Fall 2020

An overview of disease detection among ruminants in Afghanistan: Findings from two studies involving Veterinary Diagnostic Laboratories and Butcher Shops

Susan Chadima
Iowa State College Veterinary Medicine

An Overview of Disease Detection Among Ruminants in Afghanistan: Findings from Two Studies Involving Veterinary Diagnostic Laboratories and Butcher Shops

by Susan Chadima, DVM

Detection of Small Ruminant Diseases in Afghanistan 2015-2017: A Review of Field Reported and Laboratory Confirmed Data

Abstract

Over 70% of the population of Afghanistan is considered rural and largely dependent on livestock for their lives and livelihoods, particularly small ruminants (sheep and goats). Despite the presence of conflict and invasion since the 1970s, the country has developed a veterinary infrastructure to provide a mechanism for disease reporting and laboratory confirmation of disease presence. Records from the central veterinary diagnostic laboratory's information management system from 2015-2017 were analyzed to establish the status of reporting and testing procedures in sheep and goats. Of 1706 records for sheep and 962 for goats, 565 and 237 had positive findings of disease, respectively. Records with positive findings underwent case-by-case review to identify specific laboratory confirmed diseases and extract checklists of clinical signs observed in the field and suspect diseases recorded prior to laboratory submission. Presence of intestinal parasites was the most common laboratory diagnosis in sheep, followed by Peste des Petits Ruminants (PPR), *Clostridium perfringens*, and sheep & goat pox; among goats, the most commonly diagnosed were PPR, sheep & goat pox, and *Mycoplasma* species. In 79% of sheep and 84% of goats, no clinical signs were entered, and suspect diagnoses were entered for 44% of sheep and 78% of goats. Small sample sizes and painstaking, time-consuming, case-by-case review needed to process records for analysis were limiting factors in the current report. Making improvements at each step of the laboratory documentation system will help ensure that useful summary output is readily available for monitoring and evaluating purposes in the future.

Introduction and Background

The ability to accurately diagnose and confirm the presence of disease in animals is the foundation for successful disease treatment, control, and prevention. This is of even greater importance in countries with limited veterinary infrastructure, such as Afghanistan. The ability of government to facilitate reporting of serious animal disease, provide a mechanism for disease reporting, and develop high quality laboratory services is a crucial component of the veterinary public sector role.

Afghanistan is a Central Asian country that has been in continuous conflict since being invaded by the Russians in December 1979. The Russian occupation lasted from 1979-1989, followed by the overthrow of the Afghan communist government in 1992. Civil war continued between various mujahideen factions culminating in the rise of the Taliban, who seized control of the capital, Kabul, in 1996 and formed a new government, the Islamic Emirate of Afghanistan, which controlled most, but not all, of the country. The United States invaded Afghanistan after September 11, 2001, and removed the Taliban government. The country's government was reestablished as the Islamic Republic of Afghanistan, a fragile structure largely supported and financed by the international community. In the intervening years, the Taliban presence has increased throughout the country and large portions of the 34 provinces are under their control. As of 2020, peace talks between the United States and Taliban are being held with uncertainty as to the ultimate outcome.

Afghanistan is landlocked, covering an area of approximately 252,000 square miles, roughly the size of Texas, with an estimated population of 37 million, the majority of whom are illiterate. Over 70% of the Afghan population is considered rural and largely dependent on livestock for their lives and livelihoods, with some farmers also growing crops [Wikipedia, 2020]. An estimated 50% of Afghans live in poverty and 45% of the population is considered food insecure [CSO, 2018]. About 8-10% of the population is classified as Kuchi nomadic pastoralists [DCA, 2020] who migrate seasonally with their large flocks of small ruminants, following traditional routes that extend across political boundaries from Pakistan on the east, through Afghanistan, and to Iran and the former Soviet republics of Turkmenistan, Uzbekistan, and Tajikistan on the western and northern borders. The most remote part of the country is the Wakhan Corridor, a narrow strip of land in the most northeastern part of the country located between Pakistan and China. The one-eighth of the land that is arable is largely used for subsistence farming, including animal husbandry [Dupree *et al.*, 2020].

Survey data to estimate Afghanistan's livestock population were collected by the United Nations Food and Agriculture Organization (FAO) in 2003 and updated by the Afghanistan Living Conditions Survey in 2017 [CSO, 2018]. That survey reported 3.4 million cattle, 9.8 million goats, 22 million sheep, 1.6 million donkeys, 180,000 camels, 140,000 horses, and 10.3 million poultry. These numbers are used for reference and planning purposes today, but accuracy is unknown due to the impact of drought, most recently in 2018, resulting in massive livestock losses.

Veterinary Services

An assessment conducted in 1987 found that veterinary services outside of major cities were virtually nonexistent, and veterinary medicines and vaccines were not available. No government service was present in the rural areas and service delivery structures were destroyed, accompanied by an exodus of the few veterinary doctors or assistants who had worked there [Schreuder, 2015]. In this veterinary vacuum, the Dutch Committee for Afghanistan (DCA) developed a 6-month training curriculum for veterinary paraprofessionals, called paravets (PV), to provide basic veterinary services in their home village areas, including deworming, vaccination, diagnosis, and treatment for basic disease conditions [Schreuder and Ward, 2004].

Following the collapse and overthrow of the Taliban government in 2001, the country faced the daunting task of rebuilding all government ministries, crafting new laws and regulations, recruiting staff, offices, and equipment, and establishing the role of government in providing public sector services. The Directorate of Animal Health (DAH) was established under the General Directorate of Animal Health and Production (GDAHP) within the Ministry of Agriculture, Irrigation and Livestock (MAIL). Technical and administrative support for the DAH to develop regulatory authority and technical capacity was primarily provided through the Animal Health Development Programme (AHDP), which was implemented in two phases from 2006 to 2016 with funding provided by the European Union [Schreuder *et al.*, 2015]. AHDP was embedded within the DAH and provided direct technical assistance for the following:

- Construct and staff the Central Veterinary Diagnostic and Research Laboratory (CVDRL) as well as regional and provincial veterinary laboratories.
- Establish a Central Epidemiology Department (CED) and supporting provincial epidemiology offices.
- Establish the Sanitary Mandate Contracting Department (SMCD) responsible for administering the Sanitary Mandate Contracting Scheme (SMCS) for animal disease reporting.
- Establish the Department of Veterinary Public Health (VPH).

As of 2019, the majority of clinical veterinary services in Afghanistan have been delivered by PVs working from small, privately owned, fee for service, 1 or 2 room clinics called veterinary field units (VFU) [Schreuder, 2015]. DCA's 2019 nationwide assessment of 994 VFU staff revealed that 67% of the VFUs are staffed by PVs; the remaining 33% are staffed by university graduate DVMs or Veterinary Assistants who receive two years of training at an agricultural institute [DCA, 2019].

While the VFUs are independently owned and operated, most continue to have some affiliation or support from non-governmental organizations (NGOs), through donor funded programs, in addition to governmental oversight. VFU staff typically maintain registries documenting the number of animals treated, medications dispensed, and basic syndromic descriptions of diseases diagnosed. Summaries of the registries are regularly provided to the supporting NGO, which also reports summaries of organizational and VFU activities to the DAH at the MAIL. Some VFUs are contracted by the DAH to participate in the SMCS, which is MAIL's official passive surveillance system for monitoring and reporting animal diseases from the field.

Veterinary Diagnostic Laboratories

Government veterinary diagnostic laboratory services are provided without charge; there are no officially recognized private veterinary laboratories in Afghanistan. Laboratory facilities have been built, equipped, and staff training provided largely by third party donors, including the European Union, USAID, US Department of Defense and Department of State, FAO, World Bank, and others.

The CVDRL is located in Kabul, the capital of Afghanistan, and has eight sections: Pathology, Hematology, Virology and Serology, Bacteriology, Hematology, Parasitology, Drug and Vaccine Analysis, and Veterinary Public Health. CVDRL also maintains a teaching laboratory for training regional and provincial laboratory staff and university level veterinary students. A laboratory information management system (LIMS) was introduced at CVDRL in 2014 for data management. Six regional veterinary laboratories are located in the provinces of Herat, Nangarhar, Balkh, Kunduz, Kandahar, and Kabul. The regional laboratories in turn support 15 smaller provincial level laboratories that are capable of providing basic services and initial processing of samples to be sent to the regional laboratories or to CVDRL. CVDRL also maintains strong links and cooperation with the Ministry of Public Health (MoPH) and the Central Public Health Laboratory (CPHL).

A new laboratory building for CVDRL was completed in 2009. Since then, staffing, training, expertise, and technical capacity have all increased. Programs such as proficiency testing conducted in partnership with the international World Organisation for Animal Health (OIE) reference laboratories, and technical assistance from international laboratory experts have served to maintain quality control standards while expanding the range of laboratory services provided.

LIMS is used to record samples submitted, tests performed, results, and conclusions. There is no charge for sample submission or testing. Clients of CVDRL include VFUs, farmers, government customs department, government farms, NGOs, and universities.

Testing ranges from a single sample submitted by a farmer to serosurveillance studies conducted by the DAH and various partners. Each sample is assigned an identification code. Documentation for submitted samples is variable, and information regarding a specific sample may or may not be accompanied by a Disease Report and Sample Form (DRSF), which may or may not be filled out in either Dari or Pashto (both official languages of Afghanistan) or occasionally in English; the LIMS database is designed to be used in Dari and English. Sample quality is variable and may be impacted by the quality of sample storage prior to laboratory submission and duration of time between sample collection and laboratory submission, which may range from days to weeks.

Animal Disease Reporting

Afghanistan's CED was established within the DAH in 2006 and is responsible for surveillance and reporting of animal and zoonotic disease surveillance and disease outbreak response. CED maintains a list of notifiable diseases, including those both known and not known to be present in Afghanistan. This list is used as the basis for official disease reporting both within the country and internationally. The CED reports on the status of notifiable diseases in Afghanistan semiannually to OIE via the World Animal Health Information System (WAHIS) interface. Reporting to OIE is done immediately in the event of an exceptional epidemiological event.

In 2010, the SMCS passive disease reporting system was initiated as a trial in 7 of Afghanistan's 34 provinces. Under the SMCS, private VFUs are selected and contracted on behalf of the government to report suspected presence of prioritized diseases and submit samples for laboratory confirmation within the VFU's clinical practice area. In 2014, the DRSF (Disease Report and Sample Form) was designed and implemented so that a single uniform procedure could be used both for disease reporting to CED and laboratory sample submission to CVDRL. The DRSF includes sections to list the main clinical signs observed, the clinically suspected disease, as well as a section for laboratory testing information.

In 2016, there were 310 VFUs in 29 of 34 (85.3%) provinces contracted under the SMCS. Funding to support the SMCS contracts was provided by three sources: the government of Afghanistan MAIL budget, FAO, and the World Bank's National Horticulture and Livestock Program (NHLP). Reimbursement to the VFUs was based on quality of samples and reporting as well as the monetary value of different diseases according to CED's disease priority list.

In addition to the SMCS, CED receives reports of animal diseases directly from provincial veterinary officers (PVO) and provincial veterinary epidemiology officers (PVEO), VFUs, NGOs, and farmers. Disease reports from non-SMCS contracted VFUs and NGO reports to the DAH frequently do not use the DRSF format. Disease reports and/or laboratory samples submitted by individual animal owners may not be accompanied by a submission form at all. Many reports to the CED and CVDRL from all sources tend to be based on syndromic observations in the field (sudden death, abortion, respiratory, etc.), rather than listing specific differential disease diagnoses. The situation can be complicated by the use of local names for observed clinical diseases that may not translate readily into medical terminology.

Information recorded in LIMS is currently the only source of data to help establish and quantify the presence of suspected and confirmed animal diseases in Afghanistan. An initial review of these data was done to develop a baseline understanding of disease presence.

Present Study

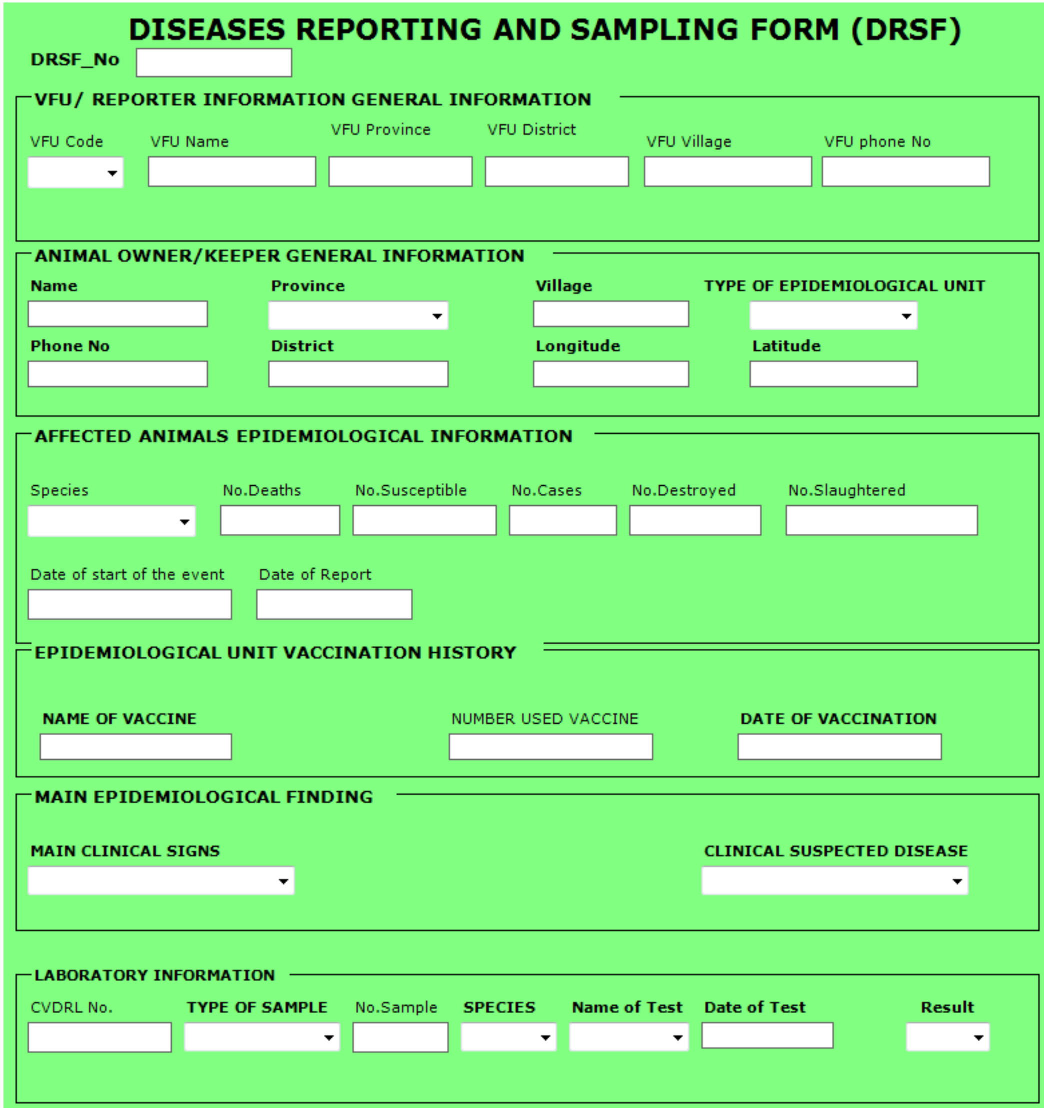
It is against the backdrop of political instability, fragile security, devastated veterinary infrastructure, and socioeconomic hardship that the mechanisms and results of livestock disease identification, reporting, and confirmation have been investigated. It is the small ruminant population (sheep and goats) that is of greatest importance to rural Afghan animal owners and was the focus of interest in this study. The existing infrastructure for collecting and analyzing samples sent to CVDRL and for recording in LIMS for the years 2015-2017 was used to address the following questions in sheep and goats:

- What diseases were identified based on laboratory testing?
- How often were the diseases identified?
- How do clinical signs observed and suspect diagnosis recorded in the field compare with diagnosis based on laboratory evidence?
- How significant and useful are the findings?

Materials and Methods

All CVDRL records from 2015-2017 were obtained through access to the LIMS for further processing. Only records related to sheep and goats were examined for analysis. Focusing on CVDRL data had the advantage of including documentation of observed clinical signs, suspected diseases, and laboratory-confirmed disease diagnoses for those samples that were submitted using the DRSF (Figure 1).

Figure 1. Disease Reporting and Sampling Form (DRSF)


DISEASES REPORTING AND SAMPLING FORM (DRSF)

DRSF_No

VFU/ REPORTER INFORMATION GENERAL INFORMATION

VFU Code | VFU Name | VFU Province | VFU District | VFU Village | VFU phone No

ANIMAL OWNER/KEEPER GENERAL INFORMATION

Name | Province | Village | TYPE OF EPIDEMIOLOGICAL UNIT

Phone No | District | Longitude | Latitude

AFFECTED ANIMALS EPIDEMIOLOGICAL INFORMATION

Species | No.Deaths | No.Susceptible | No.Cases | No.Destroyed | No.Slaughtered

Date of start of the event | Date of Report

EPIDEMIOLOGICAL UNIT VACCINATION HISTORY

NAME OF VACCINE | NUMBER USED VACCINE | DATE OF VACCINATION

MAIN EPIDEMIOLOGICAL FINDING

MAIN CLINICAL SIGNS | CLINICAL SUSPECTED DISEASE

LABORATORY INFORMATION

CVDRL No. | TYPE OF SAMPLE | No.Sample | SPECIES | Name of Test | Date of Test | Result

A spreadsheet with 10,039 records was obtained. Identifying data for each record included: date the sample was received, province of origin, job code (purpose for testing), customer/client, and animal species. Disease related data included: clinical sign(s) observed in the field, suspected disease(s), and confirmed diagnoses based on laboratory testing results. These three fields were entered as unformatted text in the database, with variations in grammar, spelling, and order listed. The data were imported into SAS software version 9.3 (SAS Institute Inc., Cary, NC) for processing and analysis.

Figure 2 shows the effect of data processing to create the final analysis data set. Of the original 10,039 records, 2,948 were from sheep or goats. Records submitted by the VPH for the purpose of testing products of animal origin, such as milk, were deleted, reducing the sample to 2,268 records. Finally, for this study, only records identifying disease presence according to laboratory testing and diagnosis were of interest, which yielded 802 records for analysis.

Figure 2. Sample Size at Each Step of Data Processing

N = 10,039 records in LIMS 2015-2017

- 1,971 (20%) Sheep
- 977 (10%) Goats
- 3,957 (39%) Cows
- 2030 (20%) Other included fowl, canine, feline, equine, camel
- 1104 (11%) Missing species identification

N = 2,948 records in the two small ruminant species of interest

- 1,971 (67%) Sheep
- 977 (33%) Goats

N = 2,268 after excluding samples for product testing

- 1,706 (75%) Sheep
- 962 (25%) Goats

N = 802 observations with laboratory confirmed diagnoses

- 565 (70%) Sheep
- 237 (30%) Goats

Results

Description of Laboratory Submissions

Animal species, province of origin, customer type, and job source are summarized for the original 10,039 records submitted to the laboratory to provide additional understanding of the context for sample submission and disease diagnosis (Table 1).

Samples from sheep and goats accounted for 30% (2,948 combined) of the total submissions, compared to 67% (31.8 million combined) in a total estimated livestock population throughout Afghanistan of 47.4 million livestock animals in 2017 [CSO, 2018].

Table 1. Distribution of Key Characteristics for 10,039 Records		
Species	20% (1,971)	Sheep
	10% (977)	Goats
	39% (3,975)	Cows
	20% (2,035)	Other (fowl, camels, dogs, deer, horses or donkeys, cats, fish, rabbits)
	11% (1,099)	Missing
Province	42% (4,263)	Kabul
	6% (570)	Panjshir
	6% (559)	Nangarhar
	4% (430)	Logar
	4% (371)	Parwan
	4% (355)	Kapisa
	34% (3,491)	Other 28 provinces <4% each
Customer	55% (5,496)	VFU
	21% (2,103)	Owner
	14% (1,356)	Customs
	3% (349)	Kabul University Department of Veterinary Medicine
	7% (735)	Missing or other
Job	54% (5,452)	SMCS
	27% (2,749)	Routine screening
	17% (1,673)	VPH to test products of animal origin
	2% (165)	Missing or other

Six (18%) of Afghanistan's 34 provinces accounted for 66% of the total submissions, with Kabul province alone accounting for 42%. The other 5 named provinces listed in Table 1 are located continguous to Kabul and accounted for 26% of laboratory submissions. The remaining 28 (82%) provinces accounted for 34% of submissions.

The majority customer of laboratory services was VFU (55%), and the majority of submissions were made under the SMCS disease reporting program (54%). Almost all VFU submissions (97%) were done under the SMCS program, and 91% of submissions made directly by the animal owners were for routine screening. The VPH Department is responsible for 76% of testing done for the Customs Department to monitor imported or exported products of animal origin.

Findings in Sheep and Goats

LIMS records from 1,706 sheep and 962 goats were examined for a laboratory confirmed diagnosis. Some records had multiple diagnoses for an individual animal, yielding a total of 730 diagnoses in 565 sheep and 251 diagnoses in 237 goats. The list of confirmations included 39 specific disease entities as recorded in the LIMS records. The laboratory confirmed diagnoses were combined into 8 groups – General Bacterial group, *Mycoplasma* spp. group, *Pasteurella* spp. group, Abortion group (the database only included bacterial causes), Intestinal Parasites group, Internal Parasites (other, non-intestinal) group, Anemia group, Foot & Mouth Disease (FMD) group (by type) – and 5 individual diseases – Anthrax, *Clostridium perfringens* (Enterotoxemia), Peste des Petits Ruminants (PPR), Rabies, and Sheep & Goat Pox. The General Bacterial group included positive findings of *Escherichia coli* (*E. coli*), *Streptococcus*, and other bacteria not classified elsewhere.

The number of animals diagnosed and percent affected based on the sample of tests submitted for 1,706 sheep and 962 goats are given in Table 2.

Table 2. 39 Laboratory Confirmed Diseases Diagnosed in Sheep and Goats		
Grouped and Specific Disease Diagnoses	**Sheep (1,706)**	**Goats (962)**
General Bacterial Group (1 or more of the following)	54 (3.2%)	7 (0.7%)
Escherichia coli spp.	39 (2.3%)	6 (0.6%)
Streptococcus spp.	6 (0.4%)	0 (---)
Other	10 (0.6%)	1 (0.1%)
Mycoplasma Spp. Group (1 or more of the following)	3 (0.2%)	34 (3.5%)
Contagious caprine pleuropneumonia (CCPP)	2 (0.1%)	21 (2.2%)
Mycoplasma arginini	0 (---)	9 (0.9%)
Mycoplasma ovipneumonia	1 (0.1%)	4 (0.4%)
Pasteurella Spp. Group (1 or more of the following)	34 (2.0%)	5 (0.5%)
Hemorrhagic septicemia	1 (0.1%)	0 (---)
Pasteurella haemolytica/Mannheimia haemolytica	18 (1.1%)	4 (0.4%)
Pasteurella multocida	1 (0.1%)	0 (---)
Pasteurella unspecified	14 (0.8%)	1 (0.1%)
Abortion Group (1 or more of the following)	38 (2.2%)	15 (1.6%)
Brucellosis	34 (2.0%)	8 (0.8%)
Q fever (Coxiellosis)	4 (0.2%)	7 (0.7%)
Intestinal Parasites Group (1 or more of the following)	139 (8.1%)	16 (1.7%)
Coenurosis	1 (0.1%)	0 (---)
Dicrocoelium	36 (2.1%)	3 (0.3%)
Dictyocaulus	2 (0.1%)	0 (---)
Eimeria	15 (0.9%)	3 (0.3%)
Marshallagia spp.	16 (0.9%)	1 (0.1%)
Moniezia	9 (0.5%)	0 (---)
Nematodirus	23 (1.3%)	2 (0.2%)
Paramiphistomum	1 (0.1%)	1 (0.1%)
Strongylus spp.	95 (5.6%)	11 (1.1%)
Trichostrongylus spp.	20 (1.2%)	3 (0.3%)
Trichuris	23 (1.3%)	3 (0.3%)
Internal Parasites Group (1 or more of the following)	16 (0.9%)	3 (0.3%)
Fasciola hepatica	9 (0.5%)	1 (0.1%)
Hydatid cyst	6 (0.4%)	2 (0.2%)
Muellerius	1 (0.1%)	0 (---)
Anemia Group (1 or more of the following)	42 (2.5%)	4 (0.4%)
Anaplasma	13 (0.8%)	3 (0.3%)
Anemia unspecified	19 (1.1%)	1 (0.1%)
Babesia	2 (0.1%)	0 (---)
Theileria	27 (1.6%)	0 (---)
Foot & Mouth Disease (FMD) Group (1 or more of the following)	31 (1.8%)	17 (1.8%)
FMD type A	4 (0.2%)	7 (0.7%)
FMD type O	19 (1.1%)	10 (1.0%)
FMD type Asia 1	4 (0.2%)	1 (0.1%)
FMD (type unspecified)	4 (0.2%)	1 (0.1%)
Anthrax	17 (1.0%)	6 (0.6%)
***Clostridium perfringens* (Enterotoxemia)**	69 (4.0%)	12 (1.2%)
Peste des Petits Ruminants (PPR)	100 (5.9%)	76 (7.9%)
Rabies	3 (0.2%)	1 (0.1%)
Sheep & Goat Pox	62 (3.6%)	42 (4.4%)
TOTAL CVDRL CONFIRMED DIAGNOSES	**730**	**251**

Figure 3 graphically shows the distinct species differences across percentages of confirmed diagnoses among sheep and goats in the LIMS data set.

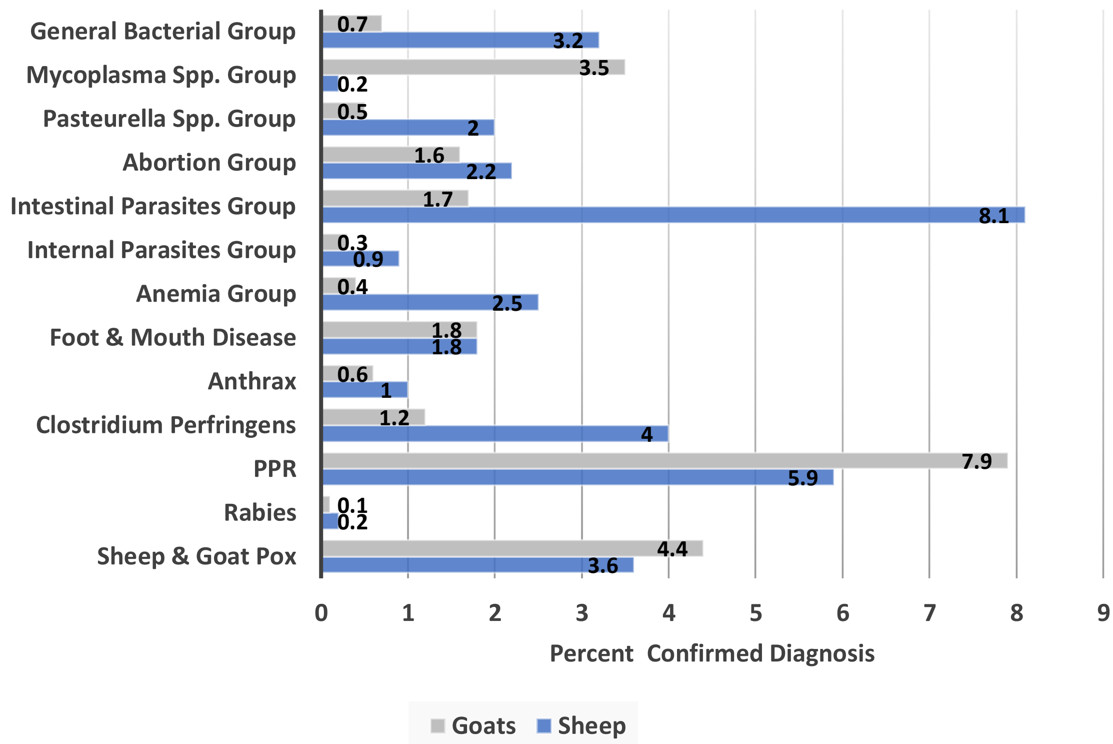


Figure 3. Percentages of Laboratory Confirmed Diagnoses in 1,706 Sheep and 962 Goats

Intestinal Parasites was the most common laboratory diagnosis in sheep, and when combined with the Internal Parasite category, accounted for 155 of the positive diagnoses in sheep but only 19 in goats. PPR (100, 76) and Sheep & Goat Pox (62, 42) were next most common laboratory confirmations in both sheep and goats, respectively. Mycoplasma associated diseases were more frequent in goats (34) than sheep (3), due to the larger number of Contagious Caprine Pleuropneumonia (CCPP) cases confirmed in goats (21). Confirmed diagnoses for abortion were mainly due to Brucellosis in both sheep (34) and goats (8). *Clostridium perfringens* (Enterotoxemia) was confirmed more frequently in sheep (69) than goats (12). *C. perfringens* was the only Clostridial disease confirmed in the data set. Pasteurella, Anthrax, and Anemia were more frequent in sheep than goats. The Anemia group included the blood parasites Anaplasma, *Babesia*, and *Theileria*, but also the clinical finding of anemia itself. FMD was confirmed in 31 sheep and 17 goats, but this was the same percentage (1.8%) of the respective species samples. Confirmed cases of Rabies were low – 3 sheep and 1 goat.

Further analysis was performed on the 565 sheep and 237 goats that had a positive laboratory confirmed diagnosis to evaluate clinical signs and suspect diseases observed and reported from the field. For each laboratory submission, observers in the field could write in multiple clinical signs and multiple suspect diagnoses, but there were many instances where no information was entered (Table 3).

Table 3. Frequency Distribution of Number of Clinical Signs, Suspected Diseases, and Final Laboratory Confirmed Diagnoses Recorded in Sheep and Goats			
		Sheep (565)	**Goats (237)**
Number of Clinical Signs	0	448 (79%)	199 (84%)
	1	43 (8%)	19 (8%)
	2-4	74 (13%)	19 (8%)
Number of Suspected Diagnoses	0	314 (56%)	53 (22%)
	1	242 (43%)	181 (77%)
	2	9 (1%)	3 (1%)
Number of Laboratory Confirmed Diagnoses	1	436 (77%)	224 (95%)
	2-6	129 (23%)	13 (5%)

Clinical Signs: In 79% of sheep and 84% of goats, no field observed clinical signs were entered. For the records that did list clinical signs, the number of clinical signs recorded ranged from 1-4 for each record.

Suspect Disease: Up to 2 suspect disease diagnoses were entered for 251 (44%) of the sheep records and 184 (78%) of the goat records.

Confirmed Diagnoses: The majority of sheep (436, 77%) and goats (224, 95%) had a single laboratory confirmed diagnosis recorded, but in some cases, up to 6 diagnoses were made per individual sheep and up to 3 diagnoses per goat.

Data extracted from case-by-case review identified 18 clinical signs observed in the field and 14 suspect disease diagnoses made prior to laboratory submission. Table 4 gives frequencies and percentages by species, based upon the analysis data set of 565 sheep and 237 goats with laboratory confirmed presence of disease. Each of the 802 individual records was reviewed in order to account for variations in spellings and terminology for clinical signs and diseases, which were recoded into a structured checklist format.

Table 4. Observed Clinical Signs and Suspect Diagnosis in Sheep and Goats		
18 Clinical Signs Observed in the Field	**Sheep (565)**	**Goats (237)**
Abortion	11 (1.9%)	11 (4.6%)
Anemia	1 (0.2%)	0 (---)
Anorexia	47 (8.3%)	9 (3.8%)
Death	30 (5.3%)	13 (5.5%)
Diarrhea	23 (4.1%)	5 (2.1%)
Discharge from nose or mouth	10 (1.8%)	3 (1.3%)
Edema	7 (1.2%)	1 (0.4%)
Facial sores	1 (0.2%)	0 (---)
Fever	30 (5.3%)	6 (2.5%)
Hematuria	2 (0.4%)	0 (---)
Lameness	6 (1.1%)	1 (0.4%)
Low production	1 (0.2%)	2 (0.8%)
Respiratory, cough	22 (3.9%)	6 (2.5%)
Sickness	6 (1.1%)	2 (0.8%)
Stomatitis	1 (0.2%)	0 (---)
Tremor	5 (0.9%)	2 (0.8%)
Weakness	26 (4.6%)	5 (2.1%)
Weight loss	2 (0.4%)	1 (0.4%)
14 Suspect Diagnoses Made Prior to Lab Submission		
Anthrax	17 (3.0%)	5 (2.1%)
Brucellosis (Rose-Bengal test)	19 (3.4%)	4 (1.7%)
Contagious caprine pleuropneumonia (CCPP)	4 (0.7%)	33 (13.9%)
Clostridium perfringens (Enterotoxemia)	2 (0.4%)	0 (---)
Foot and mouth disease (FMD)	31 (5.5%)	17 (7.2%)
Hemorrhagic septicemia	7 (1.2%)	2 (0.8%)
Hydatid cyst	3 (0.5%)	3 (1.3%)
Parasites – blood	1 (0.2%)	0 (---)
Parasites – internal	2 (0.4%)	0 (---)
Pasteurella	12 (2.1%)	2 (0.8%)
Pest des Petits Ruminants (PPR)	95 (16.8%)	76 (32.1%)
Pox	63 (11.2%)	44 (18.6%)
Q fever	1 (0.2%)	0 (---)
Rabies	3 (0.5%)	1 (0.4%)

Anorexia (47 sheep, 9 goats), death (30 sheep, 13 goats), fever (30 sheep, 6 goats), weakness (26 sheep, 5 goats), coughing (22 sheep, 6 goats), diarrhea (23 sheep, 5 goats), and abortion (11 sheep, 11 goats) were the most common clinical signs reported for CVDRL submitted samples that had a positive laboratory diagnosis.

The most common suspect diagnoses from the field prior to laboratory confirmation were PPR (95 sheep, 76 goats) and Pox (63 sheep, 44 goats), followed by FMD (31 sheep, 17 goats), Brucellosis (19 sheep, 4 goats), Anthrax (17 sheep, 5 goats), CCPP (4 sheep, 35 goats), and Pasteurella (12 sheep, 2 goats).

For three laboratory confirmed diagnoses, Anthrax, PPR, and *E. coli*, additional analysis was performed to evaluate and compare the clinical signs and suspect diagnoses reported in the field with the laboratory confirmed final diagnosis.

Anthrax: Anthrax was confirmed by laboratory testing in 17 sheep and 6 goats. Table 5 below gives the field observed clinical signs and suspect diagnoses recorded prior to lab submission. The percentages are based upon number of animals with confirmed diagnosis – 17 sheep and 6 goats. Only 1 of the records for confirmed anthrax had a clinical sign recorded; that clinical sign was death in a goat. For 15 of the 17 sheep and 5 of the 6 goats with laboratory confirmed anthrax, the suspect diagnosis was correctly identified as anthrax. Although this report focuses on examination of laboratory confirmed diagnoses, additional review was performed for the Anthrax negative test results. A total of 275 sheep and 85 goats were tested for Anthrax, with 258 (94%) negative and 17 (6%) positive results in sheep and 79 (93%) negative and 6 (7%) positive results in goats.

Table 5. Clinical Signs and Clinically Suspected Diagnosis in Laboratory Confirmed Anthrax Cases

Clinical Signs Observed in the Field	**Sheep (17)**	**Goats (6)**
Death	0 (---)	1 (16.7%)
Suspect Diagnosis Made Prior to Lab Submission		
Anthrax	15 (88.3%)	5 (83.3%)
Contagious caprine pleuropneumonia (CCPP)	1 (5.9%)	
Hemorrhagic septicemia	1 (5.9%)	
Pasteurella	1 (5.9%)	

Peste des Petits Ruminants (PPR): PPR was confirmed by laboratory testing in 100 sheep and 76 goats. No clinical signs were reported in the records with a confirmed PPR diagnosis. Table 6 below lists the suspect diagnoses recorded prior to lab submission for the PPR positive laboratory cases; 91% of sheep and 98.7% of goats that were laboratory confirmed as PPR positive cases also had a pre-submission indication of PPR as the suspect disease.

Table 6. Clinically Suspected Diagnosis in Laboratory Confirmed PPR Cases

Suspect Diagnosis Made Prior to Lab Submission	**Sheep (100)**	**Goats (76)**
Anthrax	1 (1.0%)	
Brucellosis (Rose-Bengal test)	5 (5.0%)	
Contagious caprine pleuropneumonia (CCPP)		2 (2.6%)
Clostridium perfringens (Enterotoxemia)		
Foot & mouth disease (FMD)		1 (1.3%)
Hemorrhagic septicemia	1 (1.0%)	
Hydatid cyst		1 (1.3%)
Parasites – blood		
Parasites – internal		
Pasteurella		
Pest des Petits Ruminants (PPR)	91 (91.0%)	75 (98.7%)
Pox	1 (1.0%)	
Q fever		
Rabies		

Escherichia coli: *E. coli* was confirmed by laboratory testing in 39 sheep and 6 goats. For the confirmed *E. coli* cases, clinical signs were reported for 33 of 39 sheep and 4 of 6 goats. On the other hand, suspect diagnosis was entered for only 1 sheep and 1 goat. Table 7 below gives the field observed clinical signs and suspect diagnoses recorded prior to lab submission. The sources of samples associated with presence of *E. coli* were from blood (0 goats, 1 sheep), carcass (1 goat, 2 sheep), and internal organs (heart, liver, lung, spleen, or other; 5 goats, 26 sheep).

Table 7. Clinical Signs and Clinically Suspected Diagnosis in Laboratory Confirmed *E. coli* Cases		
Clinical Signs Observed in the Field	**Sheep (39)**	**Goats (6)**
Anorexia	3 (7.7%)	1 (16.7%)
Death	12 (30.8%)	1 (16.7%)
Diarrhea	2 (5.1%)	0 (---)
Discharge from nose or mouth	2 (5.1%)	0 (---)
Edema	1 (2.6%)	0 (---)
Fever	2 (5.1%)	0 (---)
Hematuria	1 (2.6%)	0 (---)
Lameness	2 (5.1%)	1 (16.7%)
Respiratory, cough	5 (12.8%)	0 (---)
Sickness	2 (5.1%)	0 (---)
Stomatitis	1 (2.6%)	0 (---)
Weakness	0 (---)	1 (16.7%)
Suspect Diagnoses Made Prior to Lab Submission		
Clostridium perfringens (Enterotoxemia)	1 (2.6%)	0 (---)
Hemorrhagic septicemia	0 (---)	1 (16.7%)

Discussion

Findings are reported from information recorded as part of regular documentation procedures for samples from livestock submitted to the Central Veterinary Diagnostic and Research Laboratory (CVDRL) in Kabul, Afghanistan, from 2015 through 2017. The data are not only observational, but also lack an imposed sampling scheme or uniform structured recording procedures. However limited, these records are the only available source of data on livestock disease presence available from this war-torn region whose people depend upon their herd animals for both food and livelihoods. The information presented provides initial evidence to support the documentation and quantification of both suspected and laboratory confirmed diseases of concern (through entry on DRSF and in LIMS) in the country.

With appropriate limits to interpretation, this study provides insights into the suspected and documented presence of livestock diseases in Afghanistan, with a particular focus on small ruminants (sheep and goats). Disease reporting and documentation face many logistical and technical hurdles, including disease recognition and sample collection in the field, inadequate protocols for the handling and transport of specimens to CVDRL, and inconsistencies in reporting formats and data management.

Observations made in the field regarding identification of clinical signs and suspect diagnoses, as well as laboratory testing results and interpretations, were entered as text fields without uniform standards of terminology, spelling, grammar, or language. This presented a particular challenge that became evident during the initial cleaning and analysis of the data.

Records from the field are hand written in Dari or Pashto using Arabic script and translated into English for entry in the LIMS. Many of the samples directly submitted to CVDRL are provided by animal owners with limited literacy. For example, in the review of sample submissions, 26 different spellings for Anthrax as a suspect diagnosis were found.

The results of this report are skewed towards Kabul, where CVDRL is located, and five neighboring provinces, rather than being representative of the entire country. This is likely due to the lack of an official or reliable laboratory transport system, as well as the challenges of collecting and transporting samples and forms from remote and insecure provinces. Diagnostic services at the five regional laboratories outside of Kabul at the time of this study were largely limited to fecal examinations and basic hematology to check for the presence of blood parasites. Those data were not available in LIMS nor included in this report.

Sheep and goat samples (20% and 10%) were under represented in submissions to CVDRL compared to their proportion of the total livestock population (estimated 46% and 20%, respectively), while cattle samples were over represented (39% of samples, estimated 7% of livestock). It is difficult to specify the contributing causes for this disparity. Factors may include the larger economic value of a cow compared to a sheep or goat, a greater concentration of cattle closer to the markets and processing facilities around Kabul, and concomitantly greater access to veterinary services for cattle owners. The majority of the small ruminant population remains in the most rural parts of the country with more limited access to veterinary services let alone laboratory diagnostic services. It can take days to weeks for a sample from a distant or insecure province to reach CVDRL. There is limited funding available for sample transport under the SMCS, and there may be no funding to transport privately submitted samples. There is little incentive for private VFUs to collect and submit samples if results cannot be received within a reasonable timeframe, if there is not an economic model to facilitate sample collection and transport, and if there is no recognized financial benefit to the VFU or animal owner to do so.

The DRSF form was created with the intent to communicate and share standard reporting information about suspect diseases in the field to both the CED and CVDRL. Distribution of the DRSF was limited to VFUs contracted under the SMCS. The value of using a uniform format such as the DRSF is (1) to create a database that documents what clinical signs are being observed in the field along with the clinically suspected disease and (2) to link these observations to the laboratory confirmed disease diagnosis. Such information is valuable to identify not only what diseases are present, but also to identify training needs for field disease recognition and treatment recommendations. This goal is difficult to achieve when clinical signs and clinically suspected diseases are not consistently entered on the reporting forms as is the case in this study, where approximately 80% of sheep and goats with a positive confirmed laboratory diagnosis had no clinical signs reported, and 56% of sheep and 22% of goats had no suspect disease listed for the same laboratory confirmations. There is a need for the DRSF to be widely distributed to all VFUs and other veterinary service providers in order to standardize disease reporting and sample submission information for all laboratory samples, and by all users of the government's veterinary laboratory services.

Laboratory confirmations demonstrated the presence of a wide range of animal diseases, from Intestinal Parasites to serious diseases associated with economic and production impacts such as *Pasteurella*, Pox, and Enterotoxemia, as well as diseases of transboundary and public health concerns such as PPR, Brucellosis, FMD, and Rabies.

Intestinal Parasites. The presence of Intestinal Parasites was the most common disease diagnosed in sheep. Veterinary services provided in the field by paravets have largely focused on preventive health by vaccinating against common livestock diseases and administering anthelmintics against nematodes (Intestinal Parasites) and liver flukes (*Fasciola hepatica*, Internal Parasites group) [Schreuder *et al.*, 1996]. The 2017 study reported by Bartels *et al.* demonstrated consistently improved reductions in livestock mortality and increased offtake in sheep and goats utilizing these same paravet services [Bartels *et al.*, 2017]. The preponderance of confirmed laboratory parasite confirmations and the documentation of increased productivity in sheep and goats receiving anthelmintics illustrate the important role parasitism plays in small ruminant health, as well as the need for accurate laboratory parasite diagnosis.

Strongylus spp. were the most commonly identified Intestinal Parasite in both sheep (95) and goats (11), but taxonomic classification was limited to “strongyle” and did not differentiate individual species. Worldwide, *Haemonchus contortus* is considered to be the most notable intestinal parasite of sheep and goats [Delano *et al.*, 2002] for causing severe anemia, protein loss, and death. It can be an acute or chronic problem. In the event of hyperacute disease, death may occur within one week of heavy infection without significant clinical signs [Fox, 2014]. *H. contortus* was reported to be present in Afghanistan in 1976 [Barus *et al.*, 1976] (prior to the Soviet invasion), and is listed as “known to occur in Afghanistan” in the 2008 Illustrated Manual of Infectious Diseases of Livestock in Afghanistan [Mobini *et al.*, 2008]. It is reasonable to theorize that *H. contortus* continues to be represented among the *Strongylus* spp. identified at CVDRL.

Anemia was confirmed in 42 (2.5%) of the sheep with a positive laboratory diagnosis, with 19 (1.1%) of those cases being unspecified as to cause. The focus in the laboratory was to examine blood for blood parasites. It is worth considering that perhaps a large proportion of cases with a history of common clinical signs such as anemia, death, diarrhea, edema, weakness, and weight loss could be infected with *H. contortus*. FAMACHA© scoring is a readily available and easy to use tool to evaluate anemia in the field. The tool is used for estimating the level of anemia in sheep and goats associated with *H. contortus* infection and can guide deworming recommendations [Jazac *et al.*, 2016]. FAMACHA© score cards are not widely available nor routinely used in Afghanistan.

Anthelmintic resistance is a major problem among small ruminants in much of the world [Bosco *et al.*, 2020]. Anthelmintic resistance has not yet been recognized in Afghanistan, but there is no reason to believe it will not become a problem in the country as access to veterinary services improve and the use of anthelmintics becomes more widespread. The ability to identify and quantify specific parasite presence and monitor anthelmintic efficacy requires access to reliable veterinary diagnostic services. Further research is needed to identify and quantify the presence and prevalence of *H. contortus* and other specific intestinal worms in Afghanistan, to evaluate the effectiveness of deworming protocols used in the field, and to monitor the development of anthelmintic resistance.

Anthrax. The disease is caused by the spore forming bacterium *Bacillus anthracis* in grazing animals when they ingest sufficient quantities of spores from contaminated pastures. The clinical course of disease can range from peracute to chronic. It is the peracute form, when animals are found suddenly dead without a history of clinical illness, that is most dramatic. Anthrax is also a serious zoonotic disease. Humans may become infected if exposed to tissues from infected animals, contaminated animal products (wool), or direct exposure to anthrax spores [Mobini *et al.*, 2008]. Anthrax has long been recognized as an important disease in Afghanistan. As such, it is the primary veterinary vaccine produced at the Animal Vaccine Production Lab (AVPL) in Kabul; 1.5 million doses of vaccine were produced and distributed in 2015.

For the 565 sheep and 237 goats with a positive laboratory confirmed diagnosis, death was the most frequently reported clinical sign in sheep (30, 5.3%) and goats (13, 5.5%). Anthrax was listed as a suspect diagnosis in 17 sheep (3.0%) and 5 goats (2.1%). On further review of the anthrax confirmed 17 sheep and 6 goats, the suspect (field) diagnosis was correct for 15 of the 17 sheep (88.3%) and 5 of the 6 goats (83%), suggesting that VFU staff correctly diagnose the disease in the field and are familiar with anthrax's characteristic clinical signs. Surprisingly, only 1 animal in this group of anthrax positives, a goat, listed a field observed clinical sign (death).

CVDRL tested a total of 275 sheep and 85 goats for anthrax with 94% and 93% negative results, respectively. The high level of agreement between suspect and confirmed in the positive anthrax animals suggests the need to further examine the 258 (94%) sheep and 79 (93%) goats that were tested for anthrax but had negative results. Such an examination may reveal whether the disease is over diagnosed in the field. This information would be of importance for training veterinarians and paravet staff, particularly those who participate in the SMCS. Anthrax must always be considered in cases of acute death, but not all animals that die unexpectedly will die due to anthrax.

The SMCS system for contracted VFUs to report diseases and submit laboratory samples may inadvertently introduce bias because of the financial structure for reimbursing VFUs. VFU staff are paid, not only for participating in the SMCS, but are also given a payment based on the fee assigned on the CED and CVDRL list of 33 priority diseases. For example, a suspect Rabies case is reimbursed at 500 Afs for the DRSF and 750 Afs for the brain tissue sample. Anthrax is reimbursed at 400 Afs for the DRSF and 400 Afs with the delivery of a DRSF and blood smears to CVDRL. Blood smears, or cotton swabs dipped in blood and allowed to dry, are the optimal samples for laboratory testing. These sample can be readily collected in the field and easier to transport than other tissues.

When the SMCS was initiated in 2010, it was intended to provide a reimbursement mechanism for the contracted VFUs in order to encourage participation. Prior to 2001, all veterinary services were government sponsored and provided free of charge to animal owners, and veterinary staff received a government salary. Part of the transition in rebuilding all veterinary services since that time has been to develop privatized, fee for service VFUs and the parallel development of government public sector laboratory, epidemiology and public health departments. The intent with the SMCS contracts was to attempt to reimburse veterinary staff who no longer received previously accustomed government financial support while building new public-private partnerships.

Among the many challenges facing accurate disease recognition, reporting, and confirmation, it is important to acknowledge that payment for submitting samples based on a prioritized fee schedule could introduce bias. On the one hand, payments may encourage more active engagement by VFU staff in the field and inspire the search for diagnostic answers for clinical problems. On the other, there is the potential that veterinary staff could over diagnose higher priority diseases in the field, without sufficient evidence to support an initial clinical diagnosis. This may obscure adequate observations that are necessary to determine the true presence, or prevalence, of important diseases.

<u>Peste des Petits Ruminants (PPR)</u>. PPR, also known as sheep and/or goat plague, is a highly contagious viral disease affecting domestic and wild small ruminants. Caused by the PPR virus, a Morbillivirus in the *Paramyxoviridae* family, PPR is closely related to Rinderpest ("cattle plague") which was declared eradicated by the OIE in 2011. PPR can infect up to 90% of an animal herd when first introduced and can kill up to 70% of infected animals. Young stock are most severely affected. Clinical signs may include fever, depression, anorexia, ocular and nasal discharge, erosive swellings in the mouth, diarrhea, pneumonia, and abortion [OIE[a], 2020].

PPR was first identified in 1942, and today more than 70 countries across Africa, the Middle East, Asia, and Europe are infected. These countries are home to approximately 1.7 billion small ruminants, or about 80% of the global sheep and goat population. Worldwide, PPR is estimated to cause annual economic losses of up to USD 2.1 billion, which can cause a devastating impact on millions of poor rural families dependent on their flocks of sheep and goats for both their lives and livelihoods [FAO, 2020]. As an economically important and transboundary animal disease, outbreaks of PPR must be reported to OIE.

The importance of PPR to the small ruminants and animal owners of Afghanistan is readily apparent. Many of the clinical signs associated with PPR are also shared by other common diseases present in the country, such as FMD, CCPP, Contagious Ecthyma (Orf), Bluetongue, Pasteurellosis, and sheep or goat pox [Balamurugan *et al.*, 2014]. Therefore, accurate laboratory confirmation of suspected PPR cases is essential. PPR was the most common suspect diagnosis in both sheep (95, 16.8%) and goats (76, 32.1%) for all cases submitted to CVDRL. For the confirmed cases, the agreement between field suspected diagnosis and laboratory confirmation of PPR was even higher, with 91 sheep (91.0%) and 75 goats (98.7%) being correctly identified prior to laboratory testing.

However, similar to the situation with anthrax, an initial review of the PPR negative test results suggests that PPR may be over diagnosed in the field. Of the 420 total sheep and goats tested for PPR, 176 (42%) were positive and 244 (58%) were negative. For the vast majority of these records, no other test or test result was recorded in LIMS; therefore, the actual diagnosis for cases with a negative PPR test result remains unknown.

The DRSF form used for laboratory sample submission provides a single text box to enter clinically suspected disease(s). Changing the form to include listing multiple clinically suspected diseases could broaden consideration of differential diagnoses and suggest additional testing for the sample at CVDRL to increase the opportunity for determining a definitive positive diagnosis. Accurate disease diagnosis is the foundation of veterinary training, animal owner education, and the provision of treatments and disease control programs in the field.

Rinderpest was the first, and is the only, disease to be eradicated in animals [Ochmann and Behrens, 2018]. Because of the close relationship between PPR and Rinderpest, and because vaccination for PPR is successful in providing long term protection against infection, it is possible to work towards global eradication of this disease. In 2015, OIE and FAO launched the PPR Global Control and Eradication Strategy (PPR GCES). Successful disease eradication requires addressing policy, scientific, and technical challenges, including refining laboratory testing and improving epidemiological understanding of the virus [Njeumi *et al.*, 2020]. The Global Strategy includes three components: (1) a technical step-wise approach to control and eradicate the disease, (2) the strengthening of veterinary services in order to be able to carry out the technical component, and (3) the control of other priority small ruminant diseases together with PPR [OIE[b], 2020].

As of 2017, through a project implemented by FAO, Afghanistan had reached Stage 1 (Assessment) and initiated Stage 2 (Control) of the four-stage control pathway. Continued training at the field level, timely disease reporting, and accurate sampling accompanied by laboratory diagnosis will advance Afghanistan's own PPR control strategy and improve recognition and confirmation for all veterinary diseases.

E. coli: A review of the clinical signs and suspect diagnoses for the cases of confirmed *E. coli* differs significantly from that for anthrax and PPR. Whereas for anthrax and PPR, minimal or no clinical signs were recorded and there was a high level of agreement between the suspect and confirmed diagnosis, the situation is the opposite for the animals confirmed to have *E. coli*. Of the 39 sheep, 28 had one or more of a wide variety of clinical signs recorded, including death in 12 animals (30.8%). In the six goats with confirmed *E. coli*, four clinical signs were recorded – anorexia, death, lameness, and weakness. Conversely, for the *E. coli* confirmed cases, only one suspect diagnosis of *Clostridium perfringens* was made in a single sheep and Hemorrhagic septicemia in a single goat. A variety of tissues were tested (cultured) for the presence of the bacteria. *E. coli* is a main bacterial inhabitant of the intestinal tract in most mammalian species and usually does not cause disease, but is readily spread through fecal contamination [Fairbrother and Nadeau, 2006]. Over 700 antigenic types (serotypes) of *E. coli* have been recognized, some pathogenic but most not [Todar, 2020]. *E. coli* infection can cause particularly severe clinical disease in young sheep, including watery mouth, navel ill, joint ill, scour, meningitis and septicemia [Stevenson, 2017]. Finding a culture result of *E. coli* positive in CVDRL submitted samples may not be sufficient to confirm that *E. coli* infection is responsible for the clinical illness observed. Advanced testing for *E. coli* serotypes and close examination of each individual case report with *E. coli* positive findings may be required to understand the actual role *E. coli* has in sheep and goat diseases in Afghanistan.

Conclusion

The opportunity to review this data set has provided a unique overview opportunity to document suspected and confirmed diseases of sheep and goats in Afghanistan. It has also provided initial insights into Afghanistan's veterinary infrastructure and the vital role of the CVDRL in providing accurate disease diagnosis and confirmation. Analysis of the existing data to identify potential weaknesses and offer suggestions for change is a critical component of providing feedback and working toward continuous quality improvement. It should not be regarded as criticism of Afghanistan's complex government veterinary services that have achieved notable success since the construction of CVDRL in 2009, the initiation of the SMCS disease reporting system in 2010, and the introduction of systematized record keeping (LIMS) in 2014. This study has focused on the available data and has not discussed many of the operational challenges such as intermittent electrical supply to the CVDRL and Animal Health Directorate, staffing shortages, inadequate government funding, subsequent reliance on donors to help cover the costs of basic reagents and supplies, and the need to maintain adequate information technology support and upgrades. These all exist in a country that continues to suffer from 40 years of conflict and political instability.

This study does offer some observations and suggestions to improve disease reporting, data entry, and data management. This is done with the intent of advancing the ability to perform accurate analyses and extract meaningful results, in order to improve the quality of conclusions about diseases present in the country. In turn, this data based information can provide insights into training needs and disease control program management. Such output helps professionals and organizations make informed decisions about resource allocation and implementation of procedures. This is particularly important in Afghanistan, where the majority of the government budget, development, and humanitarian aid programs are dependent on foreign donors. On November 24, 2020, the international community pledged USD 12 billion in civilian aid to Afghanistan over the next 4 years [Reuters, 2020]. How much, if any, of that funding will be directed to the livestock sector and animal health services is uncertain.

It is of the utmost importance that recommendations for improvements in Afghanistan's veterinary infrastructure, be made in a collaborative manner. This requires consideration for the respective roles of government, private sector VFU staff, and NGO support programs, if change is to be successful. The use of a standard disease reporting format that is widely available to all individuals and organizations involved in animal health will benefit both CVDRL and CED, and improve communication about disease incidence and prevalence. The existing DRSF may benefit from review and revisions. The use of "tick boxes" and dropdown lists on forms and in LIMS could help eliminate the many data entry errors due to spelling inconsistencies. "Conclusions" in LIMS could be standardized and result templates developed to more easily record the entries and insure that the conclusion reflects and agrees with the test results for a given sample. There has been inconsistency in recording diagnoses – sometimes the name of a disease or clinical sign is used (Anemia), sometimes an etiologic agent (*Clostridium perfringens*), and sometimes the use of outdated nomenclature (*Pasteurella haemolytica* rather than *Mannheimia haemolytica)*. This requires ongoing training, education, and revisions of approach. One of the most challenging problems is the lack of consistent and timely reporting from all provinces of Afghanistan, and lack of a reliable transport system to deliver time sensitive samples to the CVDRL. It is equally important that laboratory results be provided in a timely manner back to the field. Electronic reporting systems have been used to great advantage in other parts of the world. Electronic reporting could be of great benefit in Afghanistan. Its implementation will require a thoughtful, comprehensive, and well supported approach in order to be effective.

Making improvements at each step of the laboratory documentation system will help ensure that useful summary output is readily available for monitoring and evaluation purposes. The ability to implement a structured sampling scheme across species, province, customer type, and purpose can better facilitate tracking the effectiveness of prevention and treatment interventions. This is especially important when evaluating national disease control programs, such as for PPR. Small sample sizes, and the painstaking case-by-case review needed to process records for analysis were a limiting factor in the current study. Developing survey instruments to collect uniform data from checklists of clinical signs and diseases using standard terminology would facilitate addressing many of these weaknesses, and create opportunities to perform subgroup analysis.

Despite the weaknesses observed and questions raised in the present study, it is with admiration and appreciation for the professionalism, commitment, and hard work of Afghanistan's veterinary professionals that this study is reported.

References

Balamurugan V, Hemadri D, Gajendragad MR, Singh RK, Rahman H. Diagnosis and control of peste des petits ruminants: a comprehensive review. Virusdisease 2014; 25(1):39–56; doi: 10.1007/s13337-013-0188-2, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3889233/.

Bartels CJM, Fakhri AQ, Shams MH, Briscoe RP, Schreuder BEC. Livestock mortality and offtake in sheep and goat flocks of livestock owners making use of services offered by paravets in West Afghanistan. Preventive Veterinary Medicine 2017; 146:79-85.

Barus V, Amin A, Blazek K, Moravec F. Nematodes parasitizing domestic ruminants in Afghanistan. Folia Parasitol (Praha) 1976; 23(3):207-216.

Bosco A, Kießler J, Amadesi A, Varady M, Hinney B, Ianniello D, Maurelli MP, Cringoli G, Rinaldi L. The threat of reduced efficacy of anthelmintics against gastrointestinal nematodes in sheep from an area considered anthelmintic resistance-free. Parasites & Vectors 2020; 13:457; https://doi.org/10.1186/s13071-020-04329-2.

Central Statistics Organization. Afghanistan Living Conditions Survey 2016-17. Kabul, CSO, 2018; https://washdata.org/sites/default/files/documents/reports/2018-07/Afghanistan%20ALCS%202016-17%20Analysis%20report.pdf.

Delano ML, Mischler SA, Underwood WJ. Biology and diseases of ruminants: sheep, goats, and cattle, chapter 14 in Laboratory Animal Medicine (2nd ed.), Fox JG, Anderson LC, Loew FM, Quimby FW (editors), 2002, Academic Press; https://doi.org/10.1016/B978-012263951-7/50017-X.

Dupree L, Ali M, Weinbaum MG, Dupree NH, Petrov VP, Allchin FR. Afghanistan, published by Encyclopedia Britannica, August 17, 2020; https://www.britannica.com/place/Afghanistan, accessed November 05, 2020.

Dutch Committee for Afghanistan. Livestock Program Annual Report 2019; https://dca-livestock.org/wp-content/uploads/2020/03/200316-DCA-Annual-Report-2019-webversie.pdf.

Dutch Committee for Afghanistan. Community Livestock and Agricultural Program – Livestock Development Among the Kuchi; https://dca-livestock.org/kuchi/, accessed November 2, 2020.

Fairbrother JM, Nadeau E. *Escherichia coli*: on-farm contamination of animals. Rev Sci Tech 2006;25(2):555-569.

Food and Agriculture Organization of the United Nations (FAO). Peste des petits ruminants; http://www.fao.org/ppr/background/what-is-ppr/en/ accessed November 28, 2020.

Fox MT. Gastrointestinal parasites of sheep and goats, in Merck Veterinary Manual, 2014; https://www.merckvetmanual.com/digestive-system/gastrointestinal-parasites-of-ruminants/gastrointestinal-parasites-of-sheep-and-goats.

Mobini S, Brown C, Amir DM, *et al*. Illustrated Manual of Infectious Diseases of Livestock in Afghanistan, 2008, Boca Publications Group, Inc.; https://ag.fvsu.edu/application/files/3715/3512/4963/illustrated_manual_of_infectious_diseases_of_livestock_in_afghanistan-english.pdf.

Njeumi F, Bailey D, Soula JJ, Diop B, Tekola BG. Eradicating the scourge of Peste des Petits Ruminants from the world. Viruses 2020; 12(3):313; doi: 10.3390/v12030313.

Ochmann S, Behrens H. How Rinderpest was eradicated. Our World in Data 2018; https://ourworldindata.org/how-rinderpest-was-eradicated#:~:text=Together%20with%20the%20development%20of,animal%20disease%.

Office International des Epizooties[a] (OIE; aka World Organisation for Animal Health). Peste des petits ruminants (PPR); https://www.oie.int/doc/ged/d13983.pdf accessed November 28, 2020.

Office International des Epizooties[b] (OIE; aka World Organisation for Animal Health). Peste des petits ruminants portal; https://www.oie.int/en/animal-health-in-the-world/ppr-portal/gsce/ accessed November 28, 2020.

Reuters. Foreign aid to Afghanistan could reach $12 billion over four years, some with conditions. US News and World Report, November 24, 2020; https://www.usnews.com/news/world/articles/2020-11-24/international-donors-meet-to-pledge-billions-in-aid-to-afghanistan.

Schreuder BEC, Moll HAJ, Noorman N, Halimi M, Kroese AH, Wassink G. A benefit-cost analysis of veterinary interventions in Afghanistan based on a livestock mortality study. Preventive Veterinary Medicine 1996; 26(3-4):303-314; https://doi.org/10.1016/0167-5877(95)00542-0.

Schreuder BEC, Ward DE. Afghanistan and the development of alternative systems of animal health in the absence of effective government. Rev Sci Tech 2004;23(1):285-95; doi: 10.20506/rst.23.1.1487.

Schreuder B. Afghanistan, a 25-Years' Struggle for a Better Life for Its People and Livestock: The Story of DCA, a Small NGO with a Large and Lasting Veterinary Programme, DCA-VET/Erasmus Publishing, Lelystad/Rotterdam, 2015.

Stevenson H. *E. coli* infection in young lambs. Scotland's is part of the Scottish Rural Development Programme Farm Advisory Service, 2017; https://www.fas.scot/article/e-coli-infection-young-lambs/#:~:text=15%20February%202017-,E.

Todar K. Online Textbook of Bacteriology, 2020; http://www.textbookofbacteriology.net.

Wikipedia. Afghanistan; https://en.wikipedia.org/wiki/Afghanistan, accessed November 2, 2020.

Zajac A, Petersson K, Burdett H. Why and How to Do FAMACHA© Scoring, 2016, contribution number 5413 of the College of the Environment and Life Sciences, University of Rhode Island, in Improving Small Ruminant Parasite Control in New England, part of the USDA Sustainable Agriculture Research and Education Program (LNE10-300); https://web.uri.edu/sheepngoat/files/FAMACHA-Scoring_Final2.pdf.

Determining the Presence of Echinococcosis, Fascioliasis, and Bovine Tuberculosis Lesions in Butcher Shops in Kabul, Afghanistan

Abstract

A 2015 survey was conducted to collect and summarize initial information from butcher shops in Kabul, Afghanistan, related to the presence of carcass lesions associated with three zoonotic diseases: hydatid cyst disease (*Echinococcosis*), liver flukes (*Fascioliasis*), and bovine tuberculosis in sheep, goats and cattle. The survey was jointly designed by the Afghanistan Ministry of Agriculture's Veterinary Public Health Department (VPH) and the Central Epidemiology Department (CED). Hydatid cysts were reported in 149 out of 7869 sheep (1.89%), 8 out of 3173 goats (0.33%), and 18 out of 3163 cows (0.57%). Liver flukes were reported in 19 sheep (0.24%), 2 goats (0.083%), and 9 cows (0.28%). A single tubercle was found. The observational study may have underestimated the true prevalence of *Echinococcosis* and *Fascioliasis* in Afghanistan's ruminant population due to weaknesses and potential biases in the sampling procedure. Hydatid cyst disease, liver flukes, and bovine tuberculosis are serious diseases of both animals and people. Additional research is recommended to determine the true prevalence of these diseases and develop effective prevention and control programs.

Introduction

The presence of zoonotic diseases in domesticated herd animals not only causes significant economic and production losses in livestock, but also presents risks to human health through consumption of animal products or environmental exposure. The problem is exacerbated in developing countries like Afghanistan, where reliable information about prevalence of these diseases is scarce. The objective of the project was to collect and summarize initial information from butcher shops in Kabul City, the capital of Afghanistan, related to the presence of carcass lesions associated with three zoonotic diseases: hydatid cyst disease (*Echinococcosis*), liver flukes (*Fascioliasis*), and bovine tuberculosis.

The Study Environment

Afghanistan is a landlocked country that has been in continuous conflict since 1978, starting with internal fighting that prompted the entry of Soviet troops in 1979 [1]. Iran and Pakistan are Afghanistan's largest geographic neighbors, and the country also shares short borders with Turkmenistan, Uzbekistan, Tajikistan and China. Trade routes through border countries and the large-scale movement of animals by migratory nomadic Kuchi pastoralists present challenges for disease recognition and control programs [2]. Reviews of the presence of animal disease in Afghanistan's neighboring countries, particularly Iran and Pakistan, with more developed veterinary and meat inspection infrastructure, can provide support for inferring the presence of similar zoonotic diseases in the country [3-4].

Afghanistan is estimated to have a total population of 38 million people, approximately 70% of whom live in rural villages, where they are directly dependent on agriculture and animals for nutrition and livelihoods [5]. The livestock population is estimated at 30 million small ruminants (sheep and goats) and 6 million cattle [6].

Meat and animal products are a preferred and primary source of dietary protein as incomes allow. In 2018, the total production of meat for Afghanistan was reported to be 330,636 tons [7]. A majority of meat is purchased directly from hundreds of small local butchers located throughout the city. Cattle, sheep and goats are slaughtered early in the morning, and meat is sold the same day. Carcasses are hung in small shops facing the street, and the desired quantity of meat is cut off, placed in plastic bags, and provided to the consumer, usually without refrigeration and frequently without running water. Disposal of offal and carcass remains is not well regulated. Veterinary public health food safety and meat inspection services are rudimentary.

Meat inspection is a basic tool to help ensure that meat is safe and wholesome for human consumption. In developing countries such as Afghanistan, it can provide initial insights regarding the presence and prevalence of zoonotic disease. Due to the ongoing conflict in the country since 1979, little is known about the true presence or prevalence of zoonotic diseases. Official meat inspection is conducted by the Veterinary Public Health (VPH) Department under the Ministry of Agriculture, Irrigation and Livestock (MAIL), with rudimentary staff and resources largely limited to the municipality of Kabul City.

An investigative article in the Pajhwok Afghan News, Afghanistan's largest independent news agency, on February 19, 2018, reported on meat sold in Kabul and documented a lack of slaughter houses, testing equipment for basic hygiene, and procedures for how animals should be slaughtered [8]. By and large, animals are slaughtered at home or in shops, and identification of a healthy versus a sick animal is left to the butcher's expertise. The consequences of the current situation are measured by the prevalence of zoonotic disease vectors in livestock and their transmission to and health implications for the human population.

The Diseases

Hydatid cyst disease (*Echinococcosis*), liver flukes (*Fascioliasis*), and bovine tuberculosis (BTB) are known to be present in Afghanistan. Both *Fasciola hepatica* and *Echinococcus* are zoonotic diseases that are present throughout the world, particularly Africa, Europe, Asia the Middle East, Central and South America. They are more common in underdeveloped countries and those with low levels of veterinary services [9]. Presence of these diseases in ruminant livestock causes economic and production losses to farmers in addition to being a potential source of disease transmission to humans.

- *Echinococcosis* (hydatid cyst disease or hydatidosis) is caused by infection with the larval stage of *Echinococcus granulosus*. Dogs are the definitive host for this tapeworm, while cattle, sheep and goats are common intermediate hosts. Humans usually become infected through the accidental consumption of soil, water or food that have been contaminated by fecal material from an infected dog. Cysts containing the larval parasites can potentially grow to a large size over a period of years, and symptoms are dependent upon the cyst location in the body. Feral dogs regularly roam the streets of Kabul, and offal from butchered animals is readily available for them to ingest, continuing the cycle of infection. Postmortem carcass findings suggestive of hydatid cyst disease include the presence of fluid filled cysts that are 5-10 cm in diameter or larger. The cysts are characterized by the presence of a concentrically laminated thick outer layer within which is a germinal layer. The cysts can be located in any tissue, but are most commonly found in the liver, lungs, heart, spleen, kidneys, and less commonly in muscle and brain. Carcasses with evidence of *Echinococcosis* that are emaciated, edematous, or with evidence of muscle lesions must be condemned and destroyed. Otherwise the carcass is approved, but with affected viscera or other tissue condemned and destroyed. Burying of affected tissue is not sufficient since it may be retrieved by dogs [10].

- *Fascioliasis is a parasitic disease of ruminants caused by trematodes of the genus Fasciola. The most important species are F. hepatica (the common liver fluke or sheep liver fluke) and F. gigantica. Liver flukes are a common disease of ruminants, particularly sheep, and can cause significant production and economic losses [11].* People usually become infected by eating raw water plants contaminated with immature parasite larvae, although it may be possible for human infection to occur from eating raw or undercooked liver that contains immature forms of the parasite. The immature larval flukes migrate through the intestinal wall, the abdominal cavity, and the liver tissue into the bile ducts, where they develop into mature adult flukes. Postmortem carcass findings indicative of liver fluke disease include the presence of flukes in enlarged and thickened bile ducts and in the liver parenchyma, hepatic abscesses, bile duct calcification, black excrement from the flukes in liver, lung, and diaphragm, hemorrhagic tracts from migrating immature flukes in the lungs and liver, and icterus due to liver damage. Emaciated carcasses with severe fluke infestation should be totally condemned. Carcasses without emaciation may have a favorable judgement and only the affected tissue trimmed and condemned [10]. Clinically, problems with liver flukes are recognized by veterinary services providers throughout the country, especially in damp areas [12].

- Bovine tuberculosis (BTB), caused by *Mycobacterium bovis*, is a chronic bacterial disease of cattle and is also zoonotic. *M. bovis* is present worldwide and well documented in Central Asian countries. *M. bovis* is closely related to *M. tuberculosis*, the primary causative agent of tuberculosis in humans. *M. bovis* primarily affects cattle, but can be transmitted from animals to humans through aerosol, direct contact, or the consumption of raw milk. Clinically infected cattle develop a chronic debilitating disease and form tubercles. Postmortem carcass findings suggestive of BTB most commonly include the presence of the tuberculosis granulomas in the lymph nodes of the head, lungs, intestine and carcass with well-defined capsules enclosing a caseous mass with a calcified center. Nodules may also be found on the pleura and peritoneum, and in the liver, spleen or kidney. Bronchopneumonia can be found, but is less specific [10].

Carcasses with suspected tuberculosis lesions require additional postmortem testing, such as confirmation with Ziehl-Neelsen acid fast staining or bacterial culture.

While the prevalence of these diseases are well documented in other countries, there is no reliable information about prevalence in Afghanistan. Therefore, there was a need for a specific study to determine the presence and extent of these diseases in order to provide appropriate treatment and response methods. The descriptive survey study was designed by the Animal Health Development Programme II (AHDP) in cooperation with the Directorate of Animal Health at Afghanistan's MAIL and involved the departments of Veterinary Public Health (VPH), Epidemiology (CED), and the Central Veterinary Diagnostic and Research Laboratory (CVDRL).

The goal of this study was to provide preliminary epidemiological evidence of these diseases by administering a survey of butcher shops to estimate the prevalence of hydatid cyst disease, liver flukes, and BTB in slaughtered livestock in Kabul butcher shops, using meat inspection as a tool to identify disease presence. Having these data would facilitate initial estimates regarding presence of these diseases in Afghanistan's livestock population, improve knowledge about these diseases from a public health perspective, and provide justification to support the development of prevention and control measures. As noted in other reports [13], the information is needed not only to improve our understanding of the scope of the problem, but to provide important insights into the challenges of designing and implementing procedures and strategies to ensure the collection of accurate and reliable data to guide the efforts of government, non-governmental organizations (NGOs), and donors working in Afghanistan's animal health sector.

Research Methods

Study Design

A convenience sample of observational data was collected from August 8 to October 23, 2015. Twelve trained meat inspectors were directed to record their observations during the course of visiting local butcher shops to perform routine monitoring in all 17 districts of Kabul City on two days of each week. The inspectors were officers of the VPH Department under MAIL. They performed regular meat inspections, including examining slaughtered animal carcasses looking for gross evidence of abnormalities suggesting disease presence (cysts, flukes, and tubercles) according to the Food and Agriculture Organization (FAO) of the United Nations meat inspection standards for developing countries [10]. The inspectors completed a survey at each butcher shop recording the date and number of carcasses inspected by animal species (sheep, goats, and cattle) and number with visual evidence of cysts, liver flukes, or tubercles. For completing surveys, the inspectors received no compensation or incentive beyond the regular daily stipend for food allowance and travel.

Sample

A total of 2,352 visits were recorded over the 77-day period, representing about nine surveys per inspector per day. The same butcher shop may be represented multiple times. Due to inconsistent spelling of shop owner names, it was not possible to determine the exact number of different butcher shops included in the survey. A total of 13,478 carcasses (7,896 sheep, 2,419 goats, and 3,163 cattle) were observed and inspected.

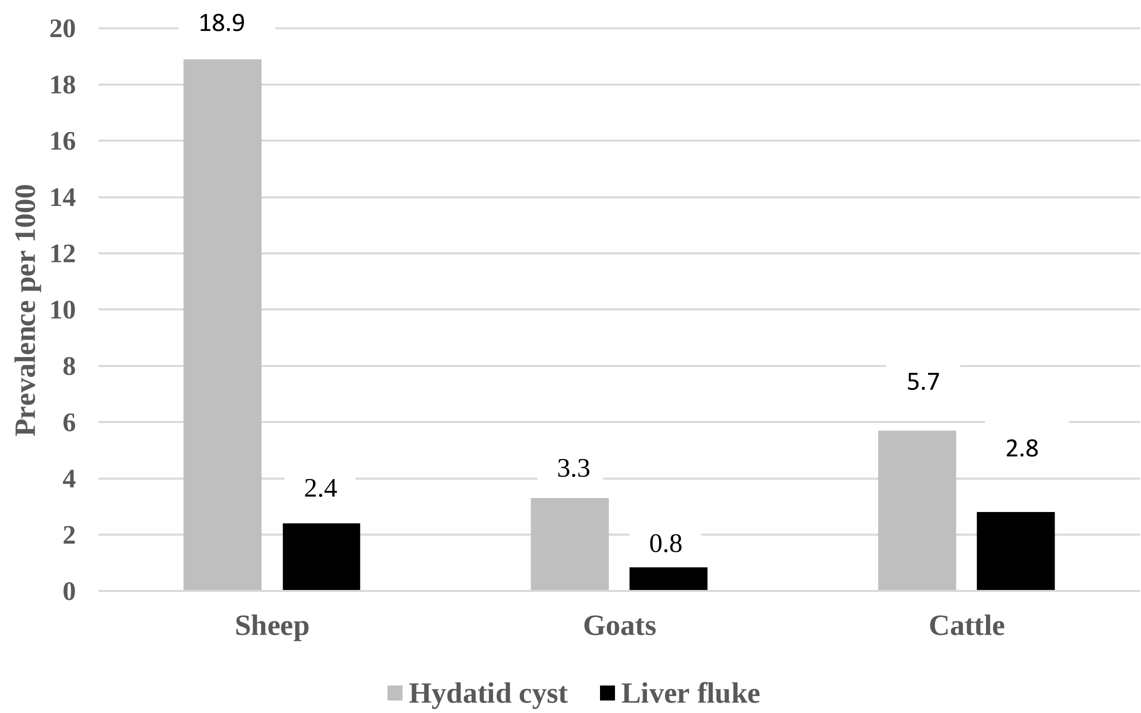
Statistical Methods

Prevalence of slaughter carcass lesions was determined based on number of positive findings out of total observations by species. Data from all dates and from all butcher shops were combined to calculate an overall prevalence.

Results

Only one tubercle in one cow was recorded. Hydatid cysts were reported in 149 out of 7869 sheep (1.89%), 8 out of 3173 goats (0.33%), and 18 out of 3163 cows (0.57%). Liver flukes were reported in 19 sheep (0.24%), 2 goats (0.083%), and 9 cows (0.28%). Figure 1 shows comparative slaughter lesion prevalence per 1000 animals of hydatid cysts and liver flukes by species.

Figure 1. Presence of Hydatid Cysts and Liver Flukes by Species



Discussion

The study was conceived to obtain preliminary data about the presence of the three diseases of interest in ruminants slaughtered in Kabul City. It also represented an initial cooperative research effort by the VPH and CED departments to establish the feasibility of jointly designing a study that could be implemented under the authority of the Animal Health Directorate and provide meaningful results. Beginning in 2002 after the fall of the Taliban, it was necessary to construct a functioning government veterinary sector from the ground up. The international community provided necessary funding and technical support to advance the capacity of MAIL's public sector role regarding food safety and veterinary public health, diagnostic laboratory, epidemiology, and the development of standards for reporting disease regulation and control. Baseline data are required before being able to monitor changes and trends over time, particularly in response to prevention and treatment initiatives.

The main objective was to document evidence of three zoonotic diseases in Afghanistan and quantify as accurately as possible. While reports of other diseases are available [14-15], no recent prevalence data from Afghanistan for the three diseases of interest were found.

It is likely that the results in the current study underestimate the true prevalence of *Echinococcosis* and *Fascioliasis* in Afghanistan's ruminant population. The data represent a sample of only those animals butchered and sold to the public in Kabul City. The observations were made and data collected from a relatively small set of animals sent to market in a specific geographic location during a limited period of time. An underlying selection bias of only observing animals healthy enough to be sent to market means that we are unable to infer true disease prevalence in the general livestock population. The sampling distribution may not be reflective of the general animal population and disease prevalence throughout the country, regional differences, or animals too ill to be marketed. Animals with early disease may not have characteristic lesions present, lesions could be too small to be appreciated, and lesions could be missed by the inspectors. Animals with advanced disease, may not survive to slaughter.

There were other weaknesses in study design that could have introduced unknown biases. A random sampling scheme was lacking. Multiple animals were included in the survey from the same butcher shop on the same or on repeated visits to the same shop; although the survey included recording the butcher's name, inconsistencies in spelling and documenting the full name meant records could not be definitively matched for the same butcher. There were language and logistical barriers to recording and entering data, information was not collected that would allow tracing back to the original source or herd. The analysis therefore treated the sample data as independent observations. This meant that analysis involving measures of variability and hypothesis testing could not be performed. For these reasons, the data are considered preliminary regarding disease presence and preparatory to more conclusive study.

The prevalence of hydatid cyst disease (cystic *Echinococcus* or CE) and liver flukes (*Fascioliasis*) reported here may not accurately reflect disease prevalence in slaughtered cattle, sheep, and goats. It should not be considered reflective of disease prevalence in Afghanistan's ruminant livestock population. Comparison of these data with reports from various sources and locations in other countries reveal broad disparities in findings and methods that impede the ability to interpret across reported data.

This study found a prevalence of hydatid cyst disease lesions in carcasses ranging from 0.33% in goats, to 0.57% in cattle, to 1.9% in sheep. CE is found worldwide and confirmed in other slaughterhouse studies. CE prevalence was reported at 20% to 95% from animals in slaughterhouses in hyperendemic areas of South America [16]. A study done in the Lombardy region of Italy demonstrated prevalence of hydatid cyst disease varying from 0.36% in sheep, to 0.29% in cattle, to 0% in goats [17]. Of interest is the highest percentage of lesions in sheep as in the current study. Differences in the prevalence of CE in various studies can be due to the differences in climatic conditions in each region, which could affect the viability of parasite's eggs, the frequency of infected final hosts, livestock farming in each region, level of contact with dogs, and occupation of the population under study [18].

Because additional data on incidence and prevalence of CE in livestock and humans in Afghanistan are lacking, it is informative to examine the situation in neighboring Iran and Pakistan. Data on CE prevalence in Iranian livestock are difficult to find, but several reports are available on prevalence in people, including a 2019 meta-analysis estimating a disease prevalence of 5% in the human population, including urban and rural areas [19]. Humans are "accidental" intermediate hosts for the *Echinococcus* parasite. A significant level of human cases therefore infers inadequate control of the disease in both other intermediate hosts (ruminants) and the canine definitive host, largely through the dog-sheep-dog transmission cycle [16]. In Pakistan, a 2017 study of 123 butcher shops in Rawalpindi and Islamabad found an overall prevalence of CE in all slaughtered animals (sheep, goats, cattle, buffalo) of 2.7% and additionally inspected the butcher shop environment association with dogs [20]. Similar to Kabul, slaughterhouse facilities in Rawalpindi and Islamabad are rudimentary, there are few facilities for disposal of infected offal, and large numbers of stray, free-roaming dogs have easy access to infected offal from slaughtered livestock [20], thus perpetuating the *Echinococcus* life cycle and disease spread. Effective, long term prevention and control of *Echinococcosis* requires a multipronged approach including health education, hygienic slaughter facilities with proper disposal of offal that prevents access by dogs, targeting farms and communities where infected livestock are found for further follow-up, culling of older sheep, dog population management programs including routine treatment with anthelmintics (praziquantel) that kill the adult *Echinococcus* tapeworm, and the potential use of a vaccine (EG95) to protect against the development of the larval stage of *E. granulosus* in lambs [16].

The prevalence of liver fluke lesions in the current study – 0.08% in goats, 0.24% in sheep, 0.28% in cattle – is lower than those reported in 2014 from a 5-year, retrospective abattoir study of ruminants in the Iranian province of Kermanshah which documented a significantly higher level of condemned livers – 0.7% of goats, 0.8% of sheep and 1.5% of cattle [21]. A similar 3-year retrospective study done in Lorestan province of Iran in 356,605 sheep and goats documented an overall liver condemnation rate of 11.1% with *Fasciola* spp. responsible for 6.3% of the liver condemnations [22]. Data on prevalence of liver fluke lesions in butchered ruminants from Pakistan are not readily available. A 2017 study using coprological and ELISA testing in Multan, Pakistan demonstrated a 33% prevalence of *Fasciola hepatica* in the goats studied [23]. The low prevalence of lesions in the current study is in contrast with the prevalence results of the Pakistan field study and lower than expected based on discussion with Afghan veterinary practitioners. In Afghanistan, *Fascioliasis* is considered a frequent clinical finding in the field, and treatment of animals with flukicides is common. Reasons for the apparent discrepancy between the prevalence of liver fluke lesions found in the study versus expectations based on clinical field experience include factors previously discussed, but perhaps most importantly, selection bias of healthy appearing animals selected for slaughter. Because liver fluke infections are dependent on an intermediate snail host preferring moist warm environments, there are large differences in geographical prevalence in livestock populations which are reflective of environmental conditions, seasonality, and ruminant livestock husbandry practices. Yet *Fascioliasis* remains an important disease of both public health and economic significance. The current study documents the presence of liver flukes in livestock butchered in Kabul City. It is not able to provide inference regarding the actual prevalence of the problem in the ruminant population. Economic losses associated with liver fluke infection include condemnation of liver or other tissues at slaughter, loss of production including morbidity and mortality, reduction in meat, milk and wool production, reduction in growth rate, and costs of treatment and control [24].

In this report, the lack of gross lesions consistent with BTB should not be mistaken for an absence of the disease in Afghanistan. Clinical signs may not be visible in animals with early infections, and it is reasonable to assume that animals sent to Kabul meat markets would appear healthy. In most countries, the standard for BTB control programs is the tuberculin skin test, which is not available in Afghanistan. The presence of enlarged lymph nodes in a carcass raises suspicion for the presence of BTB, but their absence does not rule it out. Iran, off Afghanistan's western border, has had a BTB control plan for the last four decades and the disease remains a number one health concern for Iranian veterinary practitioners and farmers across the country [25]. BTB is present in large and small ruminants in Pakistan, which borders Afghanistan on the east; however, Pakistan does not have a national testing program in place for BTB [26]. Afghanistan lacks a national BTB program, and as of 2017, the CVDRL did not have the capacity to perform bacterial cultures for *M. bovis*. Consequently, it was not possible to do the appropriate follow-up or testing even if enlarged thoracic lymph nodes, or tubercles, had been found. A small surveillance study conducted by CVDRL on dairy farms in Kabul did find animals with positive antibody titers to *M. bovis* by ELISA testing.

The initial data and results from this study are being used to inform government programs and the work of NGOs that provide the majority of training and support for animal health programs in the field. The role of the veterinary public sector continues to be collecting observational data to document the status of disease in livestock and to evaluate the progress and success of the programs and procedures that are designed and implemented. In Afghanistan, veterinary and other animal health services are largely provided by the private sector assisted by technical and logistical support from NGOs. To develop effective zoonotic disease control programs and effective veterinary public health and food safety measures, governments must share animal disease information with private sector partners and organizations. In order to ensure high data quality and reliability and minimize sources of bias, future study investigators need to address study design issues such as a sampling scheme, barriers to data collection, need for a control group, follow-up over time, laboratory confirmation on all or a random sample of tissue collections, and training and certification according to standard procedures. The use of electronic devices that could improve accuracy of survey data collection and entry would be ideal but probably not feasible in this environment. There is also need to evaluate the use of a reimbursement incentive for data collection and whether reimbursement based on the quantity of inspection reports submitted may introduce a bias in favor of quantity over quality.

Conclusion

The findings presented are the first known documentation of the presence of lesions at slaughter for *Echinococcosis*, *Fascioliasis*, and BTB in sheep, goats, and cattle in Kabul, Afghanistan. Recognizing their presence is critical in demonstrating the need for better control and management of these diseases. This initial study creates a foundation for more in-depth research regarding the true prevalence of these diseases in the livestock population. All three diseases are associated with both public health implications and economic costs. Based on our experience with this study, effective disease management and control requires a multipronged educational and management effort, along with a trace back system to identify farms or herds of origin when animals with lesions are identified.

Acknowledgments

The author thanks the following for contributions to this research: Afghanistan Directorate of Animal Health, Ministry of Agriculture, Irrigation and Livestock; Department of Veterinary Public Health; Central Epidemiology Department; Central Veterinary Diagnostic and Research Laboratory; Dr. Ali Safar; Dr. Sayed Abul Hussain Qanee; Dr. Noorrahman Ariaie; Mr. Khaja Ahmed.

The Animal Health Development Programme II was funded by the European Union Commission in Afghanistan.

References

1. Encyclopaedia Britannica. Afghan War, published November 14, 2018, https://www.britannica.com/event/Afghan-War.
2. de Weijer F. Afghanistan's Kuchi pastoralists: change and adaptation. Nomadic Peoples, June 2007, https://www.questia.com/library/journal/1G1-182124933/afghanistan-s-kuchi-pastoralists-change-and-adaptation.
3. Sarvi S, Daryani A, Sharif M, Rahimi MT, Kohansal MH, Mirshafiee S, Siyadatpanah A, Hosseini SA, Gholami S. Zoonotic intestinal parasites of carnivores: a systematic review in Iran. Veterinary World 2018;11:58-65, EISSN 2231-0916; www.veterinaryworld.org/Vol.11/January-2018/11.pdf.
4. Abubakar M, Irfan M, Manzoor S. Peste des petits ruminants in Pakistan; past, present and future perspectives. Journal of Animal Science and Technology 2015:57, https://doi.org/10.1186/s40781-015-0066-0.
5. Wikipedia. Afghanistan, https://en.wikipedia.org/wiki/Afghanistan.

6. Central Statistics Organization. Afghanistan Living Conditions Survey 2016-17, Kabul, CSO, 2018; https://washdata.org/sites/default/files/documents/reports/2018-07/Afghanistan%20ALCS%202016-17%20Analysis%20report.pdf.
7. Knoema. Afghanistan – total production of meat. 2018; https://knoema.com/atlas/Afghanistan/topics/Agriculture/Live-Stock-Production-Production-Quantity/Total-production-of-meat.
8. Karimi MH. Meat sold in Kabul without hygiene being checked, Pajhwok Afghan News, February 19,2018; https://www.pajhwok.com/en/2018/02/19/meat-sold-kabul-without-hygiene-being-checked.
9. Centers for Disease Control and Prevention, Global Health, Division of Parasitic Diseases, U.S. Department of Health & Human Services. Parasites; https://www.cdc.gov/parasites/.
10. Herenda D, Chambers PG, Ettriqui A, Seneviratno P, da Silva TJP. Manual on meat inspection for developing countries, 1994, Food and Agriculture Organization of the United Nations Rome; http://www.fao.org/3/t0756e/t0756e00.htm.
11. Çelik ÖY, Aslan Çelik B. Investigation of the prevalence of *Fasciola hepatica* in small ruminants in the Sirrt Region, Turkey. Iran J Microbiol 2018;13(4):627-631, PMCID PMC6348205; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6348205/.
12. Ballweber LR. *Fasciola hepatica* in ruminants. In on-line Merck Veterinary Manual, 2014; https://www.merckvetmanual.com/digestive-system/fluke-infections-in-ruminants/fasciola-hepatica-in-ruminants.
13. Cardona GA, Carmena D. A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals, Veterinary Parasitology 2013;192(1–3):10-32; https://www.sciencedirect.com/science/article/abs/pii/S0304401712004943.
14. Akbarian Z, Ziay G, Noormal B, Saeed I, Hussain A, Shahab Z. Survey of the seroprevalence of brucellosis, Q fever and Crimean-Congo haemorrhagic fever in humans and livestock in Herat province, Afghanistan. South Asia Regional One Health Symposium 2013, pages 30-33, http://www.onehealthnetwork.asia/sites/onehealthnetwork.asia/files/upload/Afghanistan%20CIP_131202.pdf.
15. Niazi AU, Jawad MJ, Amirnajad A, Durr PA, Williams DT. Crimean-Congo hemorrhagic fever, Herat province, Afghanistan, 2017. Emerging Infectious Diseases 2019;25(8):1596-1598, DOI 10.3201/eid2508.181491, PMID 31216260, PMCID PMC6649324.
16. World Organisation for Animal Health (OIE). Fact sheet on *Echinococcosis* or Hydatidosis. May24, 2019; https://www.oie.int/en/animal-health-in-the-world/animal-diseases/Echinococcosis/.
17. Manfredi MT, Di Cerbo AR, Zanzani S, Moriggia A, Fattori D, Siboni A, Bonazza V, Filice C, Brunetti E. Prevalence of *Echinococcosis* in humans, livestock and dogs in northern Italy. Helminthologia 2011;48:59–66.
18. Chalechale A, Hashemnia M, Rezaei F, Sayadpour M. *Echinococcus granulosus* in humans associated with disease incidence in domestic animals in Kermanshah, west of Iran. J Parasit Dis 2016;40(4):1322–1329.

19. Mahmoudi S, Mamishi S, Banar M, Pourakbari B, Keshavarz H. Epidemiology of *Echinococcosis* in Iran: a systematic review and meta-analysis. BMC Infect Dis 2019;19(1):929. doi: 10.1186/s12879-019-4458-5. PMID: 31684882; PMCID: PMC6830007.
20. Khan A, Ahmed H, Simsek S, Afzal MS, Cao J. Spread of cystic *Echinococcosis* in Pakistan due to stray dogs and livestock slaughtering habits: research priorities and public health importance. Frontiers in Public Health 2020, https://doi.org/10.3389/fpubh.2019.00412.
21. Shahbazi Y, Hashemnia M, Safavi E. A retrospective survey of liver flukes in livestock based on abattoir data in Kermansha, west of Iran. J Parasit Dis 2016;40(3):948-853; published online 2014 Nov 22, doi 10.1007/s12639-014-0612-6; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4996225/.
22. Ezatpour B, Hasanvand A, Azami M, Anbari K, Ahmadpour F. Prevalence of liver fluke infections in slaughtered animals in Lorestan, Iran. J Parasit Dis 2015;39(4):725-529; published online 2014 Jan 22, doi 10.1007/s12639-014-0428-4; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4675594/.
23. Mahmood N, Ayaz MM, Hameed R, Nazir M, Sheikh AS, Hussain I, Ullah N, Samad N, Zaman A, Akbar A, Zheng Y. Liver fluke: ELISA diagnosis in goats in Pakistan. J Vet Med Res 2017;4(6):1094; https://www.jscimedcentral.com/VeterinaryMedicine/veterinarymedicine-4-1094.pdf.
24. Saleha AA. Liver fluke disease (*Fascioliasis*): epidemiology, economic impact and public health significance. Southeast Asian J Trop Med Public Health 1991;22:361-364; https://pubmed.ncbi.nlm.nih.gov/1822928/.
25. Tadayon K, Mosavari N, Feizabadi MM. An epidemiological perspective on bovine tuberculosis spotlighting facts and dilemmas in Iran, a historically zebu-dominant farming country. Iran J Microbiol 2013;5(1):1-13; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3577560/.
26. Ullah A, Khattak US, Ayaz S, Qureshi MS. An emerging zoonosis of bovine tuberculosis: a neglected zoonotic disease (NZD) in work-related occupational groups in Pakistan. Journal of Microbial Pathogenesis 2018;2:1; https://www.hilarispublisher.com/open-access/an-emerging-zoonosis-of-bovine-tuberculosisa-neglected-zoonotic-disease-nzd-in-workrelated-occupational-groups-in-pakistan.pdf