annually. Such herds introduce the virus into immunological naïve herds and flocks south of the Sahel with disastrous results. Sick goats and sheep generate aerosols containing infective droplets. Successful transmission therefore requires close contact between sick and healthy animals (Defra, 2005).

Kids over four months and under one year of age are most susceptible to PPR. Meanwhile, sahelian breeds of sheep and goats are believed to be more resistant to the disease than the dwarf breeds in the humid and sub humid zones of West Africa. In a particular flock, the risk of an outbreak is greatly increased when a new stock is introduced into a clean herd or when animals are returned unsold from livestock markets (Radostits *et al.*, 2000a).

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1.1.10 Economic importance:

In terms of animal health impact, PPR is considered as one of the five most feared diseases during transhumance although there are no estimates of the cost of the disease associated with transhumance. However, some cost

estimates have been given for a few countries, including Angola where the cost could be as high as EUR 8.5 million (excluding production losses) (Bernard, 2005) and Nigeria, with estimates of over N 1 million annually (Butswat and *et al.*, 2005), 20- 40 million U.S. Dollars (Adeoye, 1998)

1.1.11 Diagnosis:

1.1.11.1 Clinical diagnosis:

Incubation period is 3-10 days and clinical signs and post-mortem lesions are highly suggestive in acute and peracute cases. Subacute cases, however, are difficult to diagnose in the absence of frank clinical signs among the other members of the flock. Confirmation is readily achieved by detecting antigens in lymph nodes or tonsils collected from newly dead animals (Defra, 2005).

1.1.11.2 Acute form:

Signs generally appear 3 – 6 days after being in contact with an infected animal. A high fever above (40°C) is accompanied by dullness, sneezing, and serous discharge from nostrils and eyes. A day or two later, discrete necrotic lesions develop in the mouth and extend over the entire oral mucosa, forming diphtheric plaques. The animal is unable to eat because of sore mouth and swollen lips. Nasal and ocular discharges become mucopurulent and the exudate dries up, matting the eyelids and partially occluding the external nares (the nostrils). Diarrhea develops 3 – 4 days after the onset of fever, it is profuse and feces may be mucoid and blood tinged. Dyspnea and coughing occur later and the respiratory signs are aggravated

when there is secondary bacterial pneumonia. Erosions have been described in the vulva and prepuce. Death usually occurs within a week of the onset of illness. (Radostits *et al* 2000a).

1.1.11.3 Subacute and chronic forms:

Subacute forms are more common in sheep but they also occur in goats. The signs and lesions are less marked and a few animals may die within two weeks but most recover. Contagious ecthyma (orf) may complicate the labial lesions or develop in surviving animals. (Radostits *et al.*, 2000a).

Both subacute and chronic forms are frequent in some areas because of local breed susceptibility, with the disease taking 10-15 days of development with inconsistent symptoms and some times pneumopathy only. (OIE, 2002).

1.1.12 Samples needed:

Saliki JT. (1998) stated that the Specimens to submit include blood in EDTA anticoagulant, clotted blood or serum (if possible, paired sera), mesenteric lymph nodes, spleen, lung, tonsils, and sections of the ileum and large intestine. Swabs of serous nasal and lachrymal discharges may also be useful. All samples should be shipped fresh (not frozen) on ice within 12 hours after collection.

1.1.13 Laboratory Diagnosis:

Serological Techniques:

Competitive ELIZA is a rapid simple, specific and sensitive test for detecting PPR antibodies (Osman N. A., 2005).

1.1.14 Differential Diagnosis:

1.1.14.1 Rinderpest:

The factor that determines the value of immuno diffusion test is that a rinderpest hyperimmune serum will react with antigens of *Peste des Petits Ruminants* (PPR) virus. Should the disease for which a diagnostic confirmation is being sought be associated with bovines, then it is probably safe to assume that it is rinderpest. However, should the disease have occurred in a small ruminant species, it is more likely to have been PPR (Baldock *et al*, 1999)

A growing number of countries are simultaneously infected or at risk of infection with both viruses (Rinderpest and PPR). Under these circumstances, epidemiological features should be taken into account if a disease from which morbillivirus antigens are demonstrated is encountered. For instance, the presence of a clinical disease in sheep and goats but not in contact cattle is more compatible with a diagnosis of PPR than of Rinderpest, while a disease in cattle but not in small ruminants is more compatible with a diagnosis of rinderpest (Elhag, 1973, Govindarajan *et al.*, 1997).

However, in the final analysis it is possible for rinderpest to affect small ruminants and it is apparent that PPR is transmissible to large ruminants may

also affect a minority of individuals with a clinical disease (Elhag, 1973, Govindarajan *et al.*, 1997).

Baldock *et al* (1999) reported that, in the last decade in a number of African and Asian countries, there has been a realization that both PPR and Rinderpest were affecting the national livestock industries at the same time. As PPR in small ruminants can certainly be regarded as a rinderpest-like disease, a requirement emerged for immunocapture ELISA test which would differentiate these two diseases. This test has been introduced throughout the state disease investigation laboratories in India where it has been widely used to confirm the diagnosis of PPR in sheep and goats.

In 1997 field trials were undertaken with a chromatographic strip test, a so called “pen side Rinderpest diagnostic test” for the rapid detection of the presence of rinderpest antigens which are known to be present in ocular secretions (Baldock *et al*, 1999).

DNA probes to the N genes of rinderpest and PPR are now available and can be used to identify and differentiate between the two viruses (Diallo *et al.*, 1989). The reverse transcription polymerase chain reaction has proved a very powerful tool in rinderpest diagnosis and can be used to differentiate rinderpest from PPR (Baldock *et al*, 1999). Differentiation from Rinderpest requires isolation of the virus in cell cultures

1.1.14.2 Other diseases:

Other diseases which should be differentiated from PPR include contagious caprine pleuropneumonia, bluetongue, pasteurellosis, contagious ecthyma, foot and mouth disease, heartwater, coccidiosis and mineral poisoning and Nairobi sheep disease (Defra, 2005).

1.1.15 Control:

Control of PPR follows the procedures described by Hanson and Hanson, (1983), which essentially aim at making the population less susceptible. However, there is considerable variation in the susceptibility of animals to different diseases and the ability of animals to resist infestation with disease carrying vectors (Eaton and Gray, 1995). Variation in susceptibility to disease occurs on several levels, including between breeds, between major blood lines within breeds, between families within blood lines and between individuals within families (Raadsma, 1995).

The tissue culture Rinderpest vaccine is effective but its use in enzootic areas is not as organized as it is for Rinderpest. Kids and lambs should be vaccinated at 3-4 months of age by which time maternal antibodies would have waned (Radostits *et al.*, 2000a). A newly developed recombinant vaccinia (14) or caripox (15) viruses expressing the fusion (F) and haemoagglutinin (H) protein genes of the Rinderpest virus are also effective against PPR. Further more, a homologous PPR tissue culture vaccine produced by attenuation of a Negerian isolates in Vero cells is protective against virulent virus challenge (Radostits *et al.*, 2000a).

The tissue culture Rinderpest vaccine (TCRV) protects small ruminants against severe disease, but there are clinical problems associated with vaccination. Goats were vaccinated with a vaccinia virus double recombinant expressing the haemagglutinin and fusion genes of RPV. Although vaccinated animals developed antibodies (neutralizing and ELISA) to RPV, and not to PPRV, they were completely protected against challenge inoculation with virulent PPRV. This would indicate that protection is most probably due to cell-mediated immunity. Use of the rinderpest double recombinant vaccinia virus in areas of the world where PPRV is endemic would aid in the control and eradication of PPR. (Jones *et al.*, 1993).

Rinderpest heterogeneous vaccines protect small ruminants against PPR (Bourdin *et al.*, 1970; Naduaka and Ihemelandu, 1975). The use of chloroform inactivated RPTC vaccine successfully controlled pneumonia-enteritis complex (Naduaka and Ihemelandu, 1975). Recently, the use of RP vaccine to protect small ruminants against PPR has been contradicted because the RP antibodies produced were shown to compromise RP serosurveillance (Roeder and Obi, 1999). Butswat and *et al.* (2005) suggested that a vaccination programme against PPR at the onset of the rains may drastically reduce the incidence of the disease. Mohammed (2001) carried out a study in Ethiopia and suggested that the optimum month for launching annual vaccination campaign is September due to the peak of kidding which is confined to the months of April to August. However, since PPR is a transboundary disease, eradication campaigns through neighboring countries working in concert with one another is a necessary element of international collaboration (Baldock *et al.*, 1999).

Valuable sick animals in early stages of the disease should be isolated and given hyperimmune serum, which may be obtained from cattle hyperimmunized against Rinderpest (Radostits *et al.*, 2000a). Supportive treatment includes fluid therapy for dehydration and antibiotics to prevent secondary bacterial infections. Further more, Lesions around the eyes, nostrils and mouth should be cleaned and good nursing provided (Radostits *et al.*, 2000a).

1.1.16 Prevention of host exposure to disease agents

The disease can be prevented by not introducing new stock from unknown source,. Thus, animals returned unsold from markets should be segregated unless the entire herd or flock has been vaccinated (Radostits *et al.*, 2000a). Susceptible hosts can be protected by the use of quarantine and livestock movement restrictions to prevent animals originating from an area where the disease is known to have occurred, to mix with susceptible clean herd; or enter uninfected area. Roeder and Obi (1999) recommended quarantine measures (Movement control) with the use of ring vaccination techniques in high risk animal populations, accompanied with, proper disposal of carcasses and contact fomites decontamination.

1.2 Heartwater:

1.2.1 Definition:

Abdel Rahman *et al.* (2003a) cited that, Heartwater (*Cowdriosis*) is a rickettsial tick-borne disease caused by an obligate intracellular rickettsia, *Cowdria ruminantium* that parasitizes vascular endothelial cells, neutrophils, macrophils and macrophages (Uileberg, 1983; Mahan *et al.*, 1992, Peter *et al.*, 1995). The disease affects domestic and wild ruminants and is characterized by fever, nervous signs, hydrothorax, hydropericadium, lung oedema and frequently fatal in susceptible animals (Prozesky 1987; van Vliet, 1995; Camus *et al.*, 1996). The other names by which the disease is known are *Cowdriosis*, *Malkopsiekte*, *Pericardite Exsudative Infectieuse*, *Hidrocarditis Infecciosa*, and *Idropericardite Dei Ruminanti*(Olugasa B. *et al.*, 2004)

1.2.2 Species affected

Cattle, sheep, goats, water buffalo and white tailed deer (experimentally) are severely affected, indigenous African breeds of sheep and goats are mildly affected while Blesbok, wildebeest, eland and springbok are inapparently affected (Olugasa. *et al.*, 2004)

1.2.3 Methods of transmission:

Cowdria ruminantium is transmitted by *Amblyomma spp.* ticks. *Amblyomma variegatum* ticks are major vectors of *C. ruminantium* (Uilenberg, 1983 and

Walker and Olwage, 1987). *A. lepidum* is also an important vector of heartwater, especially in eastern Sudan (Jongejan, *et al.*, 1984).). However, few epidemiologic data exist on infection rates of *Amblyomma* spp. ticks and distribution of *C. ruminantium* in Sudan.

Olugasa *et al*, 2004 said that in endemic areas, there has been evidence of transmission of heartwater from infected cows to their calves through colostrum. Wild ruminants such as blesbok (*Damaliscus dorcas phillipi*) and black wildebeest, as well as helmeted guinea fowl, leopard tortoise (*Geochelone paradalis*) and scrub hare have been shown to harbor *C. ruminantium* sub clinically for long periods and do play a role as source of infection for ticks.

1.2.4 Occurrence:

The disease occurs throughout most of Sub- Saharan Africa (including the Sudan) and three Caribbean islands (Peter *et al.*, 1995). Its occurrence in the Caribbean and sub-Saharan Africa, except certain areas of southern Africa is due to the presence of the vectors transmitting the disease.

A disease that affected sheep and goats in Kassala province was diagnosed as heartwater (Karrar (1960); while Nasri (2003) reported that heartwater was mentioned in the reports of Sudan veterinary authority in 1903, 1928, 1929 and 1931, meaning that the disease has been known in the Sudan for a very long time.

1.2.5 Risk factors associated with Heartwater in Sudan:

Introduction of susceptible animals into heartwater endemic areas poses the highest risk of infection to the newly introduced susceptible animals. Indigenous domestic ruminants are usually resistant to heartwater (El Amin *et al.*, 1987; OIE, 2002b); while high yielding breeds from *Amblyomma* free areas or imported exotic breeds and their crosses are susceptible to the disease (Jongejan *et al.*, 1984).

In a study conducted in Kassala province, El Amin *et al.*, (1987) pointed out that; the previous belt of *A. lepidum* was shifting according to where ruminants were encountered. Additionally, desertification in Southern part of Eastern Sudan had aggravated the condition even more in recent years, resulting in ticks' re-distribution to grazing areas.

Camels rose together with sheep and goats in heartwater endemic zones were found to be more heavily infested with *A. lepidum* (Karrar, 1960). However, their role in the epidemiology of heartwater needs to be elucidated. Moreover, and according to Kock *et al.* (1995) wild healthy ruminants were proved to be good carriers of the disease.

1.2.6 Host seeking and seasonal abundance of ticks:

Hassan and Osman (2003) cited that during the host-seeking stage, ixodid ticks, either hunt or ambush their host (Wlaldde and Rice, 1982). Those which hunt (*Amblyomma* and *Hyalomma spp.*) hide quiescently in their microhabitats and are only activated by the presence of their hosts towards

which they actively run. Branagan (1974) explained that this is due to the more rapid water loss suffered by ticks on entering the raised temperature of the host environment. Quiescent adult ticks are activated by start of rainfall. In Kenya, Newson and Chiera (1989) reported that parasitic adult *A. variegatum* became abundant soon after the onset of the rains and the population remained high until the end of the rainy season. In Northern Uganda, Kaiser *et al.* (1991) observed that the onset of feeding activity of adult *A. variegatum* coincided with the onset of the wet season. There are factors influencing the fluctuations in parasitic tick population density. These include temperature, photoperiod, vegetation cover, host availability, host susceptibility, resistance to ticks, or type of husbandry (Hassan and Osman, 2003).

1.2.8 Ecology of Ixodid ticks:

In their summary, Hassan and Osman (2003) stated that the tick populations are associated with plant and animal components, and therefore combination of ecologically based strategies that focus on ticks' interaction with both plant and animal hosts offer the best promise of success for regulating tick population. Branagan (1973) established that development of ticks becomes negligible below 15°C and ceases altogether at 9°C. Sweatman, (1967) reported that below 20°C markedly extended pre-oviposition and oviposition periods occur. Moreover, he reported that humidity does not affect development as air temperature and ticks are more prolific in shaded environment regardless of season. Osman and Hassan (2003) reported that survival and development of *A. lepidum* becomes almost impossible without adequate water source for the young instars.

1.2.8 Spread of heartwater in the Sudan:

Considering the presence of vector transmitting the disease, Osman and Hassan (2003) stated that the distribution of *A. lipidum* in the Sudan is generally concentrated on the eastern part of the country from Torit and Kapoeta in the South and as far as Kassala in the Northeast. The tick is absent from Northern Province, Dar Fur, Western Kordofan, Bahr el Gazal and Equqtoria West of the Nile, where the other important species *Amblyomma vareigatum* is widely distributed.

1.2.9 Prevalence of Heartwater:

An overall prevalence of 86.4% was determined in Gadarif and Kassala in Eastern Sudan in a recent survey, using an indirect MAP- 1B ELISA to detect exposure of sheep to heartwater (Abdel Rahman *et al.*, 2003b).

1.2.10 Economic importance:

Minjauw and McLeod, (2003) stated that in most pastoralist systems, off-take from small ruminants is higher and more frequent than that from cattle, providing a ready source of cash. A high or increasing seasonal incidence of *C. ruminantium* is therefore a threat to income security. Generally he stated that, tick-borne diseases can reduce human capital of household nutrition, education, health or labors are affected, either directly or indirectly. In all systems, small ruminants are a source of cash for school fees and medical bills, so that infection of these animals by *C. ruminantium* may have a serious impact on family health or education.

1.2.13 Diagnosis:

The disease is usually suspected when associated with ticks of genus *Amblyomma*. Routine diagnosis of heartwater in the Sudan depends on clinical signs and demonstration of *C. ruminantium* colonies in brain crushed smears fixed with methanol and stained with Giemsa stain (Karrar, 1960; Osaman, 1976; Shommein and Abdel Rahim, 1977).

1.2.11.1 Clinical diagnosis:

During a limited survey in Eastern, Central and Western Sudan, three forms of the disease were recognized in sheep (Abdel Rahim, 1996). A mild form (transient febrile state) in which infected animals were unnoticed, the second form, which is hyperacute to the extent that infected animals may die without exhibiting the known clinical signs and the third one, referred to as Um Banein and Tambool type, which exhibits the classical incubation period course of the disease and clinical signs (Abdel Rahim, 1996).

In sheep and goats, the peracute form is rather common in exotic breeds. Most animals collapse suddenly and die after paroxymal convulsion (OIE, 2002). Subacute form with less pronounced signs also occurs. Postmortem changes usually include lung oedema, hydropericardium, hydrothorax and congested mucous membranes (Prozesky, 1987).

Olugasa *et al.*, (2004) stated that clinical signs and lesions of heartwater are representative of injury to the vascular endothelium and the resulting increase in vascular permeability. The incubation period ranges from 14-28 days, typically being shorter in sheep and goats than in cattle. In experimentally inoculated (i.v.) ruminants, clinical signs manifest quicker

onset [7-10 days in sheep and goats, 10-16 days in cattle]. Moreover Olugasa and *et al.*(2004) mentioned that the most common form of heartwater is the acute form. This is seen in both nonnative and indigenous domestic ruminants. Animals develop an acute high fever, loss of appetite, depression and respiratory distress and tachypnea. Nervous disorders can soon follow and be seen as excessive chewing movements, in coordination, head tilting upwards, overly rigid posture and walking with a high stepping gait. Some animals may have convulsions. Galloping movements and opisthotonus are commonly seen before death.

1.2.11.2 Histopathological Changes:

In the Sudan, Shommein and Abdel Rahim (1977) experimentally studied histiopathological changes associated with heartwater disease in goats. The authors reported marked microscopic (in the lymph nodes and a spleen in all cases of heartwater) and macroscopic changes, which included hydropericardium and hydrothorax. They suggested that the lymph nodes and the spleen might be possible sites for replication of the organism. In another study of blood chemistry, Abdel Rahim and Shommeim (1978) found that hematological changes in experimentally infected goats resulted in development of microcytic hypochromic anaemia.

1.2.11.3 Laboratory Diagnosis: Serology

Mboloi *et al.* (1999) stated that antemortem tests for detecting *C. ruminantium* include animal sub-inoculation, cell culture isolation, serodiagnostic tests, DNA hybridization, and PCR.

A serological survey using an indirect ELISA (MAP1-B) to detect exposure of sheep to heartwater in Gadarif and Kassala was carried out by Abdel Rahman *et al.* (2003a) and the results revealed an overall prevalence of 74.2% and 98.5% of heartwater in Kassala and Gadarif states, respectively. Jongejan *et al.* (1993) indicated that serodiagnostic methods, such as the indirect fluorescent antibody test, immunoblotting, and enzyme-linked immunosorbent assays (ELISA), have been hampered by cross-reactions with Ehrlichia species. However, Van Vliet *et al.* (1995) pointed out that, the use of recombinant major antigenic protein 1 (MAP1) of *C. ruminantium* has been recently introduced, and an indirect ELISA based on a specific fragment of this protein (fragment B, referred to herein as MAP1-B) has been developed.

1.2.12 Differential Diagnosis:

Radostits *et al.* (2000b) stated that in endemic areas, heartwater should be suspected in susceptible animals infested with *Amplyomma* and having a fever of unknown origin. The clinical and pathological findings are not specific and the diagnosis must be based on detection of rickettsial organisms. Moreover, the peracute form should be differentiated from anthrax and the acute form from rabies, tetanus, cerebral forms of theileriosis, babesiosis and poisoning with strychnine, lead and organophosphates. Appropriate tests, are utilized to eliminate these differentials.

Differential clinical diagnosis should be made with anaplasmosis, botulism, small ruminants' haemonchosis, plant poisoning and pesticides (chlorinated hydrocarbons) (Van de Pypekamp and prozesky, 1987)

1.2.13 Control strategy:

The possibility of control of heartwater as like other ticks and tick-borne diseases is based on the following methods:

1.2.13.1. Uses of acaricide:

The dip tank is an efficient, practical and convenient means of applying acaricide to a herd of livestock (Minjauw, B. and McLeod, A. (2003)). Control of ticks and tick-borne diseases has traditionally been based on dipping of animals using acaricides. In many countries, dipping services were provided by the State and were backed up by laws making dipping compulsory. In areas of high infestation, treatment could be provided as often as twice a week (Pegram, *et al.*, 1993).

The most widely used method for the effective control of ticks is the direct application of acaricides to host animals. However, acaricides are expensive and can be detrimental to the environment: their use should be minimized and integrated with alternative approaches (Minjauw and McLeod, 2003).

The high costs of acaricides, the development of resistance in ticks, environmental concerns and the disruption of endemic stability of TBDs are among the reasons offered as drawbacks for their use in controlling TBDs.

Indeed, in some areas, the expansion of crop production has reportedly made it difficult for owners to walk their animals to be dipped (Tama, 1989).

Hand-dressing, since each tick species has a preferred site of attachment, hand-dressing can be considered in cases where the tick burden is low and there are only a few animals to treat. This procedure involves applying acaricide to the preferred host attachment site (ears, udder, or scrotum) with a brush, sponge or piece of fabric, taking care to prevent exposure of the operator to the pesticide. Since this method reduces the number of ticks but does not leave the animal completely clean, it minimizes the detrimental effect of the ticks without jeopardizing natural immunity against TBDs. This makes it an ideal method for small production systems in endemic areas, and it is the most common form of tick control in India and in small, zero-grazed production systems in Africa (Minjauw and McLeod, 2003).

An alternative to hand spraying is the pour-on acaricide that is applied to the spine of the animal. Pour-on are solutions or suspensions of acaricides formulated with solvents and/or propellants which, when poured along the back line of a treated animal, spread and disperse over the hair and skin. The active ingredients in pour-on formulations are generally pyrethroids (e.g. flumethrin, cypermethrin or deltamethrin), but although some use macrocyclic lactones such as ivermectin or moxidectin, these latter compounds have a systemic effect, and ticks are only affected when they suck the blood of a treated animal. These products therefore do not prevent tick damage or rapid inoculation of the host with tick-borne pathogens. However, most of these products are also effective against endoparasites. (Minjauw and McLeod, 2003).

A drawback is that, ticks are only affected after ingesting blood; therefore pour-ons are not effective against tick damage or against the rapid transmission of disease. Furthermore, among small ruminants the efficacy of pour-ons can vary with the breed treated. It is suggested that the greatest differences would be between hairy and woolly breeds. For instance, Kock *et al.*, (1996) found that Angora goats were afforded least protection by the application of pour-on deltamethrin, where as Dorper sheep obtained greater protection and Merino were the best protected. Differences in the density of hair follicles and the amount of grease in the fleece may play a role, but the factors involved require further investigation.

Hand dressing is another common method of applying acaricides. However, adverse human health impacts may occur with the use of the treatment. Awumbila (1996) expressed fear regarding the risks to health caused by lack of protective clothing as well as the lack of a withdrawal period for milk after treatment.

1.2.13.2. Chemotherapy:

Treatment of clinical cases of heartwater is only successful if applied within 2 days of the onset of fever. In practice by the time the disease is recognized it will be too late for the infected animals, but others within the flock can be given protective treatment. Tetracyclines are therapeutically and prophylactically effective (Bell-Sakyi *et al.*, 1996). It is also possible to use drugs to protect susceptible animals that are introduced to an endemic area, aiming to allow the natural disease challenge to stimulate immunity in the animals. Minjauwand and McLeod (2003) said that *Cowdria ruminantium*

infections are generally treated with tetracycline antibiotics, which can also be used prophylactically during peak periods of *Amblyomma* activity.

The use of ivermectin (sustained-release bolus) orally, has been shown to reduce the reproductive capacity of ticks. Administration of a bolus shortly before the peak period of tick activity could reduce tick numbers later in the season or even in the following year (Soll *et al.*, 1990).

1.2.13.3. Vaccination:

1.2.13.3.1. Live vaccine:

Minjauw and McLeod (2003) mentioned that this is based on an infection-and-treatment method, which involves inoculation with virulent sheep's blood followed by treatment with tetracyclines. Although this procedure is useful for the induction of immunity to the disease, it is far from ideal as its application is cumbersome and risky: the blood has to be administered intravenously and intensive monitoring and treatment are necessary if losses due to adverse reactions are to be avoided. The vaccine is produced by the Onderstepoort Veterinary Institute, Republic of South Africa.

1.2.13.3.2. Attenuated vaccine

Minjauw and McLeod (2003) cited that (Jongejan, 1991) who thought that a vaccine of this type was developed by attenuating *C. ruminantium* stock by *in vitro* passage through endothelial cells; virulence for sheep and goats was lost but the material induced solid immunity to homologous challenge .

The principal advantage of using attenuated material for vaccination is that no subsequent monitoring or antibiotic treatment is required. However, the level of protection against field challenge is lower than that provided by the live vaccine. Results with attenuated *Cowdria* vaccine are encouraging, but it is not known whether or not the attenuated material can revert to virulence, or be transmitted by ticks. It is therefore advisable to use it only within its region of origin.

1.2.13.3.3. Inactivated vaccine

Martinez *et al.* (1994) mentioned that this vaccine confers a certain degree of protection but more work is needed to determine its viability under field conditions.

The important observation that goats can be partially protected against disease by immunisation with inactivated *Cowdria* makes recombinant vaccines more feasible. Efforts are currently being made to develop a sub-unit vaccine, but it is unlikely that one will be available in the near future.

1.2.13.4. Resistant breeds:

It is well known that local breeds, although often with less production potential, are more resistant to tick and tick borne diseases (TTBDs) than exotics. Crossbred animals are particularly vulnerable to TBDs. The resistance of different crosses should be tested to assess their suitability for introduction in areas where TBDs are prevalent. In Ethiopia Horro-Jersey and Horro-Simmental crossbred animals have been found more tolerant of ticks than many other crosses (Ali and de Castro, 1993).

1.2.13.5. Integrated tick and Tick borne diseases (TBDs) control:

Many authors now recommend the introduction of integrated tick control programmes (Pegram *et al.*, 1993). These programmes involve the use of vaccinations, resistant breeds of livestock, the manipulation of tick populations to allow the establishment or maintenance of conditions of endemic stability and the strategic use of acaricides to control TTBDs at times of high infestation. However, the establishment of an integrated programme is no simple matter. First, an understanding of the local ecology of tick species is required to identify the times of year at which infestation will be high. Next, knowledge of the levels of challenge faced by livestock from the various parasites is needed to allow an estimation of the risk of disease and the extent to which the establishment or maintenance of endemic stability is likely. Furthermore, given the heterogeneity in the morbidity and mortality rates for many TBDs within a relatively small geographic area, control programmes would have to be specifically targeted to be appropriate to the agro-ecological zone. Information of this kind, combined with a study

of the economics of the local farming systems and the costs and availability of various control measures would enable planners to design an appropriate control strategy for TTBDs.

1.2.13.6. Alternative methods of control:

A substance known as kupetaba, a mixture of dried tobacco leaves and a mineral called 'Magadi' soda, mined near Lake Magadi in the Rift Valley Province of Kenya, was studied by Dipeolu and Nduga (1991). It was found that a 100% suspension of kupetaba kills all stages of *R. appendiculatus*. It is suggested that its low cost and easy availability in West, Eastern and Central Africa, make this substance suitable for use by resource-poor farmers. Kupetaba is easy to prepare and can be applied easily with a cloth.

Another alternative control method that has been proposed is the use of chickens as predators of ticks. In an experiment, chickens were allowed to scavenge ticks from tethered cattle. It is suggested that the numbers of ticks eaten by chickens during 3- or 4-hour periods indicate that it is likely that chickens could have a positive impact on the control of ticks (Hassan *et al.*, 1992). The release of sterile male ticks is another possible control method. While the technique could work, it is difficult to raise the massive numbers of ticks that would be required (Morrow *et al.*, 1996).

Appropriate extension activities must provide farmers with the information necessary to enable them to design and evaluate sustainable strategies suitable for the control of ticks and TBDs under their particular conditions (Minjauw and McLeod, 2003).

Chapter Two: Materials and Methods.

2.1 Study sites selection:

To fulfill the requirement of the Small Ruminants' Project (ILRI/ICARDA) three sites were selected according to predetermined criteria , namely, diversity in terms of production systems, market outlets, prevalence of poverty, dominance of sheep and goats and prevalence of major transboundary diseases. Thus, Blue Nile, Gadarif and West Kordofan States (Elkhowei area) were selected as study sites.

2.2 Study sites description:

2.2.1 Gadarif State:

Gadarif State is in the eastern part of the Sudan, bordering Ethiopia. It has an area of 75,263 squared km and located between latitude 13°- 15°N, longitude 34° – 37° E. It has a rainfall ranging from: 75 mm—1500 mm north to south. Other sources of water include Bore-holes, Deep wells, Hafiers, Dams (*Suddud*), Water pumps and seasonal streams. The State has a human population of 1,148,262. The livestock population estimates (MOAR&F, 2004) comprised of 1,976,352 sheep, 1,210,329 goats, 979,775 cattle and 181,555 camels.

2.2.2 Blue Nile state:

This state is situated in the southeastern part of the Sudan at the boarder with Ethiopia. It has an area of 84,445 square km and located between latitude: 12° 34' - 9° 30' N, longitude: 35° 15' - 33° 8' E. It has a rainfall ranging from: 500mm – 1500 mm. Other sources of water include, Blue Nile river, shallow wells, Hafiers and seasonal streams. The state has a human population of 845,512. The livestock population estimates (MOAR&F, 2004) comprised of 4,650,240 sheep, 3,379,212 goats, 3,903,233 cattle and 15,694 camels

2.2.3 West Kordofan state:

It is situated in the Western part of the Sudan. It has an area of 111,373 square km and located between latitude 14° 5' - 9° 4' N, longitude 29° 8' - 27° E. It has a rainfall ranging from 200 mm - 750 mm. Other sources of water include hand pumps, deep wells, Hafiers, seasonal streams and Kelake Lake. The state has a human population of 992,172. The livestock population estimates (MOAR&F, 2004) comprise 3,763,788 sheep, 2,017,440 goats, 3,288,394 cattle, 449,785 camels.

2.3. Questionnaire development

The following questionnaires were designed by the Small ruminant project Scientists (ILRI/ICARDA), discussed with the national research team and agreed upon to be used for data collection from identified entry points along the “market chain (villages, households/flocks ((appendix: 4), markets,

traders, slaughter houses, quarantines and veterinary infrastructure facilities) in the project selected sites. The questionnaire administrators were selected from among the staff of the Ministry of Animal Resources & Fisheries. They were trained in methods of surveys and questionnaire administration and then carried out field testing of the questionnaires at (*Elsalam*) market in Omdurman, Khartoum twin city.

Developed and structured questionnaire comprised of 262 for households (116 from Blue Nile, 101 from Western Kordofan (Elkhowie administrative unit) and 45 from Gadarif States). Questionnaires were also structured for 19 villages, (5, Blue Nile, 6 Western Kordofan (Elkhowie administrative unit) and 8 from Gadarif States), 10 veterinary facilities, 56 traders, 8 slaughter houses, 25 markets and 3 quarantines. However, for this study only the information on villages and households/flocks was used, supplemented with disease information from the other categories.

2.4.1 Methodology for serum samples Collection;

The following criteria were adopted for sampling:

- a) The target population was defined as including all small ruminants in the project sites.
- b) The study population was identified to include the small ruminants that had not been vaccinated against PPR.

2.4.2 The sample size determination.

The three study states were taken as clusters with known population of small ruminants. Sampling with probabilities proportional to number of small ruminants in each state (i.e. probability sampling) was used to determine the number of small ruminants (sample size, n) to be included in the study in each state. The sample size determined, thus for each state was, Blue Nile, 280, Gadarif 105 and W. Kordofan, 215 samples, giving a total of 600 animals (sheep and goats)

The within each state number of small ruminants in each locality was selected conveniently (convenient sampling). Thus, the distribution within each state was as follows,

Blue Nile State: (280): Elrusseries locality, 70, El damazine locality, 65, Gessan locality, 80, and Bau locality, 65 sheep and goats

Gadarif state: (105): Galabat locality, 55 and Hawata locality, 50 sheep and goats.

West Kordofan state (Elkhowei Administrative Unit): (215): Murkab village, 41, Elsharif Elnaji village, 40, Elkhowei village, 45, Fakoki village, 46 and Taiba village, 43, giving a total of 215 animals.

The total number sampled and bled for sera for all the three states was 600.

2.5 Competitive Enzyme Immunosorbent Assay for Detection of Antibody to PPR Virus:

2.5.1 Reagents used for c-ELIZA test:

Antigen, Control Sera(Bovine), Monoclonal Antibody, Anti-Species Conjugate, Coating Buffer, Wash and Blocking Buffer Base, Blocking Detergent, Substrate, Chromogen and Reconstitution Diluent's were the reagents used (See appendix: 1). PPR ELIZA Kit (BDSL, 2006.) was used for detection of antibodies to PPR virus in the test sera.

2.5.2 Assay Procedure:

An aliquot of reconstituted PPRV antigen stock was gently mixed to ensure uniform dispersion. Immediately, A working dilution of PPRV antigen in coating buffer in 1:100 volume, for 1 plates, 6ml of coating buffer + 60 µl of antigen stock (6ml of working dilution per micro plate) was Prepared. The working antigen dilution was mixed gently and 50 µl volumes of the working dilution of RPV antigen dispensed into all 96 wells of the flat bottom microplates. The sides of the microplates were taped to ensure that the antigen was evenly distributed over the bottom of each well. The microplates were covered and placed on an orbital plate shaker at +37°C for 1 hour and then incubated for overnight at +4°C.

The blocking buffer was prepared 100 ml of PBS (0.01 M PBS) + 100 µl of tween 20 + 300 µl of normal bovine serum (C-control serum) to prepare 100ml blocking buffer for 8 plates.

The microplates were washed 3 times by using the washing buffer. The test sera and all 3 control sera (C+ +, C+ and C-) were agitated gently to ensure homogeneity. 40 μ l volumes of blocking buffer were dispensed to all 96 wells of all the microplates. According to the plate layout (Appendix: 3), 10 μ l volumes of pre-diluted test and control sera were added to the appropriate wells. 10 μ l of blocking buffer were added to the monoclonal antibody control (Cm) wells and 60 μ l blocking buffer to the conjugate control (Cc) wells. A working dilution (1:100) of the monoclonal antibody in blocking buffer for all the plates (6ml of working dilution per plate i.e the same portion as for antigen dilution) was prepared immediately. 50ul volumes of the working dilution of the monoclonal antibody were added to all the wells of the microplates except the conjugate control (Cc) wells following the plate layout (A1, A2). The microplates were covered and incubated at +37°C on an orbital plate shaker for 1 hour with continuous shaking.

Immediately before the end of the serum/monoclonal antibody incubation, a working dilution (1:1000) of the conjugate prepared in blocking buffer, for 1 plate 6 μ l of conjugate was added to 6ml blocking buffer. After 1 hour of serum incubation, the microplates were removed from the incubator and the microplates were washed. Immediately after washing, 50 μ l volumes of the working dilution of conjugate were added to all 96 wells of the microplates. The sides of the microplates were tapped The microplates were covered and incubated for 1 hour at +37°C with continuous shaking.

Immediately before the end of the conjugate incubation, a working dilution of the substrate/chromogen solution prepared, for 1 plate, 6 ml of chromogen stock (OPD) solution, 24 μ l of substrate stock (H₂O₂) was added. After 1

hour of conjugate incubation, the microplates were washed. 50 µl volumes of the substrate/chromogen solution was added to each well of the microplate, The plates were incubated at room temperature for 10 minutes and 50 µl volumes of the stopping solution were added to the wells of the microplates. The microplates were read using 492 nm filter and the software ELIZA.

2.6 An indirect MAP-1B ELIZA for detection of Cowdriosis:

2.6.1 Procedure:

Of the 620 small ruminants serum collected, only 320 (200 sheep and 120 goats) samples were tested for cowdriosis (antibodies).

The procedure of Mboloï and *et al.* (1999) and van Vliet et al (1995), with some minor modifications was used. Thus, one hundred microliters per well was used in all the steps described below. MAP1-B antigen was diluted in 1:600 in coating buffer containing Carbonate / Biocarbonate buffer (Fluka Biochemika). A capsule was dissolved in 100 ml water, giving a 0,05 M Solution PH 9.6 (25C) and immobilized onto 96-well ELISA plates (Microlon Multibind immunoassay plates; Greiner Labortechnik, Alphen aan den Rijn, The Netherlands) by incubation for 1 hour at 37°C and then stored overnight at 4°C. Plates were washed and incubated for 20 min. at 37°C with blocking buffer (phosphate-buffered saline [PBS], pH 7.3, supplemented with 0.1% Tween 20 and 1% skimmed milk [PBSTM]).

Plates were washed three times with PBS (Flip the fluid from the plate and plot it dry on absorbent towels) supplemented with 0.1% Tween 20 (PBST)

and subsequently incubated with sera (diluted at 1:200) in PBSTM for 1 hour at 37°C. All samples were analyzed in duplicate on the same plate.

Plates were washed three times with PBST and incubated for 1 hour at 37°C with rabbit anti-sheep antibodies conjugated with horseradish peroxidase (Nordic, Tilburg, The Netherlands) that was diluted in PBSTM (rabbit anti-sheep antibodies, 1:1000 dilution. The plates were washed three times with PBST, and freshly prepared citrate phosphate buffer with ABTS substrate (see appendix: 2) were added. Color development was allowed for 30 minutes incubation in the dark, and absorbance was measured at 450 nm, using a spectrophotometer (Labsystem, Multiskan, RC, Finland). Each plate contained two positive and three negative reference serum sample. The means of the duplicate measurements were calculated, and the average optical density was determined

2.6.2 Determination of the cutoff for the indirect MAP-1B ELIZA:

The cutoff point was determined, using the procedure of Mboloi *et al.* (1999) by the addition of the mean optical density (OD) values of negative control to 2 standard deviations (SD) for both sheep and goat. OD values of samples that were equal to or greater than the cutoff point value were considered positive for *C. ruminantium* infection.

2.7 Data analysis

SPSS software version 11.5 was used to analyze the data (Descriptive statistics including frequency and cross tabulation), Correlation, regression (to identify the risk factors), nonparametric test (Chi-Square was used to test the null hypothesis that there was no significant difference between the seopositivity in different project sites, age groups, sex and species), compare means). Moreover, Microsoft Excel windows XP was used to layout the histograms and the pie charts.

Chapter three: Results

3.1. The Households' questionnaire information:

(See appendix 4 for the questionnaire given to small ruminant producers)

3.1.1 Results of analysis of household information on small ruminants' production systems and health:

3.1.1.1 Production systems:

The Seasonal movements dominating the production system (53.2%) in the overall study area while the sedentary and permanent movement production systems are (42.5% and 4.3% respectively) (Figure 3.1)

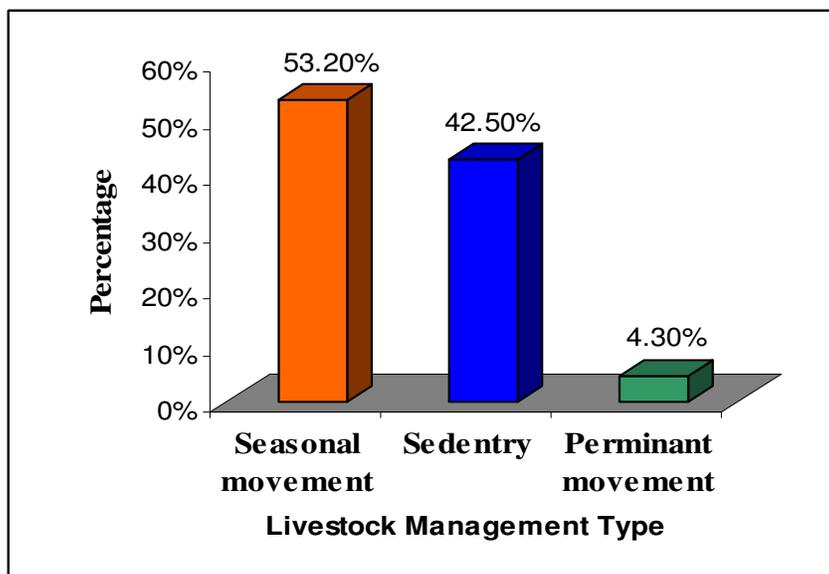


Figure 3.1 Small ruminants production systems in the overall study area

On the state level, the most dominant livestock (small ruminants) production system is seasonal movement in Gadarif and Blue Nile states while it is mostly sedentary in Western. Kordofan (Elkhowei area). (Figure 3.2)

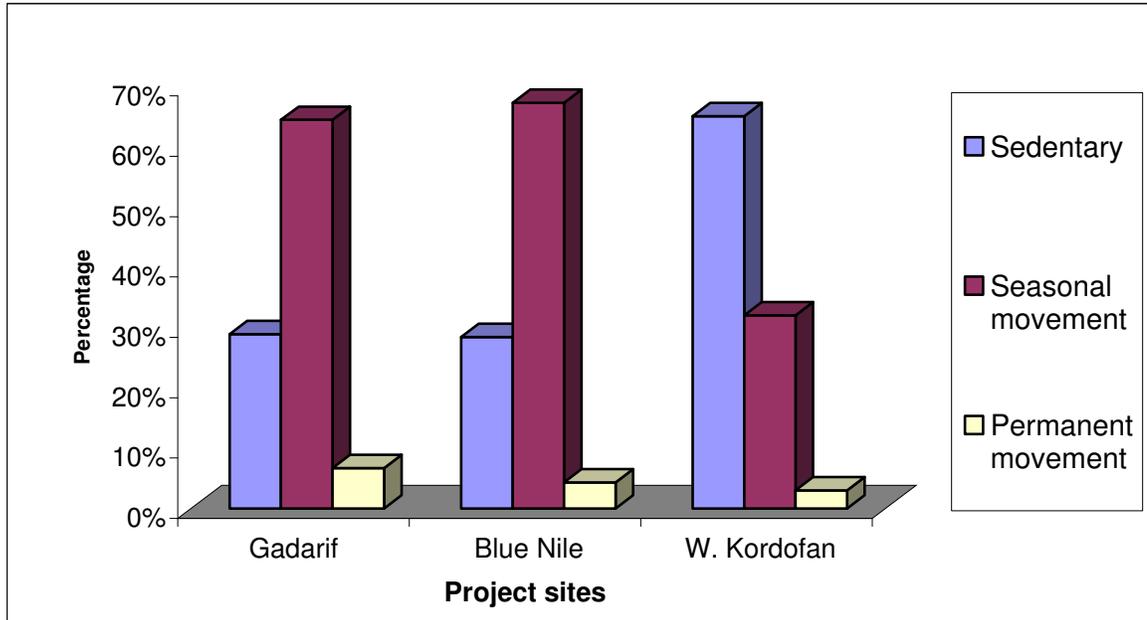


Figure 3.2 Small ruminants production systems in each project site

3.1.1.2. Herd management arrangements (labor) in each State:

In general, most households in Gadarif, Blue Nile and W. Kordofan (Elkhowei area) manage their own animals compared to those who manage own animals and animals of others for a fee, those manage own & others for a share of output, those manage portion of own & others for fee or output share and other (85.1%, 8.4%, 3.9%, 1.1%, 1.5% respectively). Moreover, there is considerable percentage of respondents who were in Elkhowei area and manage own and others for a share of output (6%) compared to 2.2% and 2.6% in Gadarif and B. Nile respectively (Figure 3.3)

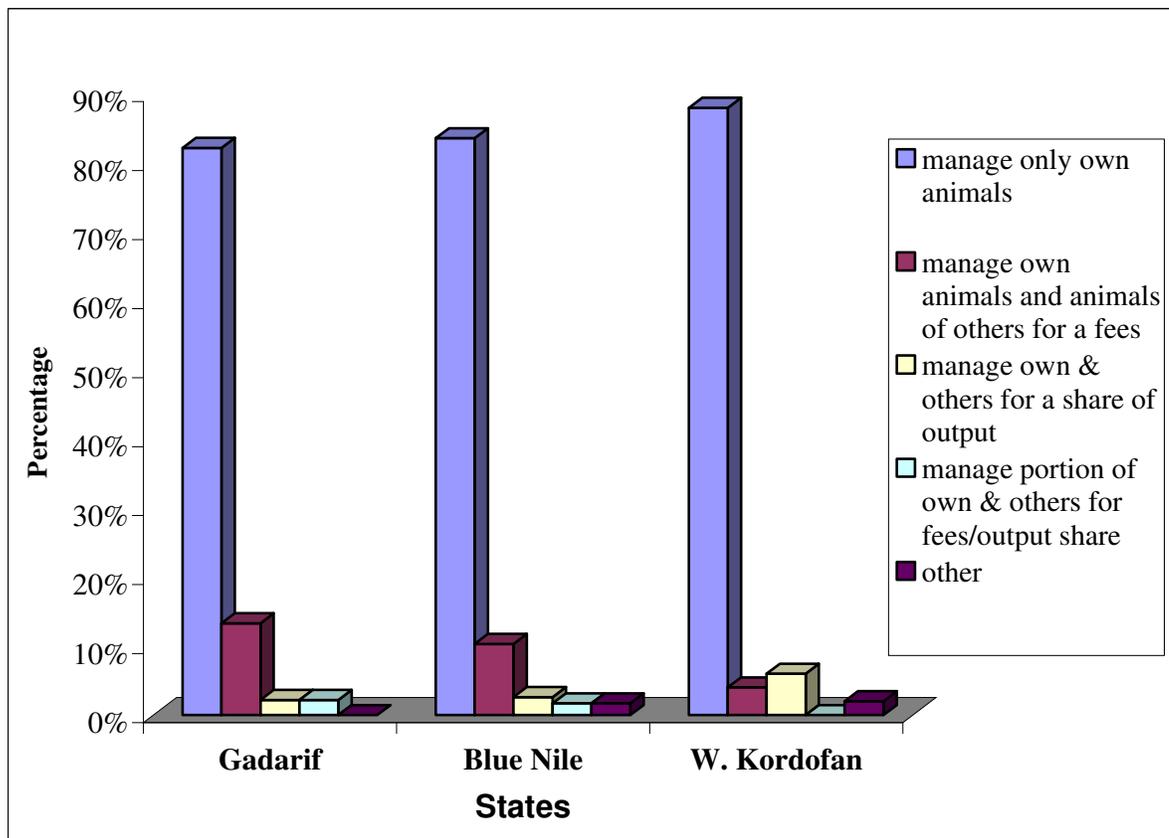


Figure 3.3 Herd management arrangements (labor) by state

3.1.2. The first most important diseases that affect small ruminants' herds in the project states in the current year (2005) by production systems:

Heartwater and PPR were more prevalent under seasonal movement production system, while pneumonia, poisoning and sheep pox were more prevalent under sedentary production system (Table 3.1)

Table 3.1 The first most important diseases that affect the herds in the current year (2005) in each livestock production system:

First most important diseases that affect your herd in the current year	Livestock production systems			Total
	Sedentary	Seasonal movement	Permanent movement	
Heart water	10	24	0	34
Pneumonia	14	6	0	20
Poisoning	17	12	2	31
PPR	15	56	3	74
Sheep Pox	21	15	3	39
Total	98	129	10	237

There was a significant association ($p < .05$), when chi square was used, between the first most important diseases that affected small ruminants' herds and production systems in the current year (2005) at 0.05 level of significance.

3.1.3. The second most important diseases during the last three years:

Sheep pox was reported as the second most important disease in the three successive years (2003, 2004, 2005) by all project areas, but within the project sites heartwater emerged the most important during the three successive years in Gadarif state, PPR emerged the first in the previous year (2004) in Blue Nile state and pneumonia in the current year (2005) in W. Kordofan state (Elkhowei area). (Figures 3.4, 3.5, 3.6 and 3.7 respectively).

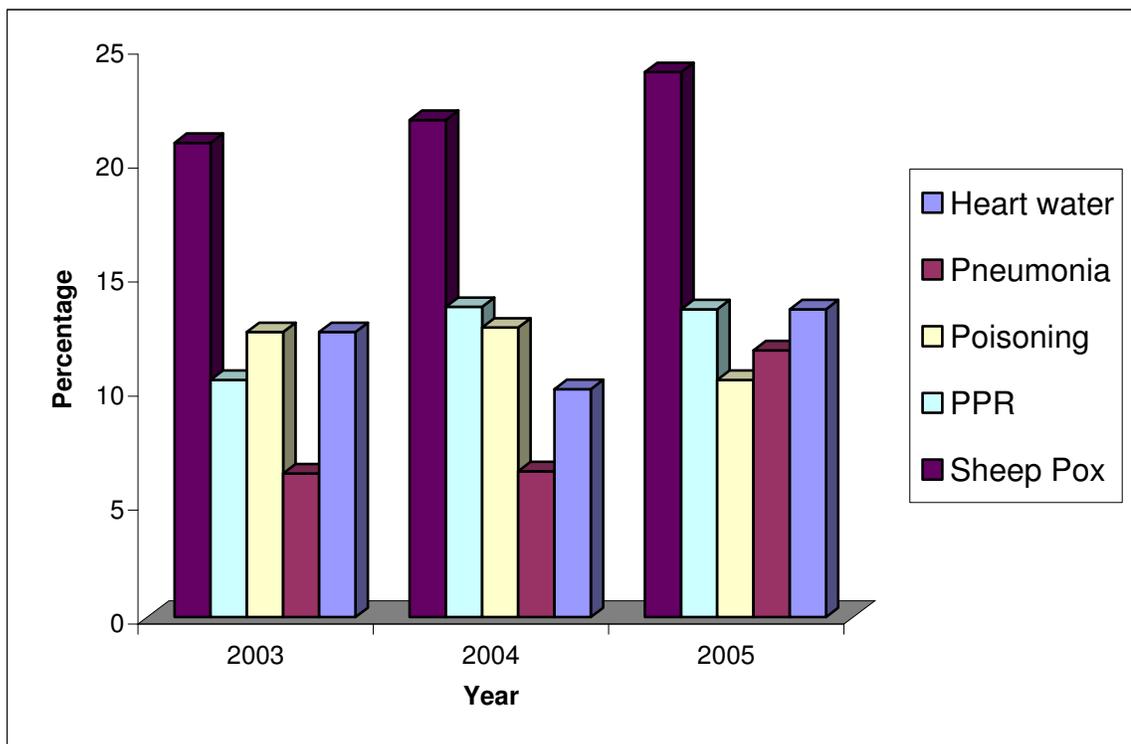


Figure 3.4 The second most important diseases during the last three years in all project areas.

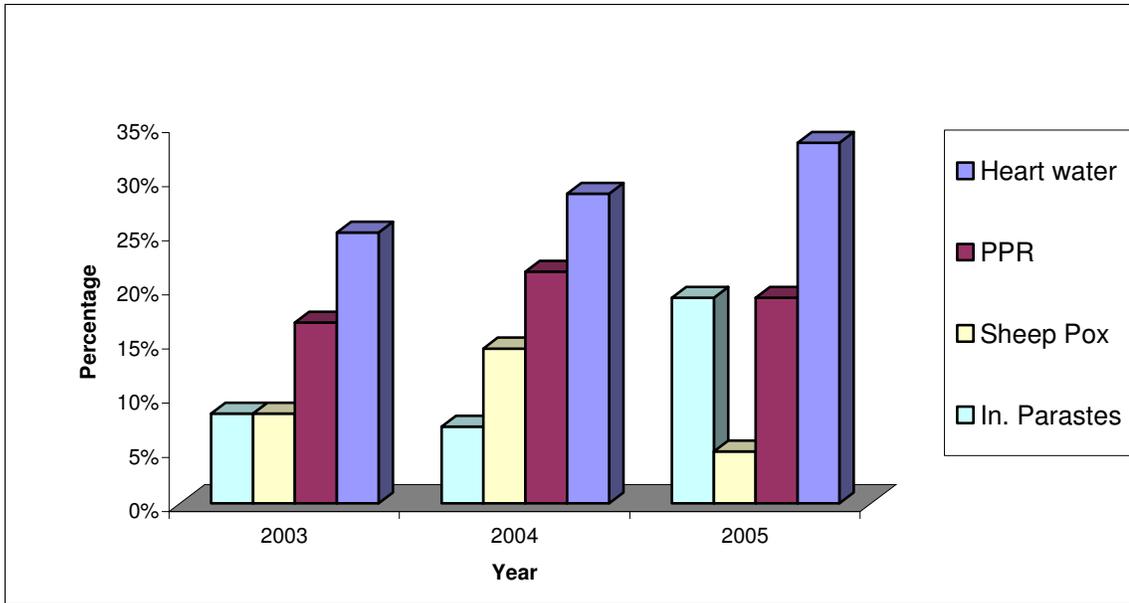


Figure 3.5 The second most important diseases during the last three years in Gadarif

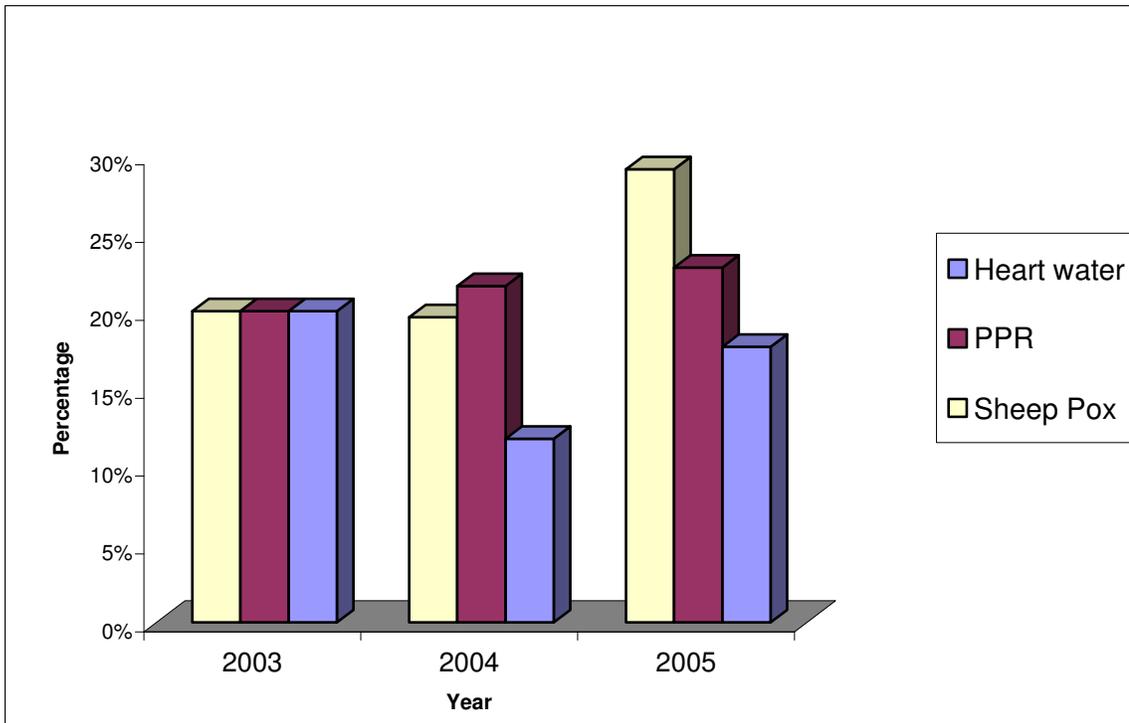


Figure 3.6 The second most important diseases in the current year in Blue Nile

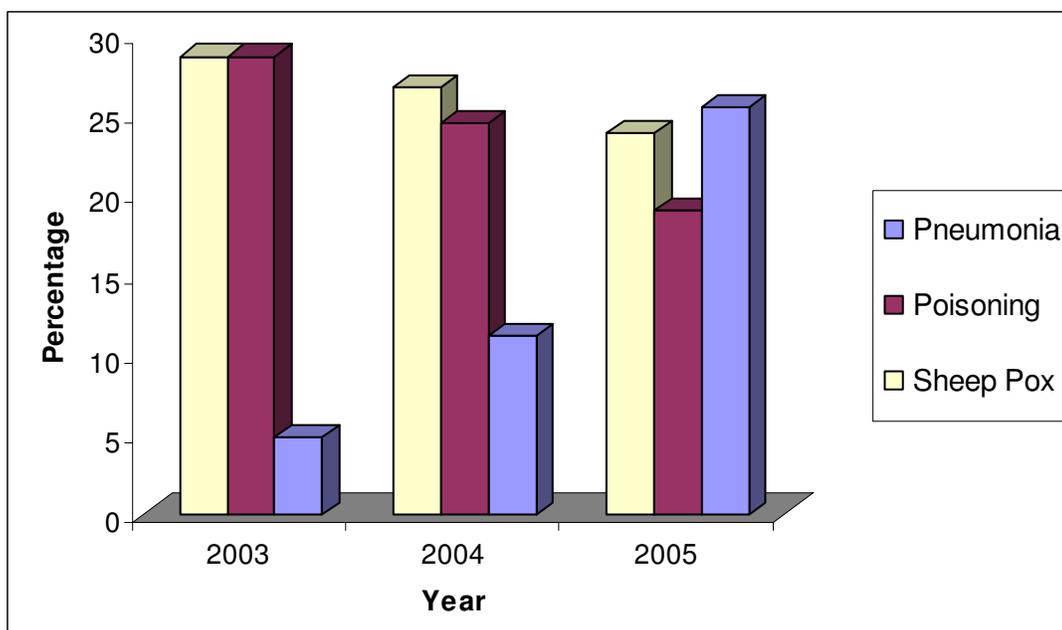


Figure 3.7 The second most important diseases during the last three years in W. Kordofan

3.1.4.1 Species affected by small ruminant's first most important diseases reported by the interviewees in the three project sites in the current year (2005):

The number of sheep and goats in the flock/ herd of respondents at time of interview was 64,690 (83%) and 13,277 (17%) respectively.

3.1.4.2 Species affected in the three successive years:

The respondents reported that sheep are more susceptible to first important diseases mentioned than goats. However, considerable numbers of respondents (15%, 58.9% and 18% for heartwater, PPR and sheep pox respectively) stated that both sheep and goats were affected equally with the

exception of poisoning which was more prevalent in sheep (19.9%) than goats. Moreover, goats were mostly affected with PPR (57.1) compared to other diseases. Sheep were mostly affected with sheep pox relative to other diseases (37%) (Table 3.2)

Table 3.2 Species affected by the first most important diseases reported by the respondents during the last three years:

Diseases	Sheep (%)	Goats (%)
Heart water	44(12.2)	1(14.3)
Pneumonia	34(9.4)	1(14.3)
Poisoning	72(19.9)	0(0.0)
PPR	77(21.3)	4(57.1)
Sheep Pox	134(37.1)	1(14.3)
Total	361(100)	7(100)

3.1.5 Small ruminant's first important diseases reported by the interviewees:

3.1.5.1 Small ruminants' health: Diseases reported by household respondents as most important in Gadarif, Blue Nile, W. Kordofan (Elkhowei area) States and in all projects States in three successive years:

3.1.5.1.1 Gadarif state:

In the current year (as of June 2005) heartwater ranked first, as reported by 51.4% of respondents, while PPR came, with 17.1%. However, in the last year (2004), the results were in reverse, with PPR ranked first by 43.6% of respondents and heartwater the second as reported by 17.9% of respondents.. In the year before the last year (2003) sheep pox was ranked first by 39.3% and PPR the second by 21.4% of the respondents respectively (figure 3.8).

3.1.5.1.2. Blue Nile state:

In the current year (as of June 2005) PPR was ranked first, (64.2%) and Heartwater the second (11.3%). In the last year (2004) PPR was also ranked first (46.4%), while Sheep pox took the second place, (29.8%) The year before last year (2003) had Sheep pox ranked first (36%), followed by PPR (22%) (Figure 3.9)

3.1.6.3. W. Kordofan state (Elkhowei area):

In the current year (as of June 2005) sheep pox was ranked first (33%), followed by poisoning as the second (28.9%). In the last year (2004), however, poisoning was reported first (31.5%), while sheep pox became second (28.3%). The ranking in the year before last year (2003) was the same as in 2005, that is, sheep pox first, with 53.6% and poisoning the second with 17.9% of respondents respectively (figure 3.10)

3.1.5.1.4. All project sites:

The ranking of diseases for all the three project sites was as of June 2005, PPR the first (31.1%) and sheep pox the second (16.4). In the last year (2004) sheep pox was ranked first (26.5%), followed by PPR (26.0%).

Results for the year before last year (2003) were Sheep pox, first (44.0%) and PPR, second one with (12.7%) (Figure 3.11)

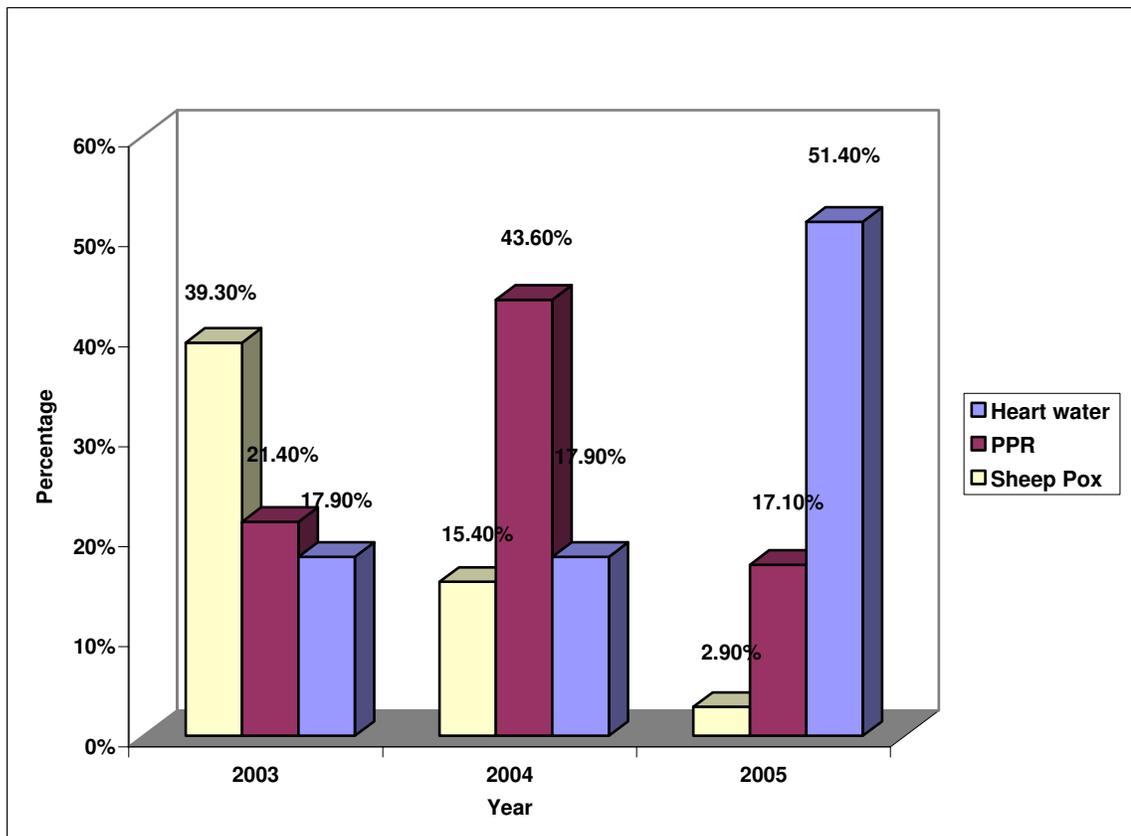


Figure 3.8 Diseases reported as first important in Gadarif State (first three diseases)

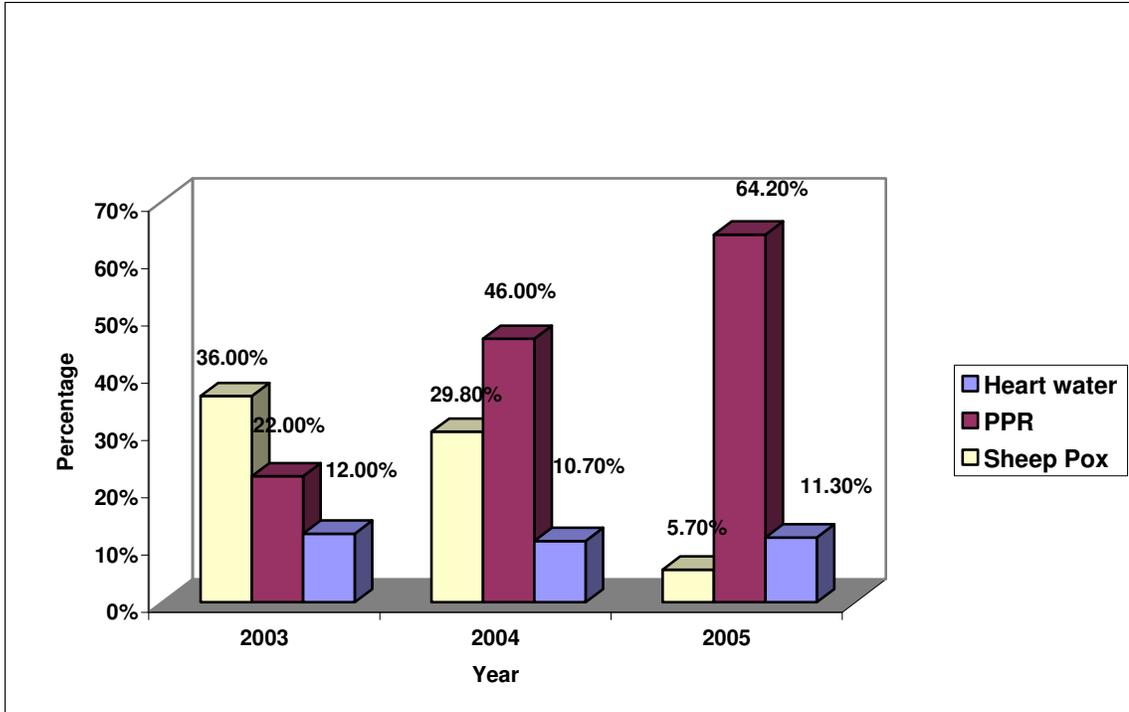


Figure 3.9 Diseases reported as first important in Blue Nile State (first three)

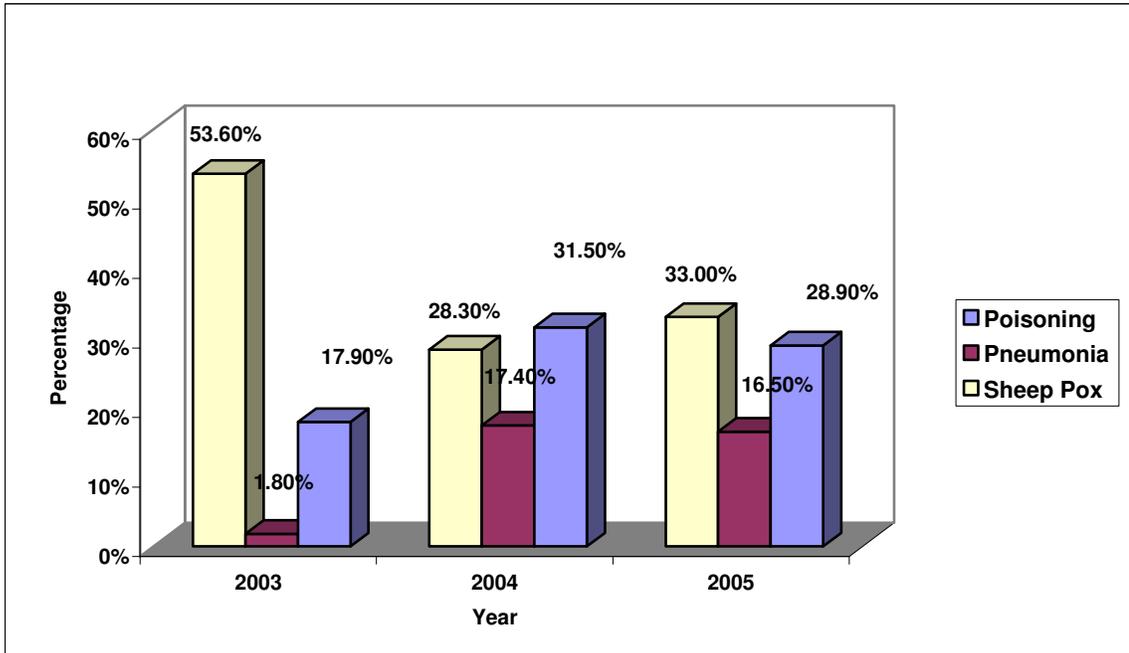


Figure 3.10 Diseases reported as first important in W. Kordofan State (Elkhowie area) (first three)

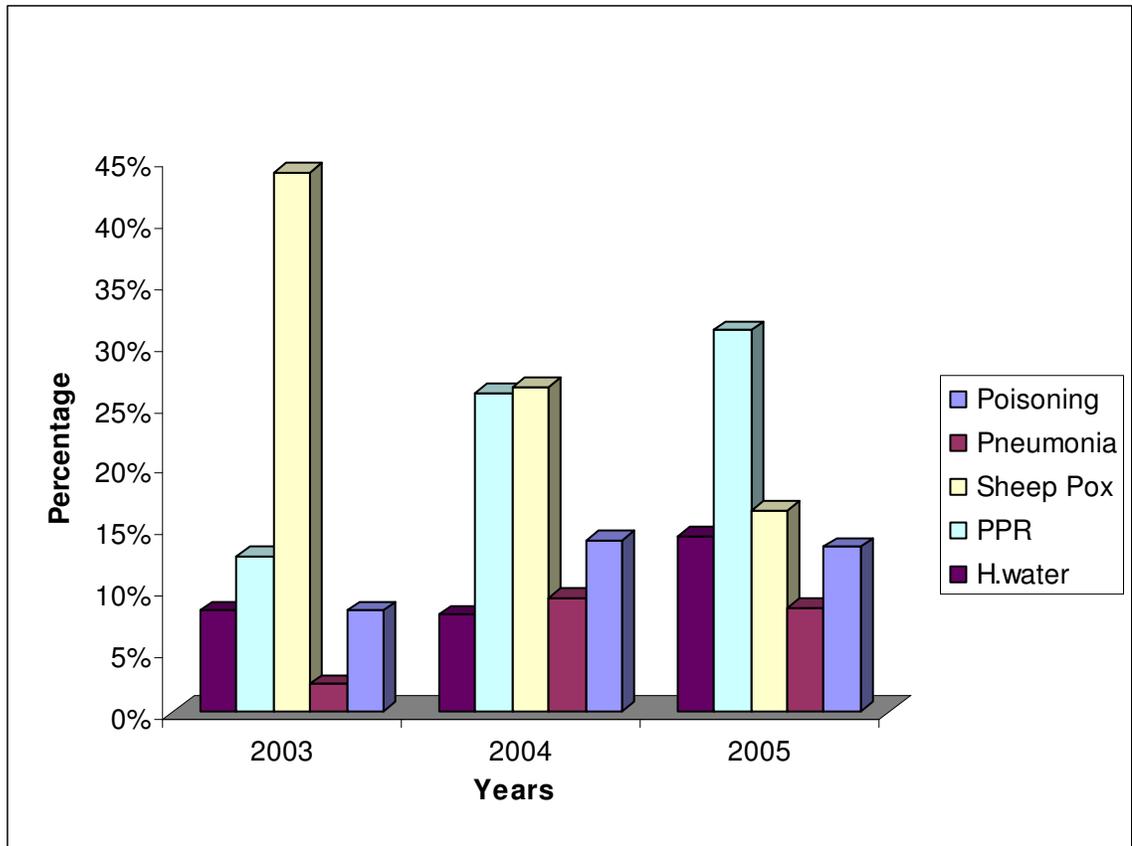


Figure 3.11 Diseases reported as first important by all project States (first three).

3.1.6 Local names of the important diseases/ conditions reported by the respondents in the project sites:

See appendix: 5

3.1.7 Effect of species and season/month:

There was a significant association ($p < .05$), when Chi square was used, between the occurrence of heartwater in Gadarif state, PPR in Blue Nile state and sheep pox in W. Kordofan state (Elkhowei area) as the first most

important diseases in the current year (2005) and season/month of the year as well as species affected.

3.1.8 Seasonality of small ruminant's most important diseases as reported by respondents in the three project sites during the last three years:

Most diseases of small ruminants reported by respondents occur during the dry season, but some (poisoning, sheep pox) peak during the rainy season; while others (heartwater and PPR) occur all year round. (Figure 3.12).

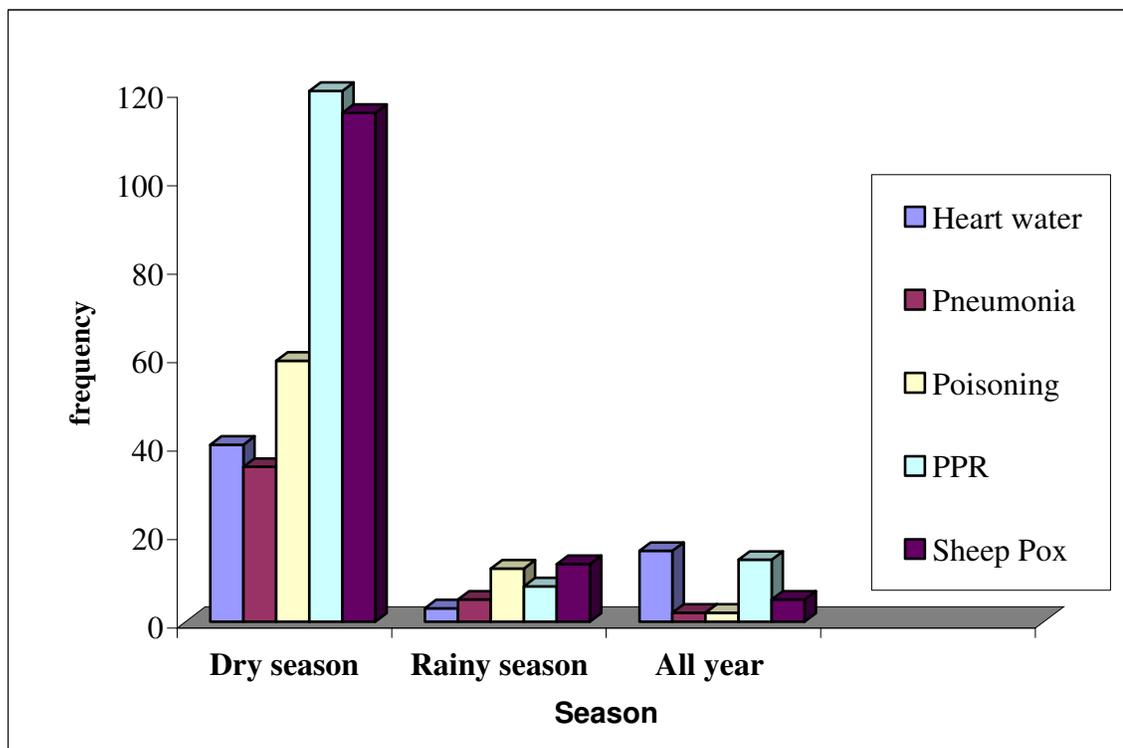


Figure 3.12 Seasonality of the first most important diseases reported by respondents during the last three years

3.1.9 Statistical analysis:

The correlation (r) between the sick animals that could not be sold at all in the current year(2005) and the number of animals affected by the first most important diseases (PPR, sheep pox, heartwater, poisoning and pneumonia) in the current year in the three project sites is significant (r =.534) at the .01 level (2 tailed t-test).(Table 3.3)

Table 3.3 Relationship between the number of sick animals and selling Status in the three project sites:

Factor	Un standardized Coefficients		Standardized Coefficients	t	P-value.
	B	Std. Error	Beta		
(Constant)	.671	.736		.912	.363
No. of animals affected by the first most important disease in the current year	.071	.009	.534	7.868	.000

a Dependent Variable: How many sick animals could not be sold at all in the current year.

The correlation (r) between the number of sick animals that could not be sold at all in the current year (2005) and number affected of PPR among the herds in the current year (2005) in the three project sites is significant (r =.821) at .01 level of significance (2 tailed t-test) (Table 3.4)

Table 3.4 Relationship between the numbers of sick animals that could not be

Sold in the current year (2005) and the number affected of PPR in the three project sites:.

Factor	Unstandardized Coefficients		Standardized Coefficients	T	P-value.
	B	Std. Error	Beta		
(Constant)	-.837	1.517		-.552	.584
No. of animals affected by the first most important disease (PPR) in the current year(2005)	.093	.011	.821	8.738	.000

a Dependent Variable: Number of sick animals that could not be sold at all in the current year (2005)

The correlation (r) between the number of sick animals that could not be sold at all in the current year(2005) and number affected of PPR among the herds in the current year (2005) in Blue Nile state is significant (r =.819) at .01 level of significance (2 tailed t-test) (Table 3.5)

Table 3.5 Relationship between Number of sick animals that could not be sold at all in the current year and No. affected by PPR in the current year in Blue Nile state :

Factor	Unstandardized Coefficients		Standardized Coefficients	t	P-value.
	B	Std. Error	Beta		
(Constant)	-.907	1.798		-.504	.618
No. of animals affected by the first most important disease in the current year	.094	.012	.819	7.952	.000

a Dependent Variable: Number of sick animals that could not be sold at all in the current year

3.1.10 Losses and case fatality rates (CFR) during the three successive years for the most important diseases ranked first and second by respondents:

During the year 2003, diarrhea, poisoning and foreign body had higher crude case fatality rates (95.74%, 60.13% and 51.61% respectively) (Table 3.6).

During 2004, diarrhea and poisoning had the highest crude case fatality rates (95.74%, and 84.4%% respectively).

During 2005, Poisoning, Foreign body and *haemorrhagic septicaemia* (H.S) complications gave the highest crude case fatality rates (83.14%, 81.81% and 64% respectively). Disease condition not known gave the highest crude case fatality rate (92.2%).

Table 3.6 Crude case fatality rates for diseases reported by household respondents as important during a three year (2003, 2004 and 2005) period:

Diseases	2003			2004			2005		
	No. Aff.	No. Died	CCFR (%)	No. Aff.	No. Died	CCFR (%)	No. Aff.	No. Died	CCFR (%)
PPR	635	220	34.64	3160	1524	48.23	4400	1495	33.98
Sheep Pox	2845	677	23.80	2694	700	25.98	2969	1199	40.38
Heart water	167	70	41.92	522	175	33.52	1886	763	40.46
For. body	31	16	51.61	0	0	0	22	18	81.82
H.S.	15	15	100	175	77	44	177	114	64.40
Botulism	40	28	70	75	49	65.33	20	13	65
Arthritis	0	0	0	35	22	62.86	2	0	0
Diarrhea	94	90	95.74	94	90	95.74	108	62	57.41
Poisoning	301	181	60.13	692	576	83.24	289	242	83.74
Mastitis	20	0	0	107	65	60.75	0	0	0
A vitamin.	0	0	0	5	0	0	100	60	60
Pneumonia	63	15	23.81	59	7	11.86	222	92	41.44
Unknown	248	43	17.34	70	47	67.14	38	35	92.10
Total	4459	1355	30.39	7688	3332	43.34	10233	4093	39.10

Generally, PPR, Sheep pox, Heart water and Poisoning are the most diseases that causes high losses in the project sites as reported by the respondents (3239 heads (36.89% of the total losses), 2576 heads (29.34% of the total losses), 1008 heads (11.48% of the total losses) and 999 heads (11.38% of the total losses) respectively) during the last three years (2003 – June 2005))

3.1.11 Economic importance of important diseases as reported by respondents:

The total cost of small ruminants that died of first and second most important diseases reported by respondents during the last three years (2005, 2004 and 2003) was valued at Sudanese Dinnar (SD) 48,452,800, 40,625,450 and 12,303,500 respectively. The equivalent values, in US dollars, estimated at \$ 201,886.7, \$162,501.8 and \$ 47,321.2 respectively. Hence, the total value of small ruminants that died during the last three years (2003 – up to May 2005) was SD 101,381,750 (equivalent to \$ 411,709.7).

Approximate value of total losses other than deaths (Abortion, milk loss and emaciation) due to same diseases mentioned above for three consecutive years (2005, 2004 and 2003), was SD 11,350,100 (equivalent to \$ 46,280.2).

Cost of drugs for treatment against same diseases identified by respondents as above over the same period (2005, 2004, and 2003) was SD 16,669,510 (equivalent to \$ 67,704.1).

Cost of fees for services for above diseases over the same three year period was SD 19,500 (equivalent to \$ 80). The combined cost due to deaths, losses other than deaths, drugs and services attributable to the first and second important small ruminants' diseases was SD. 129,420,860 (equivalent to \$ 525,774). (Table 3.7)

3.1.12 Economic importance of PPR and Heart water:

The total cost of small ruminants that died of PPR disease reported by respondents during the last three years (2005, 2004 and 2003) was valued at Sudanese Dinnar (SD) 13,674,500, 10,811,950 and 1,370,000 respectively. The equivalent values, in US dollars, estimated at \$ 56,977.1, 43,247.8 and 5,269.2 respectively. Hence, the total value of small ruminants that died during the last three years (2003 – up to May 2005) was SD. 25,856,450 (equivalent to \$ 105,494.1).

Approximate value of total losses other than deaths (Abortion, milk loss and emaciation) due to PPR disease for three consecutive years (2005, 2004 and 2003), was SD. 6,321,600 (equivalent to \$ 25,838.2).

Cost of drugs for treatment against PPR disease over the same period (2005, 2004, and 2003) was SD 5,447,050 (equivalent to \$ 22,146.9).

Cost of fees for services for PPR disease over the same three year period was SD 6,500 (equivalent to \$ 26.8). The combined cost due to deaths, losses other than deaths, drugs and services attributable to the PPR disease was SD 37,631,600 (equivalent to \$ 153,479.2). (Table 3.7).

The total cost of small ruminants that died of heartwater disease identified by respondents during the last three years (2005, 2004 and 2003) was SD 9,656,100 (equivalent to \$ 39,694.5).

Approximate value of total losses other than deaths (Abortion, milk loss and emaciation) due to heartwater disease for three consecutive years (2005, 2004 and 2003), was SD. 738,600 (equivalent to \$ 3,071.2).

Cost of drugs for treatment against heartwater disease over the same period (2005, 2004, and 2003) was SD 2,446,800 (equivalent to \$ 10,053.5).

Cost of fees for services for heartwater disease over the same three year period was SD zero. The combined cost due to deaths, losses other than deaths, drugs and services attributable to the heartwater disease was SD. 12,841,500 (equivalent to \$ 52,819.2). (Table 3.7).

Table 3.7 Value of losses resulting from deaths and other than deaths losses (abortion, milk...etc) and cost of drugs, vaccines and services due to diseases (PPR and heartwater) reported by respondents.

Value of losses and costs	The total losses and costs of the 1 st and 2 nd most important diseases reported (SD)	PPR (SD)	Heartwater (SD)
Value of dead small ruminants	101,381,750	25,856,450	9,656,100
Value of production losses (milk, abortions lost of weight, etc..)	11,350,100	6,321,600	738,600
Cost of drugs and vaccines	16,669,510	5,447,050	2,446,800
Fees for services	19,500	6,500	000,000
Total (SD)	129,420,860 (100%)	37,631,600 (29.1%)	12,841,500 (9.9%)

3.1.13 Occupation and main sources of family income reported:

71.98% of the respondents depend on livestock rearing, 22.34% on crop production, 2.86% on livestock trade, 1.03% on other/trade and business, 0.52% on services and 0.28% on remittance from family members working away from household.(Figure 3.13)

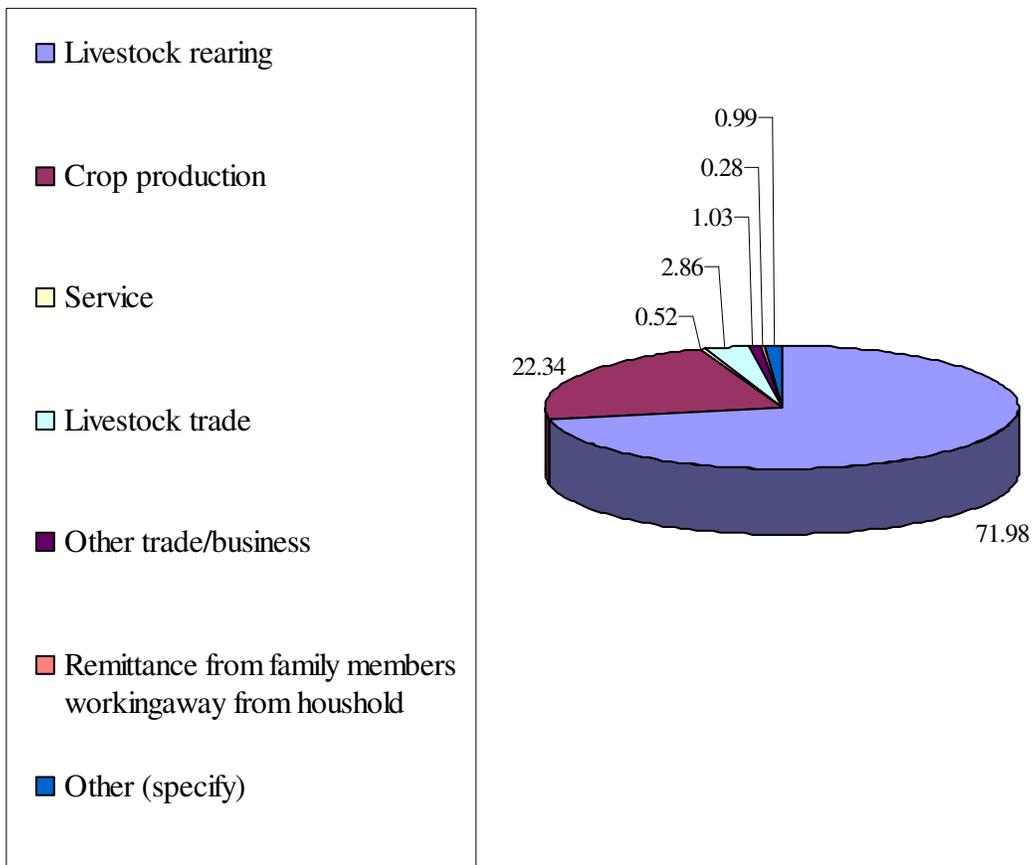


Figure 3.13 Occupation and main income reported

3.2. Village appraisal questionnaire results:

3.2.1. Important diseases reported by the respondents:

Respondents in the study villages reported the most important (based on morbidities and mortalities) diseases during the year 2005 were PPR (36.8%), sheep pox (31.6%), poisoning (10.5%), heartwater (5.3%) and others (15.8%) (Figure 3.14). Those they ranked second in the same period were pneumonia (25%), heartwater and sheep pox (18.8% each), PPR and poisoning (12.5% each) and others (43.7%). (Figure 3.15)

In 2004 however, those ranked first were sheep pox (35.7%), PPR (21.4%), heartwater and poisoning (14.3% each). (Figure 3.14)

The second important diseases in 2004 were PPR (40%), heartwater, sheep pox and poisoning (10% for each) and others (50%). (Figure 3.15)

The order for 2003 was sheep pox and PPR (33.33% each), poisoning (8.3%) and others (25%), (Figure 3.13), followed by heartwater (37.5%), sheep pox (25.0%), PPR and poisoning (12.5% each) and others (45%). (Figure 3.15)

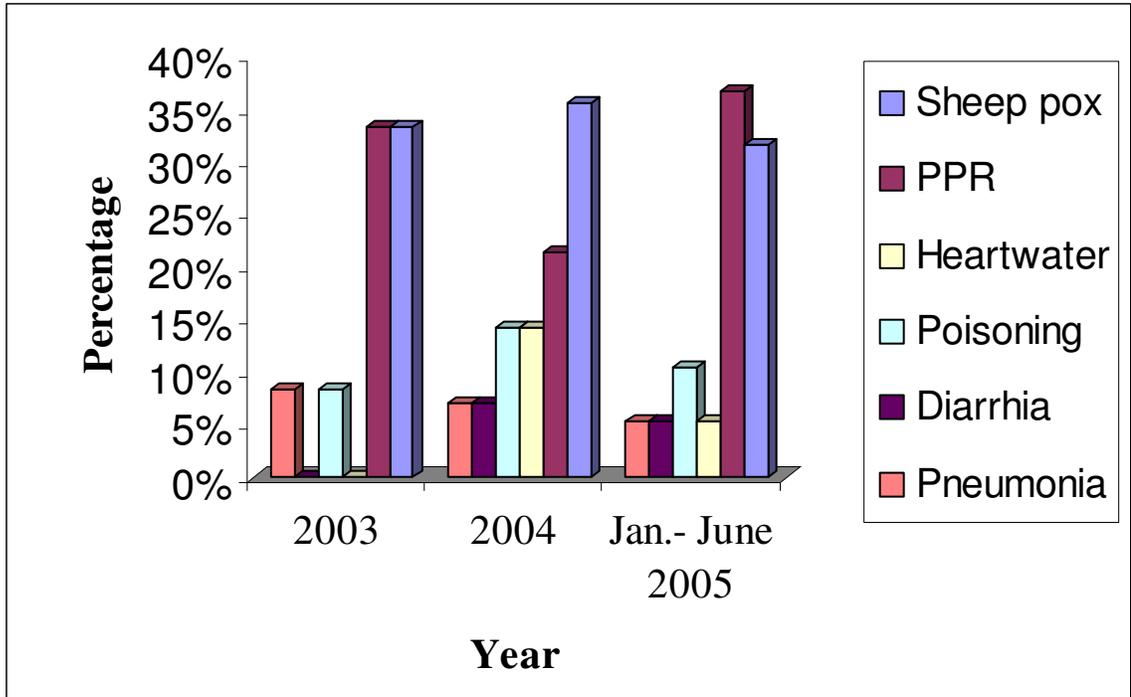


Figure 3.14 Diseases ranked first by respondents in study villages

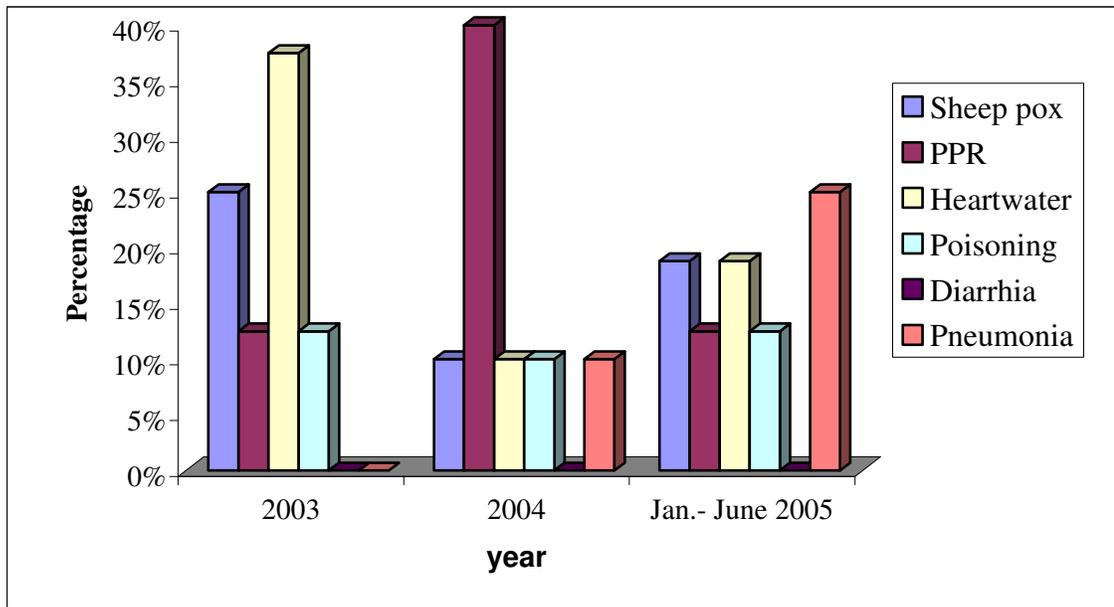


Figure 3.15 Diseases ranked second by the respondents in study villages

3.3. Serology:

3.3.1 An indirect MAP-1B ELIZA for detection of Cowdriosis antibodies.

Of the 320 small ruminants serum samples tested, (73.1%) were positive for *C. ruminantium* antibodies with seroprevalence at individual sites ranging from 12.0% to 98.5%. The highest seroprevalence was seen in the Gadarif state (98.5%) followed by Blue Nile state (93.3%) and the Lowest seroprevalence was seen in Elkhwei area (W.Kordofan state) (12.0%). (Figure 3.16).

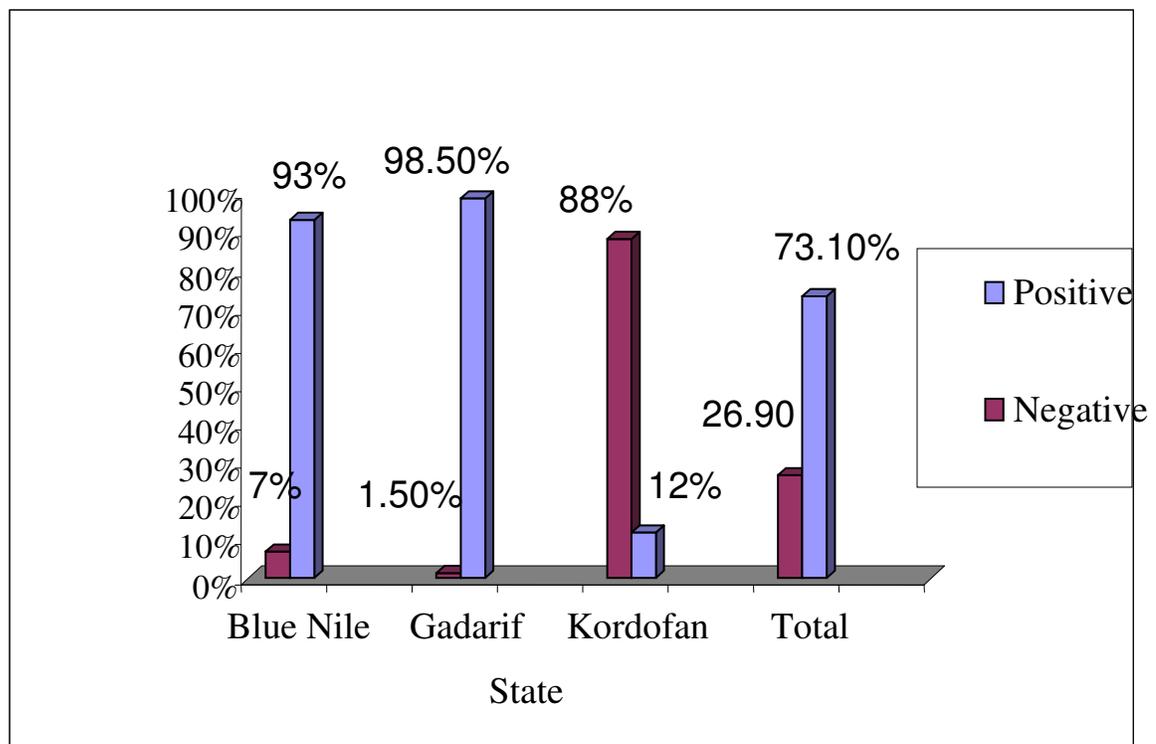


Figure 3.16 The Heartwater sero- prevalence of the samples collected from the study area:

The overall prevalence of the antibodies was 63% in sheep and 90% in goats. However, the prevalence of antibodies per species showed a different picture by state (Table 3.8).

Table 3.8 Seroprevalence of heartwater in sheep and goats tested with an indirect MAP-1B Eliza in project areas (States):

State	Sheep			Goats		
	Number tested (n)	Number positive	% positive	Number tested (n)	Number positive	% positive
Blue Nile	89	78	87.6	83	82	98.8
Gadarif	40	40	100	25	24	96.0
Kordofan	71	8	11.3	12	2	16.7
Total	200	126	63.0	120	108	90.0

In the three project sites, except for B. Nile, the overall sero-prevalence was not significantly difference in sheep and goats ($p > 0.05$). For the overall project sites there is significant difference when chi square was used ($p < 0.05$) and the odd ratio was found to be 5.28 which means that the goats had a risk factor 5.28 times that of sheep.

3.3.1.1 Sero- prevalence of heartwater in the study area by sex:

In the overall project sites, the overall sero-prevalence of heartwater showed no significant difference between males and females (sex) ($p > 0.05$) when Chi square was used. (Table 3.9)

Table 3.9 Sero-prevalence of heartwater by sex in the study area:

Study site	Males			Females		
	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve
Blue Nile	44	37	84.1	128	123	96.1
Gadarif	9	9	100	56	55	98.2
Kordofan	19	3	15.8	64	7	10.9
Total	72	49	68.1	248	185	74.6

The overall sero-prevalence of heartwater in the study states did not show, any significant difference by age when Chi square was used ($p > 0.05$), even after categorization into less than one year, one to less than two years, two to less than three years and three and above.

3.3.2 Competitive ELIZA (c- ELIZA) for detection of PPR antibodies:

Of the 600 small ruminants serum samples tested, (61.8%) were positive for *Peste des petits ruminants* (PPR) antibodies, with sero-prevalence at individual sites ranging from 28.6% to 69.3%. The highest sero-prevalence was seen in Blue Nile state (69.3%) followed by Elkhwei area in west Kordofan (68.4%), while the lowest sero-prevalence was seen in Gadarif state (28.6%). (Figure 3.17)

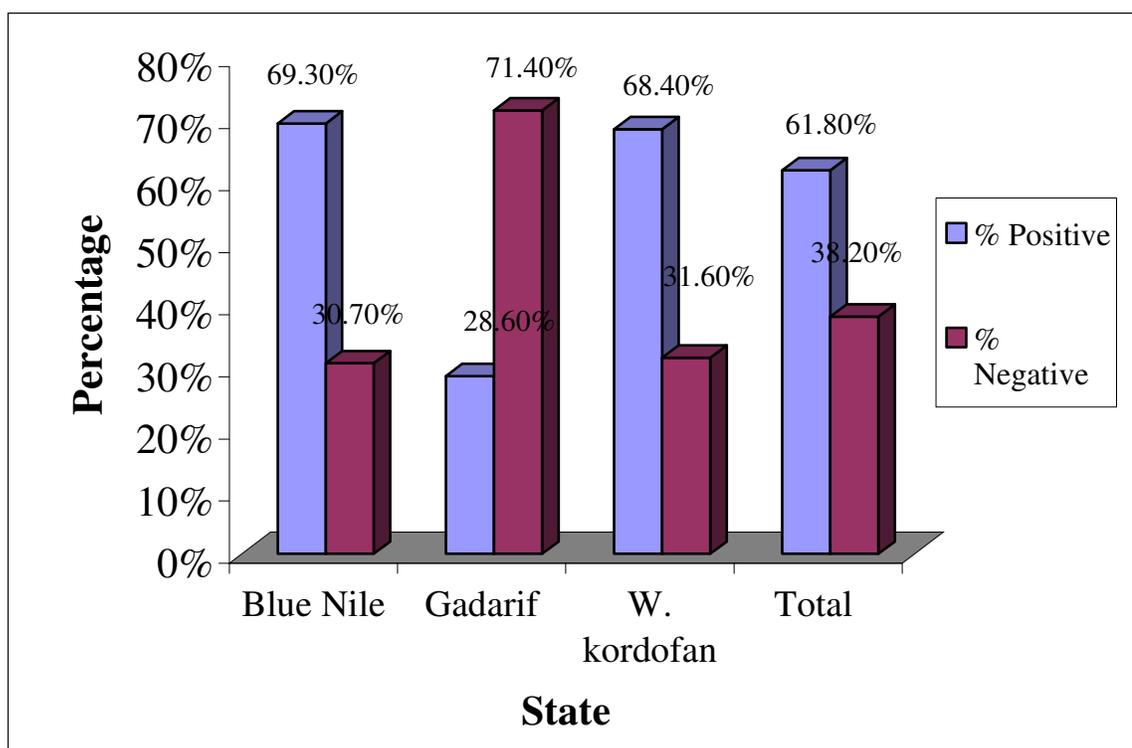


Figure 3.17 Prevalence of PPR antibodies in small ruminants sera in the study area examined by c - ELIZA

Of the 144 male small ruminants serum samples tested, (54.2%) were positive for PPR antibodies, with sero-prevalence at individual sites ranging from 10% to 64.7%. The highest sero-prevalence was seen in the Elkhwei area (64.7%) followed by Blue Nile (58.9%) while Gadarif gave sero-prevalence (10%), (Table 3.10)

Table 3.10 Prevalence of PPR antibodies in small ruminants by sex in the study area:

Study site	Males			Females		
	No. tested	No. +ve	% ive	No. tested	No. +ve	% +ve
Blue Nile	73	43	58.9	207	151	72.9
Gadarif	20	2	10	85	28	32.9
W.Kordofan (Elkhowie area)	51	33	64.7	164	114	69.5
Total	144	78	54.2	456	293	64.2

Of the 456 female small ruminants sera tested for PPR antibodies, (64.2%) were positive, with sero-prevalence at individual sites ranging from 32.9% to 72.9% (Table 3.36). The highest sero-prevalence was seen in Blue Nile State (72.9%) followed by Elkhowei area (69.5%) and Gadarif State with the lowest (32.9%). The difference in overall sero-prevalence between females and males among study states was significant when Chi square was used, and the odds ratio was found to be 1.52 which means that the female had a risk factor 1.5 times that of males (Table 3.11), while the within State significant difference was observed in Blue Nile state and Gadarif only .

Table 3.11 Statistical parameter estimates for sero-positivity risk factor for sex:

Pearson Chi sq.	P -value	SE	Odds ratio
4.72	.0298	.1938	1.52

3.3.2.1 Prevalence of PPR antibodies in small ruminants' sera in the study area examined by c – ELIZA per species

Of the 399 sheep sera from all study states were tested for PPR antibodies and 62.9% of the sera were positive. Sero-positivity by individual state ranged from 25.9% to 72.5% (Table 3.12). The highest sero-prevalence was seen in Elkhowe area (72.9%) followed by Blue Nile state (65.1%), and Gadarif, with the lowest sero-prevalence (25.9%).

Table 3.–12 Sero-prevalence of PPR in sheep and goats tested with an indirect c-Eliza in project areas (States):

State	Sheep			Goats		
	No. tested	No.+ve	% +ve	No. tested	No.+ve	% +ve
Blue Nile	152	99	65.1	128	95	74.2
Gadarif	58	15	25.9	47	15	31.9
W.Kordofan (Elkhowie area)	189	137	72.5	26	10	38.5
Total	399	251	62.9	201	120	59.7

Of the 201 goats serum samples tested, (59.7%) were positive for PPR, with sero-prevalence at individual sites ranging from 31.9% to 74.2% (Table 3.12). The highest sero-prevalence was seen in Blue Nile (74.2%) followed by Elkhowe area (38.5%) and the Lowest sero-prevalence was seen in Gadarif state (31.9%).

There was no significant difference in sero-prevalence between sheep and goats in all the study sites when Chi square was used ($p > .05$). However, there is significant difference in the Elkhwei area ($P < .05$) where the odds ratio was found to be 4.2 which means that the sheep had a risk factor 4.2 times that of goats.

There was a significant association between the age of small ruminants tested and sero-positivity status ($P < .05$) in all study areas. Likewise, there was significant positive correlation between percent inhibition (PI) which used to measure the cut off points for c ELIZA and the age of samples at the 0.01 level (2-tailed). (Table 3.12)

Table 3.13 Relationship between age (in months) of tested small ruminants and Percent Inhibition (PI) for PPR disease:

Factor	Unstandardized Coefficients		Standardized Coefficients	t	P-value
	B	Std. Error	Beta		
(Constant)	15.369	1.452		10.584	.000
PIav	.120	.020	.291	5.885	.000

a Dependent Variable: Age in months

3.3.2.2 Age profiles of the sero-positive sheep and goats to PPR infection:

The mean age of the positive sheep and goats were 27 months and 21.15 months respectively. The most frequent age (mode) sero-positive for sheep was 36 month while that for goats was 24 months (Table 3.14) indicating that goats are more susceptible than sheep.

Table 3.14 Mean age in months of sheep and goats that tested positive for PPR disease;

	Goats	Sheep
Mean	21.15	27.00
Median	18.00	24.00
Mode	24	36
Std. Deviation	12.813	14.300
Variance	164.162	204.492
Range	54	67
Minimum	6	5
Maximum	60	72

Chapter Four: Discussion:

This study was designed to determine the sero-prevalence and economic impact of the most important diseases that affect the small ruminants in Elkhwei area (W. Kordofan state), Blue Nile and Gadarif states.

Results indicated that *Peste des petits ruminants* (PPR), Heartwater and Sheep pox were the most important diseases prevalent in the study area, based on herders' perception. Hence, the study focused on the verification of PPR and heartwater by laboratory tests while, the sheep pox and other important diseases were base on the perception of the respondents (the small ruminant producers). The prevalence rates of PPR and *Cowdriosis* antibodies in non vaccinated small ruminants in the study area were determined at a fixed point in time (Smith, 2006).

In Gadarif state, the importance Sheep pox seems to decrease year by year, while that of Heartwater increases, attaining prominent importance during the year 2005. However, PPR remained the most important disease through out the three successive years, decreasing slightly in importance in the year 2004.

The picture for Blue Nile, State presented PPR with increasing importance year by year, while that for sheep pox decreases. Heartwater maintains its importance steadily through out the successive years.

In Elkhwei area (W. Kordofan), however, Sheep pox was the top most important disease in the three successive years, with slight decrease in

importance in 2004 when plant poisoning gained importance as more animals succumbed to plant poisoning. This observation was agreement with government records (Animal Health and epizootic disease control department, 2004) which reported that, in Elnuhoud locality of Western Kordofan (study area) 500 small ruminants died of suspected clostridia infection in different out breaks, which occurred simultaneously during the early stage of rainy season. Pneumonia provoked much concern among producers in 2004 and 2005. Moreover, diarrhea, together with unknown conditions, leading to sudden deaths, dullness, and abortion and circling in animals was observed. Such symptoms and laboratory results led to suspicion that PPR might have been the cause of deaths, but misdiagnosed by small ruminants; producers. Mariner and Paskin (2000) had reported that it is unusual to find that communities recognize one or two major diseases that have no name in the local language. These researchers suggested that such unknown ill-health become major problems, and then they were usually new disease.

Generally, ranking of diseases' importance by the respondents tends to agree with our laboratories results, confirming observation of Mariner and Paskin (2000) who reported that farmers are a rich source of practical agricultural knowledge and the extend of knowledge is usually related to the degree of economic dependence a society has on that activity. Moreover, the study revealed that 71.98% of the respondents report livestock rearing is the occupation and main source of income.

The observation that case fatality rate of PPR in goats was (53.3%), compared to only (39.7%) in sheep indicates that goats are more vulnerable

to PPR than Sheep. This finding is in agreement with (Lefèvre and Diallo, 1990, Roeder *et al.*, 1994) who were reported that PPRV exhibits different levels of virulence between sheep and goats. It is also agreed with Radostits (2000) findings, which indicated that case fatality rates are much higher in goats than in sheep.

Losses caused by the diseases identified in this study are not well documented. However, this study has determined the total value of losses from deaths, losses other than deaths (abortion, emaciation and milk loss), and cost of drugs, and fees for services during that last three successive years (2003, 2004, 2005) be \$ 525,774. Of these, 29.10% was due to PPR and 9.90%, heartwater. Since 72% of the respondents depend entirely on livestock rearing, these losses could be taken to be of considerable economic impact on the livelihoods of small ruminants; keepers in the project areas. Additionally, small ruminants' producers are not able to access markets for their animals because of these identified diseases. Therefore, although the total losses and economic impact may have been underestimated, the PPR is economically significant disease of small ruminants as observed by Dar *et al.*, (2002).

Results of village appraisal survey, which acted as a triangulation process confirmed the importance of PPR, Heartwater, sheep pox and Poisoning as reported by the respondents at the household level survey

Major ecological changes have occurred due to overgrazing as a result of insecurity, tribal conflict and raiding, leading to extensive animal movement and due to establishment of new quarantines. All these changes certainly

affected the distribution of diseases and the vectors which transmit them. Hence, combination of ecologically based strategies that focuses on ticks' interaction with both plant and animal hosts offer the best promise of success for regulating tick population as summarized by Hassan and Osman (2003) with respect to the tick populations which associated with plant and animal components. Moreover, when a new stock is introduced the risk of an outbreak is greatly increased as observed by Radostits *et al.* (2000a).

Semu *et al* (2005) mentioned that serological diagnosis of heartwater has been hampered by severe cross reactions with antibody responses to related ehrlichial agents. However, van Valiet *et al.* (1995) has developed a MAP-1 B indirect ELIZA that has an improved specificity and sensitivity for detection of Immunoglobulin G (IgG) antibodies to overcome this constraint, MAP-1B cross reacts only with *E.canis* and *E.chaffeensis* which do not infect ruminants).

This study revealed that *Ehrlichia ruminantium* sero- prevalence in small ruminants was highest in Gadarif state (100% for sheep and 96% for goats), a finding which is consistent with that reported by Abdel Rahman *et al.* (2003b), being 98.5% sero- prevalence in Gadarif. In Blue Nile state the sero-prevalence was 87.6% for sheep 98.8% for goats. These figures are greater than those reported by, Abdel Rahman (2003b), which was 83% and 77% for sheep and goats respectively. The changes may be attributed to the different ecological changes and the different localities covered.

In Elkhwei area sero- prevalence of heartwater was 12%, and it was not emerged as important in the household survey results in the area. This

finding may be due to the poor savanna and semi desert environment and vegetation which can not support the survival of the vector, *A. lepidum* as reported by Osman and Hassan (2003) due to lack of adequate water for the young instars, leading to less vectors population Its worth mentioning that there have been no serological studies on heartwater (cowdriosis) conducted before in Elkhwei area.

The observed sero- prevalence (90%), in goats, higher than in sheep (63%) in all the study sites, except in Gadarif state (96% and 100% respectively) appears to disagree with the findings of other researchers in other parts of Africa. For instance, in Ghana, Koney et al. (2004) reported a higher sero-prevalence in sheep than in goats. However, the results are in agreement with those reported by Abdel Raman (2006), 75% in Goats and 69% in sheep in different states in Sudan. The fact that the sero-positivity showed no significant difference between sheep and goats, except for B. Nile state putting doubt on a assumption of the significant difference in the overall project sites.

This study revealed that *Peste des petits ruminants* (PPR) sero- prevalence in small ruminants was higher in Blue Nile state (69.3%), which agreed with the findings of Osman (2005). This observation may be attributed to the characteristics of PPR as a transboundary disease and the frequent movements of animals (small ruminants) within the state and to other parts of the country. States, Blue Nile and Gadarif border Ethiopia at areas where insecurity makes veterinary services inaccessible to small ruminants' producers. Gadarif state had a lower sero-prevalence (28.6%) compared to

Blue Nile, which may be an indication of good PPR vaccination coverage in this state.

There have been no serological studies on PPR carried out in Elkhoweï area specifically before this study and so the observed seroprevalence of 68.4%, which is higher than that found by Osman (2005) (41.25%) in the same State could be explained by absence of vaccination against PPR in the Elkhowie area as reported (Hassan, 2006)

The study revealed that the overall (all three study states) sero- prevalence for Female was 64.3% while that for males was 54.2% and there was significant difference in sero-positivity between females and males small ruminants tested ($p < .05$). This finding disagreed with what had been reported by Osman (2005) that the sex of animals had no effect on the development of PPRV antibodies. The fact that small ruminants' producers keep more females for long time for breeding purposes may explain this observation. Hence, the probability for females getting exposed to PPRV throughout their life time is more than for males. However, association between PPR sero-positivity and sex of tested animals in Elkhoweï area was not significant ($p > .05$), putting doubt on this assumption.

Likewise, there was significant difference in the prevalence of antibodies to PPRV in Blue Nile and Gadarif states among different age groups. This finding disagrees with observation of Osman (2005). This can be justified by the fact that the older animals have greater probability of exposure to the PPRV throughout their life time than younger ones; interviewees stated that they usually keep older animals for breeding purposes. The fact that there

was significant correlation between Percent Inhibition (PI) average (which used to measure the cutoff point) and different age groups confirms the findings of Radostits *et al.* (2000a) that the percentage of antibodies to PPRV in small ruminants raises with age.

4.1 Conclusion and recommendations:

The study showed that PPR, Heartwater and sheep pox are the most important small ruminant diseases in Elkhwei area, Blue Nile and Gadarif states.

Small ruminants are significant components of economy and pastoralists and semi-pastoralists who small ruminants' producers are sustaining their livelihoods through small ruminants keeping. However, small ruminants' diseases have a significant impact on household food security and accessibility to local, regional and international markets, PPR and heartwater are major constraints to small ruminant rearing. However, the economic impacts of PPR and heartwater are not properly estimated, they considered as the major causes of economic loss and poor productivity in small ruminants.

Uncontrolled movement of animals in search of water and grazing and introduction of new animals into areas not known to have the disease play an important role in transmission of PPRV. The newly established quarantine in Elkhwei where more animals from far area (e.g., from Darfur region), are held could be one of the possible reasons for higher prevalence of PPRV in Elkhwei area. The conclusion from this study is that *Peste des petitis*

ruminant (PPR) is probably more prevalent in the Sudan than is known so far. The same conclusion could be made for heartwater, which has shown more prevalence in Blue Nile and Gadarif States.

Management systems, sex, age and species were found to be risk factors that influence the epidemiology of PPR. Moreover, infection with PPR and heartwater led to rejection of animals from the market and hence considered as risk factors affecting market access for small ruminants.

PPR warrants due attention for control and future eradication. Homologous PPR attenuated vaccine is highly recommended to be used to protect against virulent virus challenge in all the states covered by this study (Elkhowei area, Blue Nile and Gadarif) and elsewhere in the country for control of PPR.

The sero-prevalence of PPR which this study has demonstrated is a confirmation that the virus is circulating the study states, and possibly elsewhere in the region. Hence, the question of whether other species of animals (camel and cattle) could be infected need to be verified through research.

Improvement of field diagnostic facilities and use of c-ELISA, which is effective in the diagnosis of PPR, is recommended to improve surveillance. In addition, extension packages for small ruminants' keepers for appropriate use of communal pastures,, better recognition of diseased animals, informed usage of drugs and vaccines are highly recommended.

The occurrence and spread of PPR in areas where it was not known to exist previously should provoke an investigation into possible factors influencing disease events. Such research should explore all possible factors from the causative virus to epidemiological events. Similarly,, research into causes of diarrhea, debilitation and foreign bodies in small ruminants as a cause of their rejection from markets need to be undertaken.

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Treatment of infected heartwater cases after proper diagnosis and usage of highly effective control strategy (based on ecology) for ticks in general will be of benefit in controlling *cowdriosis*.

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4.3 Appendices

4.3.1 Appendix 1: Reagents used for c-ELIZA test:

Reagents used:

Antigen: Cell culture PPR virus antigen, lyophilized store at -20°C

Control Sera (Bovine).....

C ++, anti- PPRV antibody positive (strong).

C+, anti- PPRV antibody positive (moderate).

C -, anti- PPRV antibody negative, also used in Blocking Buffer.

All in control sera are whole bovine sera, freeze dried. Store at $+4^{\circ}\text{C}$.

Monoclonal Antibody: Mouse anti- PPR monoclonal antibody supplied as freeze dried hybridoma cell culture supernatant. Store at $+4^{\circ}\text{C}$.

Anti-Species Conjugate: Horseradish peroxidase (HRP) conjugate, rabbit anti-mouse immunoglobulin, liquid. Store at $+4^{\circ}\text{C}$

Coating Buffer: Phosphate buffered saline (PBS) powder (Sigma Aldrich Chemicals; catalogue no. 1000-3). Store dry at room temperature).

Wash and Blocking Buffer Base: Phosphate buffered saline (PBS) powder. Store dry at room temperature).

Blocking Detergent: Tween 20, liquid. Store at room temperature).

Substrate:Hydrogen peroxide tablets. Store at +4°C

Chromogen: Ortho-phenylenediamine (OPD) tablets. Store blister pack containing tablets in bottle at +4°C.

Reconstitution Diluent's: Water. Pyrogen-free, deionised water and bottle have been autoclaved to ensure sterility. Store at +4°C.

PPRV Antigen Stock

The freeze dried contents of a vial reconstituted with precisely 1 ml of the sterile water (reconstitution diluent) supplied with the kit and mixed gently until completely dissolved. The stock stored in its original vial at -20°C.

Anti-Rinderpest Monoclonal Antibody Stock

The freeze dried contents of a vial reconstituted with precisely 1 ml of the sterile water (reconstitution diluent) supplied with the kit and mixed gently until completely dissolved. The stock stored in its original vial at -20°C

Anti-Species Conjugate Stock

The rabbit anti-mouse immunoglobulin HRPO conjugate stock supplied subdivided into 500 ul aliquots in 1 ml Cryopreservation vials supplied, labeled and stored at +4°C.

Control Serum Stocks

The freeze dried contents of a vial of each control serum (C+ +, C+ and C-) reconstituted with precisely 1 ml of sterile water (reconstitution diluent) supplied with the kit agitate gently until completely dissolved. These control serum stocks stored in their original vials at -20°C

Chromogen Stock

2.2 mM OPD

One OPD tablet dissolve in 75ml of locally produced distilled/deionised water, just before substrate/ chromogen incubation step and store in the dark at 4°C.

Substrate Stock

3% (w/v) H₂O₂ (882mM)

One hydrogen peroxide tablet placed in the brown bottle_supplied and dissolved with 10 ml of locally produced distilled/deionised. This will give a 3% solution. Store at +4°C.

Coating Buffer

0.01 M Phosphate Buffered Saline, pH 7.4 +/- 0.20

The contents were dissolved of one bottle per 1 litre of locally produced distilled/ deionized water. Labelled and stored at +4°C

Blocking Buffer

0.01 M Phosphate Buffered Saline, pH 7.4 +/- 0.20 plus 0.1% (v/v) Tween 20 plus 0.3% (v/v) normal bovine serum (C- control serum). The contents were dissolved of one bottle per 1 litre of locally produced distilled/deionized water.

Wash Buffer

0.002 M Phosphate Buffered Saline, pH 7.4 +/- 0.20

The contents were dissolved of one bottle per 1 litre of locally produced distilled/ deionized water. Transferred to a wash fluid container with a tap to which tubing may be attached and further dilute with the addition of 4 litres of distilled/deionised water, mix well and store at room temperature for no longer than two weeks.

Stopping Solution

1M Sulphuric Acid

Slowly add 55 ml of concentrated sulphuric acid to 945 ml of locally produced distilled/deionised water. Label and store at room temperature.

Test Sera

Whole blood samples allowed to clot in the tubes into which they were collected and the serum sampled directly off the clot. Test sera stored at -20°C after they were labeled and the additional information for serum were transferred into other data sheet.

4.3.2 Appendix 2: Substrate preparation for indirect ELISA for detection of antibody to recombinant MAP1B antigen of *Cowderia ruminantium*:

1. Citrate phosphate buffer for 40 ml each:
 - a) Citric acid 1.14gm / 40 ml H₂O. Take 24.3 ml and add it to
 - b) 25.7 Di-sodium hydrogen phosphate solution that formed from 0.77 gm Di-sodium hydrogen phosphate / 40 ml.
2. Dissolve 1 tab of ABTS in 10 ml of citric phosphate buffer.
3. Add 4ul hydrogen peroxide

4.3.3 Appendix 3: Plate layout for c-ELISA to detect PPR antibodies

4.3.4 Appendix 4: The Household's questionnaire

ILRI

MARF

ICARDA

Household Survey to Identify Small Ruminant Health Constraints to Market Access in Sudan

Household ref no. _____

Sample number _____

A. Identifier

A1 State _____ Code _____

A2 Local Authority _____ Code _____

A3 Administrative Unit _____ Code _____

A4 Village _____ Code _____

A5 Coordinates A5a Latitude _____ A5b Longitude _____

A6 Name of household head :

A7 Sex: 1= Male 0= Female A8 Age (yrs) _____ A9 rs of schooling _____

A10 Name of respondent if different from household head

A11 Relation to household head: _____ Code _____

B. Household demographics other than the household head

Age group (yrs)	Number		Number currently in school		Total yrs of schooling		Number engaged in animal care and management	
	a. Male	b. Female	c. Male	d. female	e. Male	f. female	g. Male	h. Female
B1. < 5			x	x	X	x	x	x
B2 6-15								
B3 16-30								
B4 31-50			x	x				
B5 > 50			x	x				

C. Production system and herd type

- C1 Household type Code _____
 1= Only livestock rearer 2= Mainly livestock, minor crop 3= mainly crop, minor livestock 4= livestock and crop roughly equally important
- C2 Livestock management type Code _____
 1= Sedentary 2=Seasonal movement 3= Permanent movement
- C3 Herd ownership and management Code _____
 1= manage only own animals
 2= manage own animals and animals of others for a fee
 3= manage own animals and animals of others for a share of output (e.g.offspring)
 4= manage portion of own animals and let others manage some for fee or output share
 5. Other (specify) _____

D. Current ownership and management of sheep and goats by breed

Species and breed	Number Owned a	Number brought from others for management b	Number given to others for management c	Total number in flock/herd d=a+b-c
Sheep				
D1				
D2				
D3				
D4 Total				
Goats				
D5				
D6				
D7				
D8 Total				

E. Number of other animals owned by the household at present

- E1 Cattle _____ E2 Camel _____ E3 Equines _____
 E4 Poultry _____ E5 Other (specify) _____

F. Inventory changes for sheep and goats in the flock in the last 12 months

	a. Sheep	b. Goats
F1. Number of animals in the beginning (month)		
F2. Number born during the year		
F3. Number bought during the year		
F4. Number brought from others during the year		
F5. Number received as gift during the year		
F6. Number received for other reasons (specify)		
F7. Sub-total incoming(2+3+4+5+6)		
F8. Number sold during the year		

F9. Number died during the year		
F10. Number rented out during the year		
F11. Number given out as social/religious gift during the year		
F12. Number slaughtered for normal home consumption		
F13. Number slaughtered for social/religious functions		
F14. Number lost/stolen during the year		
F15 Sub-total outgoing (8+9+10+11+12+13+14)		
F16 Number at the end of the year (month ...) (F1+F7-F15)		

G. Sale of goat and sheep in the past two years

	a. Current year Month --- Month----	b. Last year
G1. Number of sheep sold		
G2. Number of goats sold		
G3. Where did you sell and how many	1. At camp/h.hold _____ 2. At market _____ 3. _____	1. At camp/h.hold _____ 2. At market _____ 3. _____
G4. What were the main reason(s) for selling animals?	1. _____ 2. _____	1. _____ 2. _____
G5. How many of the sold animals were sick/unhealthy/deformed when sold?		
G6. If G5>0, what was the health problem/disease?		
G7. Was there any problem in selling sick/unhealthy animals?	1 = yes 0 = no 9=not applicable	1 = yes 0 = no 9= NA
G8. If yes, what was/were the main problems?	1. _____ 2. _____	1. _____ 2. _____
G9. How many sick animals could not be sold at all?		
G10. If G9>0, what was the health problem/disease?		
G11. What did you do with unsold animals?		

H. Please give details about the most recent transaction of sheep and/or goats

	a. Sheep	b. Goats
H1. Month in which sold		
H2. Number of animals offered to sell		
H3. Number actually sold		
H4. If there were unsold animals, how many had health/disease problem ?		
H5. What kind of health/disease ?		
H6. What other reasons for unsold animals?		
H7. What did you do with unsold animals		
H8. Where sold? 1= at camp/h.hold 2= at market 3= on the way to market 4.= _____		
H9. Type of buyer? 1= Bush trader 2= trader 3= Consumer 4= bucher, 5= herder/farmer 6=other (specify)-----		
H10. If sold at market, type of market : 1= Local/primary 2=Secondary/Admin Unit level 3= Tertiary/State capital /export point 4= Other _____		
H11. How the animals were transported to market : 1= on hoof, 2= By transport		
H12. Travel time (hrs)		
H13. Transport cost for the animals transported to market		
H14. Transport cost for the herder/labour to take animals to market		
H15. Did you require a certificate from a vet or market official to sell the animals? 1. = yes 0 = no		
H16. If yes, did you pay a fee for such certification? 1= yes 0 = no		
H17. If yes, amount paid		
H18. Did you use a broker/middle man (sabati) : 1. yes 0 = no		
H19. If yes, how much did you pay the broker/sabati?		
H20. Did you use 'damin' (guarantor) 1=yes 0 = no		
H21. If yes did you pay the guarantor, how much?		
H22. What other taxes/fees/payments you made to sell animal and amount		
H23. Total time required to obtain certificate, negotiate with buyer and broker/middleman and complete sale transaction (hrs)		

J. List two most important diseases that affected your flock and related information for the last three years

	a. Current year Month ---- Month -- -	b. Last year	c. Year before
Most important disease	X	x	x
J1. Name of the disease			
J2. Brief description of the disease			
J3. Season/month of occurrence			
J4. Species affected			
J5. No. of animals affected			
J6. Number of animals died			
J7. Value of animals died			
J8. What other losses were encountered, e.g milk loss, abortion,			
J9. Approximate value of losses other than death			
J10. Type of treatment given			
J11. Treatment provider e.g. govt, private, traditional, drug store			
J12. Cost of drugs			
J13. Cost of fees for services			
Second most important disease	a. Current year	b. Last year	c. Year Before
J14. Name of the disease			
J15. Brief description of the disease			
J16. Season/month of occurrence			
J17. Species affected			
J18. No. of animals affected			
J19. No of animals died			
J20. Value of animals died			
J21. What other losses wer encountered, e.g milk loss, abortion,			

J22. Approximate value of losses other than death			
J23. Type of treatment given			
J24. Treatment provider			
J25. Cost of drugs			
J26. Fees for services			

K. Access to and use of veterinary services

- K1. How far is the nearest health outpost or vet assistant from your household/flock? Km -----
- K2. Most common means of travel to outpost : 1= on foot, 2= bicycle 3= transport 4= horses/mules _____ 5. other -----
- K3. Travel time required (hrs)----- K4. Cost/fare _____
- K5. How far is the nearest veterinary clinic with professional vet doctor from the household/flock? Km _____
- K6. Most common means of travel to clinic: 1= on foot, 2= bicycle 3= transport 4= horses/mules 5. other -----
- K7. Travel time required (hrs)----- K8. Cost/fare _____
- K9. How far is the nearest diagnostic lab from the household/flock? Km _____
- K10. Most common means of travel to lab: 1= on foot, 2= bicycle 3= transport 4= horses/mules ____ 5= other -----
- K11. Travel time required (hrs)----- K12. Cost/fare _____
- K13. How far is the nearest veterinary drug store from the household/flock? Km _____
- K14. Most common means of travel to drug store: 1= on foot, 2= bicycle 3= transport 4= horse/mule _____ 5.other -----
- K15. Travel time required (hrs)----- K16. Cost/fare _____
- K17. Visit by govt vet staff in last 12 months (month ----- to month -----)

	1. Local office staff	2. Local Authority staff	3. State office staff
K17a. Number of times visited			
K17b. Purpose of visit			
K17c. Paid any fee? 1= yes 0=no			
K17d. If yes, amount paid			
K17e. For what purpose?			
K17f. Nature of benefits derived from the visits			

- K18 In the past one year, how many times did you feel the need for services of a veterinarian for your flock? _____
- K19. For what purpose or problem?
K19a. _____ K19b. _____
- K20 How many times did you actually seek the services of a veterinarian ? _____
(If answer is 0, go to K30)
- K21 For what purpose or problem?
K21a _____ K21b. _____
- K22. How many times did you use following types of veterinary service staff in the last 12 months and reason for choice?
- | | 1. No of times used | 2. Reason for choice |
|--|---------------------|----------------------|
| K22a. Government vet
_____ | _____ | |
| K22b Private Vet
_____ | _____ | |
| K22c Llocal drug store staff.
_____ | _____ | |
| K22d. Community health worker
_____ | _____ | |
| K22e. Traditional medicine practitioner
_____ | _____ | |
- K23. Have you gained enough experience to sometimes prescribe drug yourself?
1=yes 0=no
- K24 If yes, how many times did you buy drugs on your own in the last 12 months?

- K25 How much did you spend on veterinary services and drugs in the past 12 months?
- | | |
|---|-------|
| K25a. On drugs prescribed by a vet: | _____ |
| K25b. On drugs prescribed by a traditional practitioner | _____ |
| K25c. On drugs based on own judgement/knowledge | _____ |
| K25d. On fees for veterinarian | _____ |
| K25e. On fees for traditional practitioner | _____ |
| K25f. Total | _____ |
- K26 Did you find the expenditure on vet service fees useful/worthwhile? 1. Yes 0=no
- K27 Reason for your answer K27a. _____
K27b _____

- K28 Did you find the expenditure on drugs useful/worthwhile? 1= yes 0 = no
- K29. Reason for answer? K29a. _____
K29b. _____
- K30 If you did not seek the services of a veterinarian when you needed or felt the need for it, what was the reason (s)
K30a. _____ K30b _____

M. Housing and feeding

- M1 Do you have animal barn/kraal/house: 1= Yes 0= no
- M2 Do you keep any animals inside dwelling house: 1= yes 0=no
- M3 If yes, what type of animals? 1=Sheep 2= goats 3= cattle 4=1 and 2
5= 1 and 3 6= 1,2,3
- M4 Main form of feeding by season 1. Dry season 2.Wet
season 1=yes 0=no
- M4a. Only open grazing on common land _____
- M4b. Grazing plus supplementation of fodder/crop residue _____
- M4c. Grazing plus supplementation of fodder/crop residue
and concentrate _____
- M4d. Stall feeding fodder/crop residue _____
- M4e Stall feeding fodder/crop residue plus concentrate _____
- M4f. Other (specify) _____
- M5. Sources of fodder/crop residue:
M5a. Own production _____% M5b. Purchase _____% M5c. Other _____%
- M6 Sources of concentrate :
M6a Own production _____% M6b. Purchase _____% M6c Other _____%

N. Occupation and main sources of family income (derive shares by using 10 bean seeds or similar units and distributing them to different items)

Score out of 10 points

- N1 Livestock rearing _____
- N2 Crop production _____
- N3 Service _____
- N4 Livestock trade _____
- N5 Other trade/business _____
- N6 Remittance from family members working
away from household _____
- N7 Other (specify) _____
- N8 Total **10**

P Land ownership in fedan (1 hectare = fedan)

P1	Cropland	_____
P2	Orchard	_____
P3	Homestead	_____
P4	Private Pasture	_____
P5	Other (specify)	_____
P6	Total	_____

4.3.5 Appendix 5: Local name of the important diseases/ condition reported by the respondents in the project sites:

Disease/ Condition	Local name	Brief description reported by the respondent	Scientific name
Abortion	Torah	Abortion	Abortion
Arthritis	Abu radaa/ Guruz	Lameness, Unable to move	Arthritis
CCPP	Abu neeni/ Abu koweris	Cough, difficulty breathing, crust in nostril,	CCPP
Diarrhea	Reet/ Khorg	Diarrhea	Diarrhea
Foreign Body	Omdaradim/ Jesingarib	Emaciation, Palpable hard body in stomach	Abomesal phytobezoars
Dullness	Dogass	Tired, depression	Dullness
H.S.	Tasamom	Bloat, swelling about the throat, sudden death	H.S
Heart water	Abu kashar/ Abu gelaib/ Khadar/ Abu dadoya	Respiratory stress, diarrhea, emaciation, nervous signs, water in heart and death	Heart water
Mastitis	Goruz/ Hadaya	Oedema of udder	Mastitis
Pneumonia	Abu feshai fish/ Om tonkul/ Iltihab	Cough, nasal discharge, difficulty breathing, off food	Pneumonia
Poisoning	Samti/ Tasamom	Bloat, diarrhea, Sudden death	Plant poisoning
PPR	Abu demayaa	Nasal discharge, lacrimation, diarrhea, death	PPR
Sheep Pox	Jadari	Skin lesions (nodule and ulceration), cough	Sheep pox
Stomatitis	Abu khadra	Errotion on tongue and gum	Stomatitis
Wounds	Dabara/ Juroah	Wound	Wound
Internal Parasites	Hulaa, Hoomra, Dedan	Odema at the jaw, diarrhea, emaciation, warms in faeces.	Heamonchosis/ Paramphistomum
Botulism	Abu regaiba/ Abu denaib	Nervous signs, death	Botulism
Abscess	Koraj	Edematous nodules at lateral side neck	Caseous lymphadenitis
Avitminosis	Aama	Blindness	Avitminosis
Foot rot	Abu dulaa	Lameness	Foot rot
Tick infestation	Gurad/ gamul	Emaciation and present of ticks on the body	Tick and lice infestation
Unknown	-	Dullness, abortion, circling, sudden death	Unknown