Prevalence and Risk Factors Of Zoonotic Protozoa Among Small Holder Livestock Farmers in Aurora Province

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INTRODUCTION

populations rise

intensification of livestock production

problem of emerging and re-emerging zoonotic diseases

EMERGING AND RE-EMERGING ZOONOSES

Important global issues on:

- public health
- sustaining food security
- biosafety both in man and animal

Special attention

- unique population affected by the livestock industry

households who engage in smallholder livestock farming

Issues on Livestock Production

Two main types of human health risks:

1. diseases transmitted from livestock to humans
   - body fluids & excreta
   - products like raw milk / eggs / meat
2. diseases transmitted from polluted environment

Situationer

- Smallholder livestock farmers in the Philippines
  - minimal methods of protection from getting infected from animal diseases
  - poorly informed on the prevention of zoonoses spread to neighboring farms and communities

Needs

As of to date, no information

- prevalence of blood, enteric and tissue protozoan infections affecting both man and animals in Aurora province
- need to determine association of factors that contribute to the risk of zoonotic disease transmission among animal handlers & livestock animal

Objectives

- Determined the prevalence (point & confidence interval) of zoonotic pathogenic protozoa among animal handlers engaged in small holder livestock farming and their livestock in Aurora province by microscopy and PCR.
  - arthropod-borne

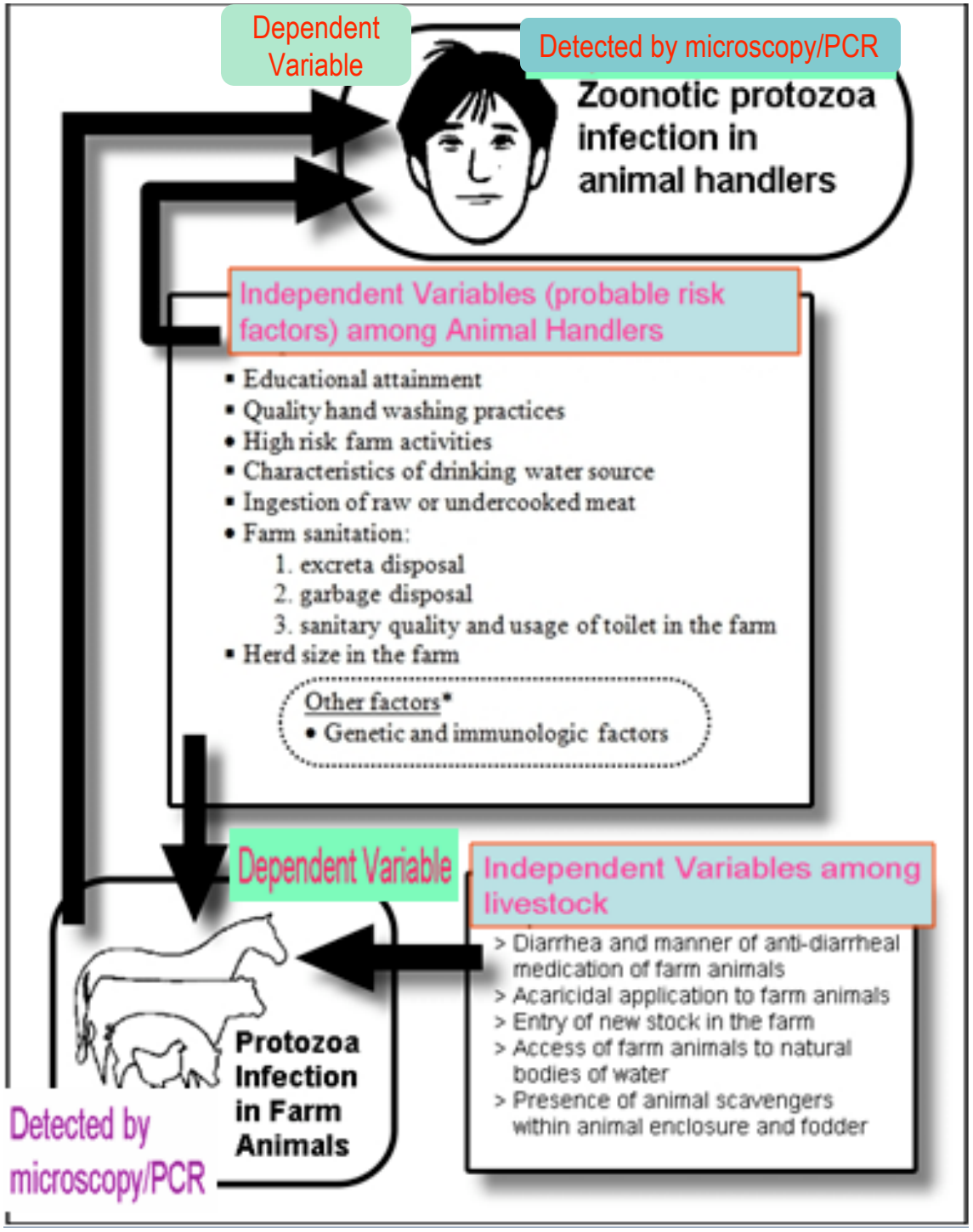
    ***Babesia microti* and *Trypanosoma evansi*;**
  - water-borne/fecal-oral borne

    Balantidium coli, Blastocystis hominis, Cryptosporidium parvum, Entamoeba histolytica/dispar, Giarda lamblia
  - food-borne

    Sarcocystis* spp, and *Toxoplasma gondii

- Determined the association of several probable exposure factors to zoonotic disease transmission between humans and their animals.
- Recommend public health measures to prevent and control these zoonotic protozoan infections.

Conceptual Framework


Dependent Variable
Detected by microscopy/PCR
Zoonotic protozoa infection in animal handlers
Independent Variables (probable risk factors) among Animal Handlers
• Educational attainment
• Quality hand washing practices
• High risk farm activities
• Characteristics of drinking water source
• Ingestion of raw or undercooked meat
• Farm sanitation:
1. excreta disposal
2. garbage disposal
3. sanitary quality and usage of toilet in the farm
• Herd size in the farm
Other factors*
• Genetic and immunologic factors
Dependent Variable
Independent Variables among livestock
> Diarrhea and manner of anti-diarrheal medication of farm animals
> Acaricidal application to farm animals
> Entry of new stock in the farm
> Access of farm animals to natural bodies of water
> Presence of animal scavengers within animal enclosure and fodder
Protozoa Infection in Farm Animals
Detected by microscopy/PCR

Materials and Methods

Study Design: cross-sectional, analytic design

Study Area: Aurora province

Baler, Ma.Aurora and San Luis- represent the most number of livestock population in the province

Study Population- Systematic sampling method was used in selecting the respondents/farm owners

Sample Size Determination:
STATCALC of EPI-INFO 6

Areas Covered

Aurora Province

Sample Size

based on the **lowest** expected prevalence of zoonotic protozoa reported in the Philippines and other Asian countries.

Sample size = power of 80 and 95% C.I.

Sample size requirement for animal handlers

Variables	n (sample size)
Prevalence of infection	284
Educational attainment of animal handler	328
Quality of hand washing practice	**360**
High risk farm activities	340
Exposure to fecal contaminated drinking water	340
Ingestion of raw and under cooked meat	340
Excreta disposal practice	340
Garbage disposal practice	**360**
Usage of sanitary toilet facility in the farm	340
Herd size of farm	**360**

For 9 possible risk exposure variables,

- **additional 10 percent of the sample size for each variable was added**
- **sample size increased by 324 more.**

Therefore, n = 684

- **number of animal handlers which represented 684 small holder farms randomly sampled from all three municipalities.**

Sample Size Requirement for Livestock in the Study

Parameters	Cattle	Swamp Buffalo	Goat	Pig	Dog	Stray Cat	Native Chicken
Total Population Size in the 3 Municipalities	2529	2973	3516	17000	15000	1000	26,161
Expected Prevalence	5%	5%	5%	25%	5%	7.3%	3.0%
Worst Acceptable Error	0.15%	0.15%	0.15%	0.15%	0.15%	0.15%	0.15%
Level of Confidence	95%	95%	95%	95%	95%	95%	95%
Sample Size	**75**	**76**	**76**	**12**	**36**	**51**	**137**

For 5 potential possible risk exposure variables,

- additional 10 percent of the sample size for each variable was added

Therefore,

- **For each type of mammalian livestock, n = 114**
- **For chickens, n = 206**

Data Collection- scheduled interview using structured questionnaire

- **Risk variables** to animal handlers
- **Risk variables** to farm animals

1. Collection of Stool Samples from farmer and livestock

Stool Processing

- FECT, Sporulation Technique
  Acid Fast Stain, DNA extraction

Stool Examination

- microscopy and PCR

2. Collection of Blood Samples from farmer, cattle, buffalo and field rats

Blood Processing

- blood smear, DNA extraction

Blood Examination

- microscopy and PCR

DATA ANALYSIS

A. Descriptive Analysis

- Frequency distribution of variables
  - STATA version 8.0 statistical software

- Prevalence of the different zoonotic protozoa according:
  1. risk variables for animal handlers
  2. farm animals by species
  - STATA version 8.0 statistical software

B. Inferential Analysis

Associations between the outcome and independent variables

1. crude analysis (odds ratio)
2. multivariate analysis using logistic regression.

RESULTS AND DISCUSSION

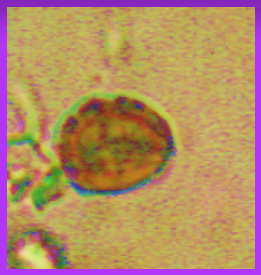
Characteristics of the Animal Handlers

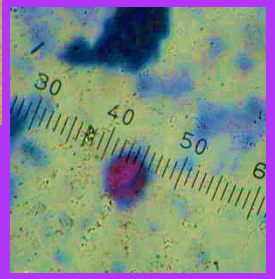
- The males dominate livestock raising throughout the three municipalities
- starting ages 37 years onwards.

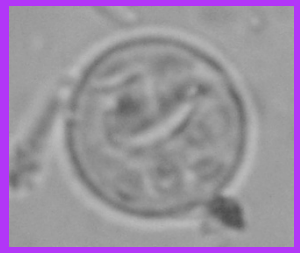
Prevalence of Protozoa Infection among Animal handlers

Four zoonotic protozoa identified in the feces of 678 animal handlers.

- *Blastocystis hominis*
- *Cryptosporidium parvum*
- *Entamoeba histolytica*
- *Giardia lamblia*







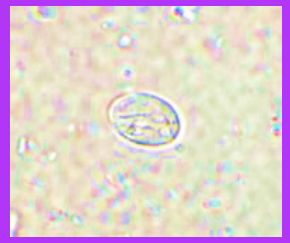
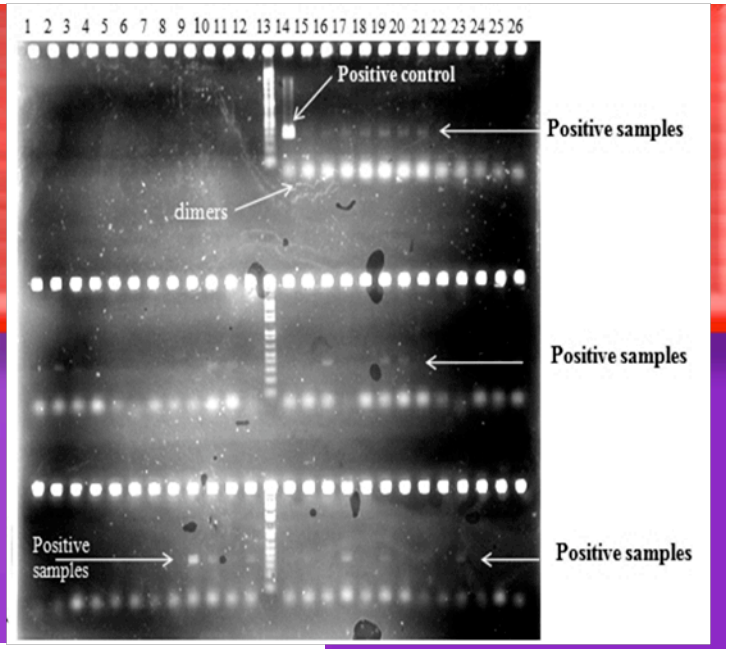


Table 1. Prevalence of Zoonotic Protozoa Among Animal Handlers by Gender



Zoonotic Protozoa	Male (N=410)			Female (N=268)		
	Prev	Lower (95% CI)	Upper (95% CI)	**Prev**	Lower (95% CI)	Upper (95% CI)
Blastocystis hominis[1]	**2.44%**	1.18%	4.44%	**1.87%**	0.61%	4.30%
Cryptosporidium parvum[2]	**20.98%**	17.13%	25.24%	**21.64%**	16.86%	27.06%
Entamoeba histolytica[2]	**0.49%**	0.06%	1.75%	**1.12%**	0.23%	3.24%
Giardia lamblia[1]	**1.22%**	0.40%	2.82%	**0.00%**	0.00%	1.37%
Plasmodium falcifarum[2]*	**0.24%**	0.01%	1.35%	**1.87%**	0.61%	4.30%
Entamoeba coli[1]**	**0.00%**	0.00%	0.90%	**0.37%**	0.01%	2.06%

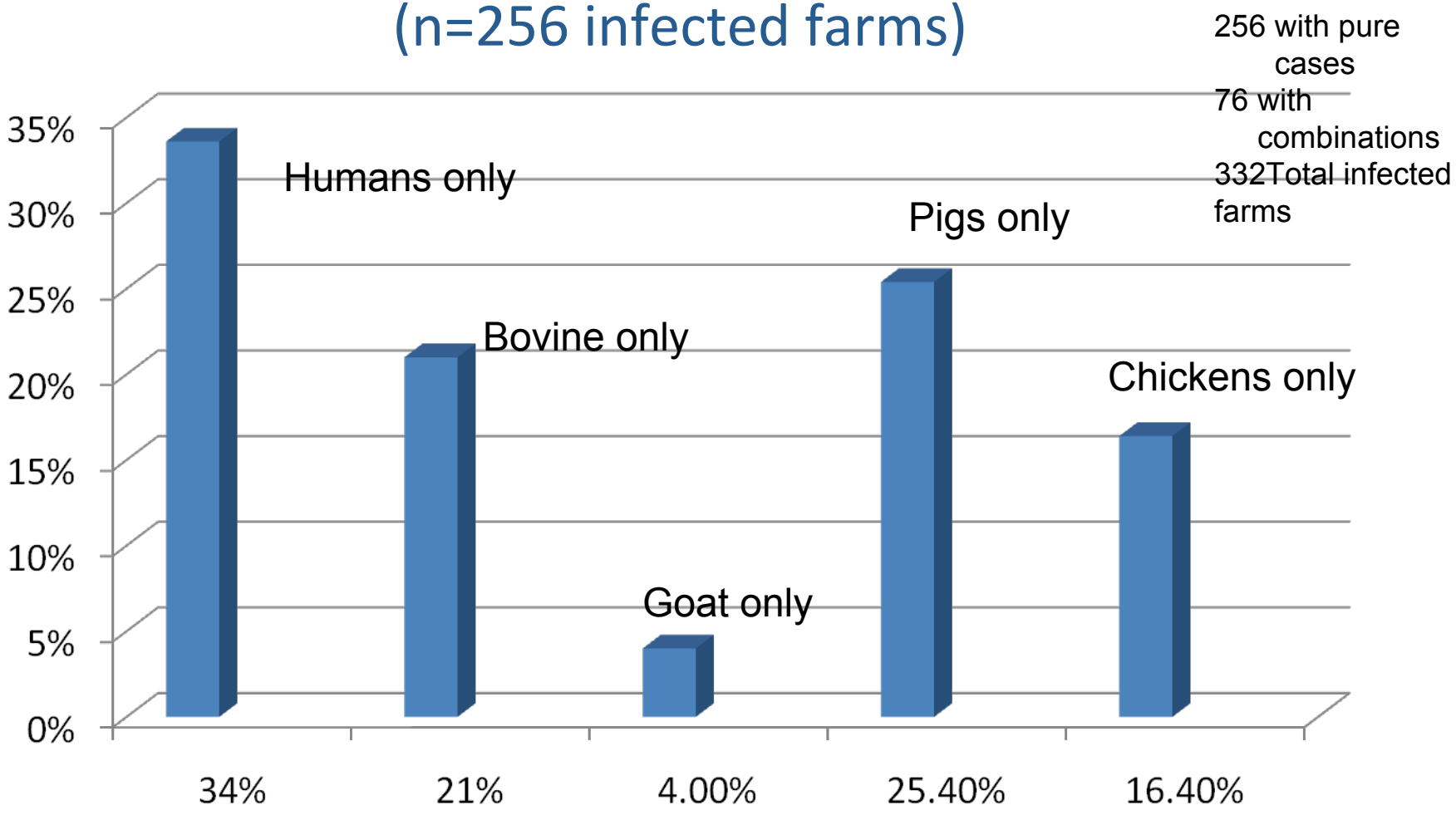
* not zoonotic, as differential diagnosis for *Babesia* spp. infection

** nonpathogenic

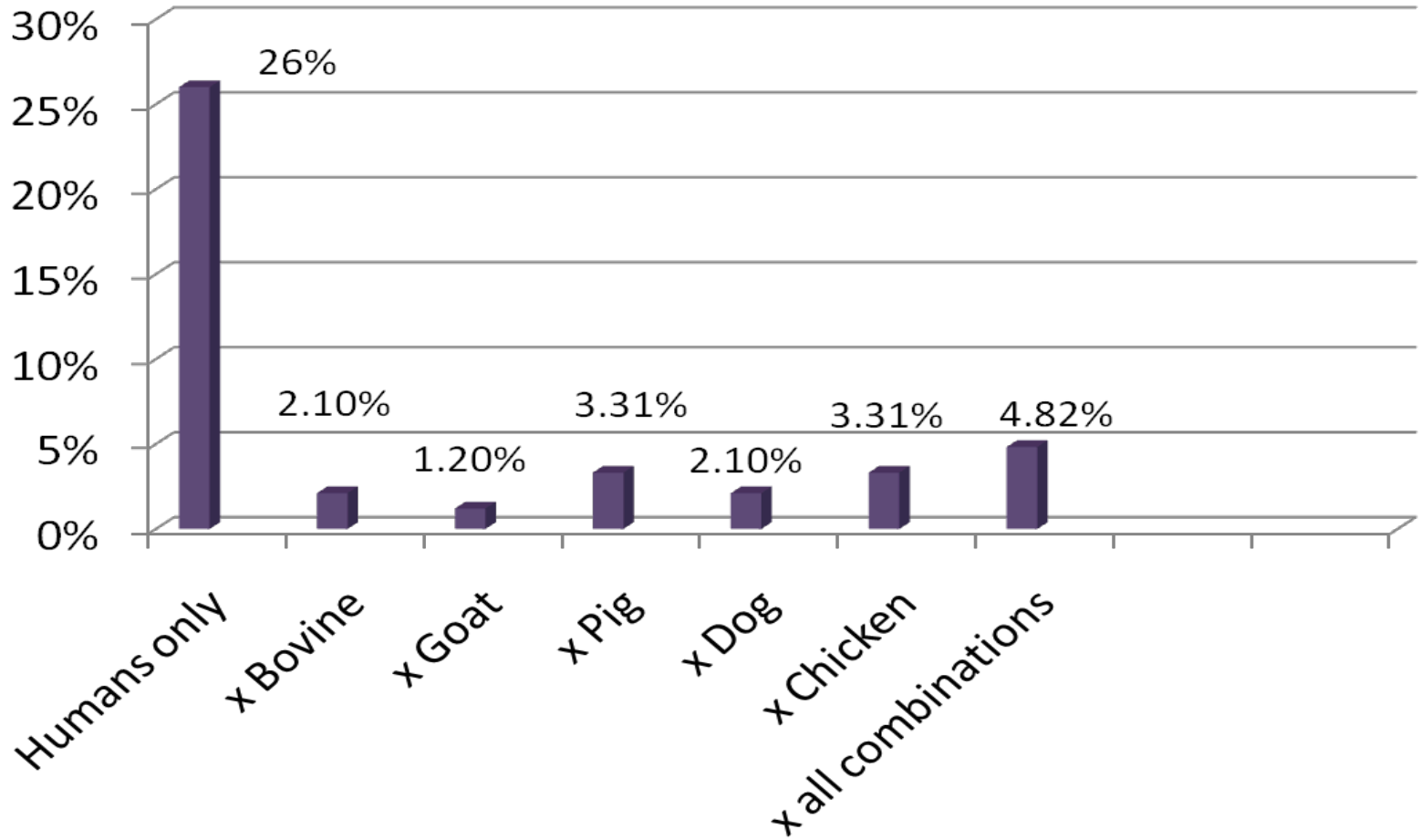
[1] superscript –examined by microscopy

[2] superscript – examined by PCR

Distribution of *Cryptosporidium* spp. Infected Farms with Pure Cases of Humans and Livestock (n=256 infected farms)

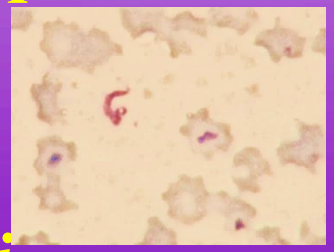


Percentage of Farms with *Cryptosporidium spp.*-Infected Humans and Their Animal Combination (n=142 infected farms)

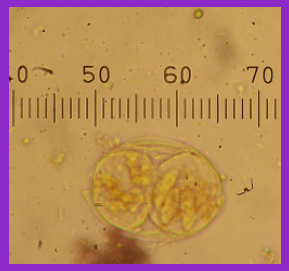


Other findings

- **The study affirmed the presence of *Plasmodium falciparum* infection and not *Babesia spp.***
- ***T. evansi* = cattle and buffaloes *(+);* animal handlers (-)**

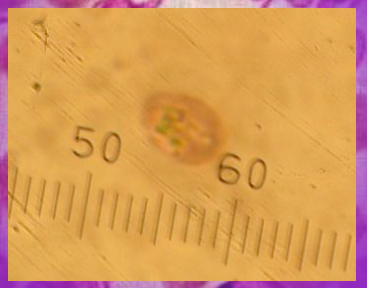


- **30% prevalence of *Toxoplasma gondii* among cats;**



animal handlers (-)

- **6.49% prevalence of *Sarcocystis spp.* infection among dogs; animal handlers (-)**



RISK FACTORS IN ANIMAL HANDLERS

Risk Factors with NO significant association with the transmission of the different zoonotic protozoa

A. Educational Attainment
B. Hand Washing Practices
C. Farm Activities *(pen cleaning, animal bathing, etc)*
D. Ingestion of Raw or Uncooked Meat
E. Manner of Excreta Disposal
F. Manner of Garbage Disposal
G. Sanitary Quality and Use of Toilet

Risk Factors with significant association with the transmission of the different zoonotic protozoa

- Characteristics of Drinking Water

  Source
- Herd Size

Prevalence of zoonotic protozoa infection among animal handlers by source of drinking water.

Source of Drinking Water	No. of Infected Animal Handlers	No. of Uninfected Animal Handlers	TOTAL	Prevalence
Public system	3	27	30	10%
Shallow Well	1	3	4	25%
Artesian Well	**103**	**351**	**454**	**22.69**
Spring, River, Creek	**53**	**148**	**201**	**26.37%**
TOTAL	157	521	678	23.15%

PREVALENCE OF ZOONOTIC PROTOZOA AMONG LIVESTOCK

General prevalence of zoonotic protozoa infection among livestock

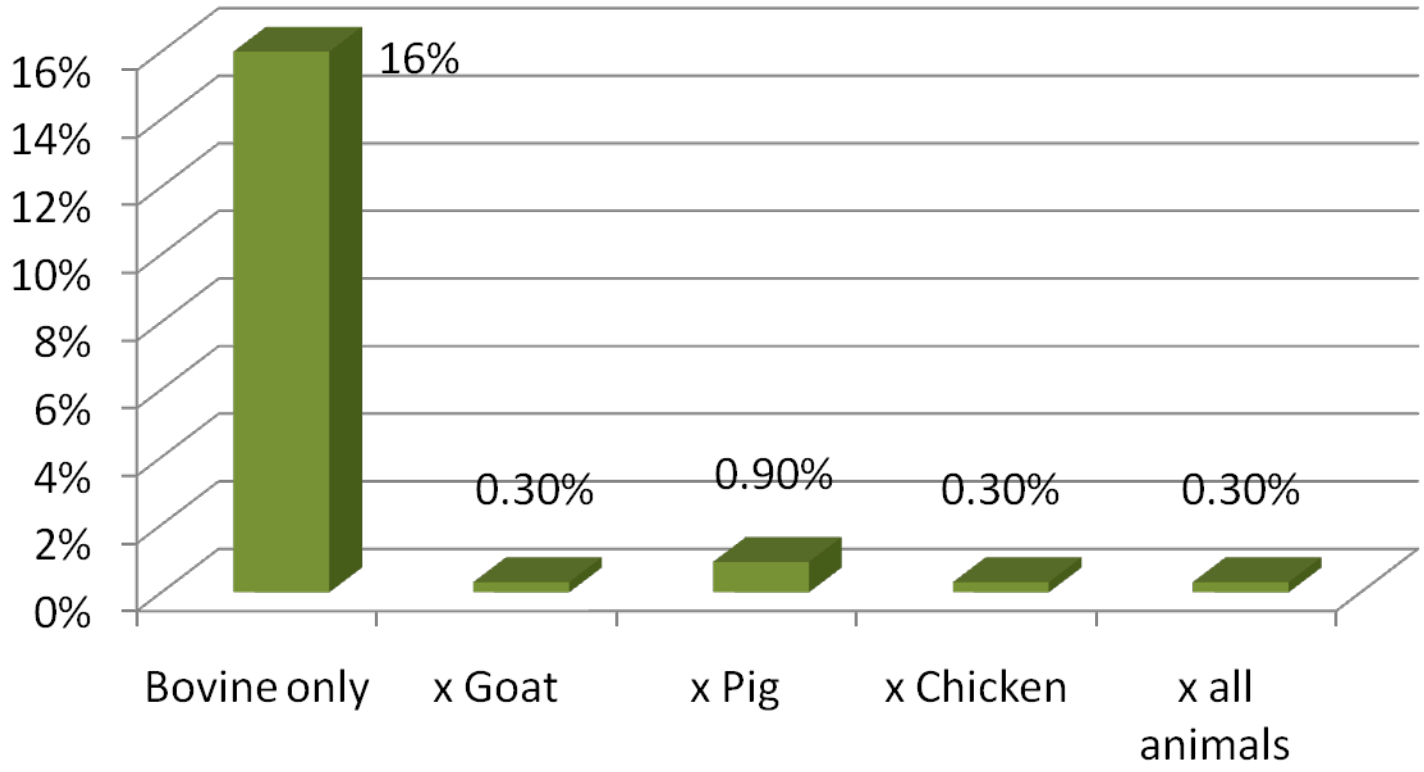
ANIMAL	INFECTED	NOT INFECTED	TOTAL	PREVALENCE %
Cattle	68	144	212	32.1
Buffalo	57	174	231	24.7
Goat	34	134	168	20.2
Pig	115	268	383	30.0
Dog	25	129	154	16.2
Chicken	130	206	336	38.7
Cat	15	38	53	28.3

Table 2. Prevalence of zoonotic protozoa by species among farm animals

Zoonotic Protozoa	Cattle	Buffalo	Goat	Pig	Chicken	Pet Dogs	Pet Cats
	Prevalence %	Prevalence %	Prevalence %	Prevalence %	Prevalence %	Prevalence %	Prevalence %
Blastocystis hominis 1	13.7	1.3	4.2	28.7	22.3	1.3.0	n.a.
Cryptosporidium spp.1	26.9	21.2	17.9	29.8	21.1	9.7	n.a.
Trypanosoma evansi 2	3.3	3.9	ϕ	ϕ	n.a.	ϕ	n.a.
Sarcocystis spp.1	ϕ	Φ	ϕ	ϕ	n.a.	6.5	n.a.
Toxoplasma gondii 1	ϕ	Φ	ϕ	ϕ	n.a.	ϕ	30.2

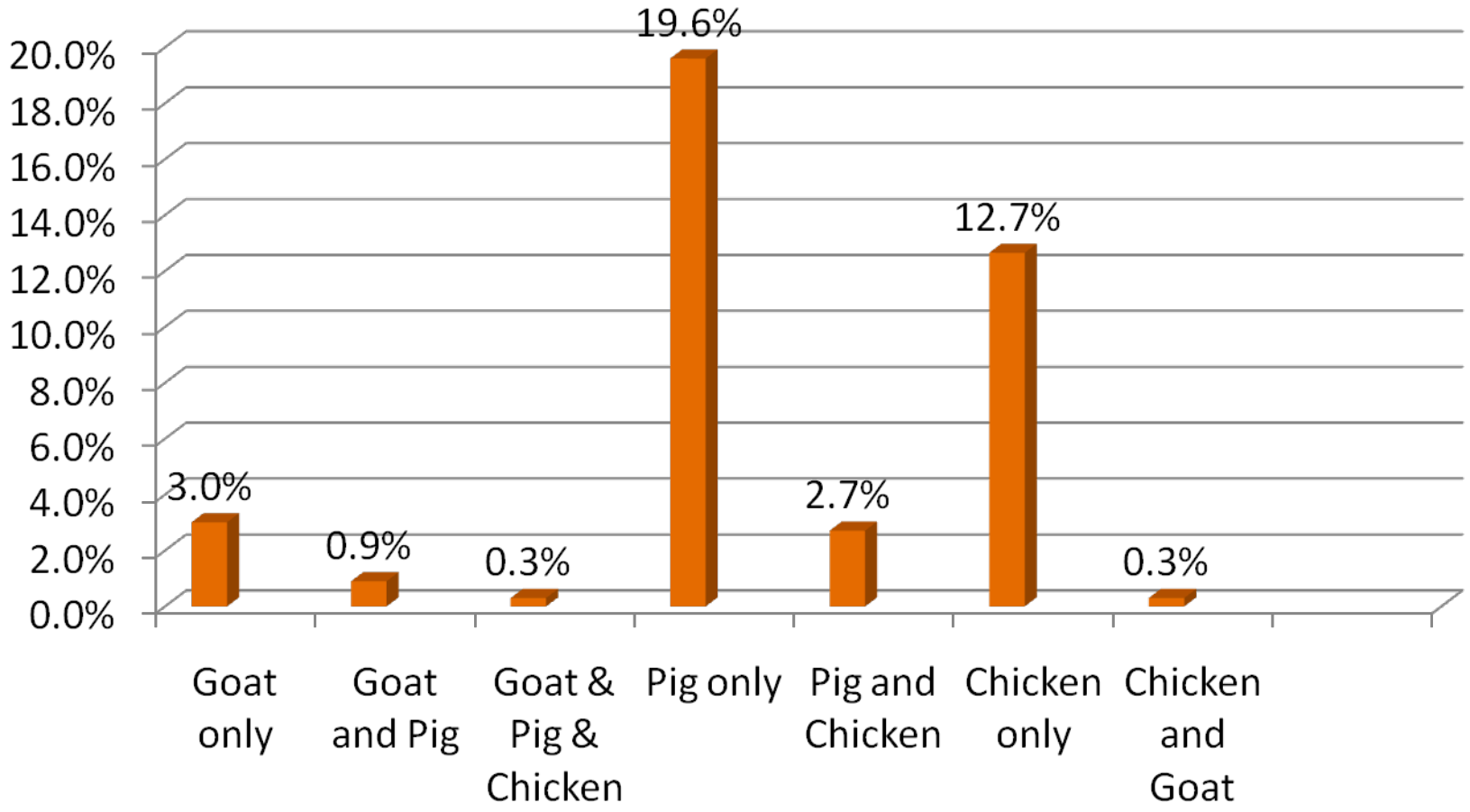
1 - microscopy, 2 – PCR, φ – did not examine, n.a. – not applicable

Percentage of Farms with *Cryptosporidium spp.*-Infected Bovine and Other Animal Combinations (n=332 infected farms)

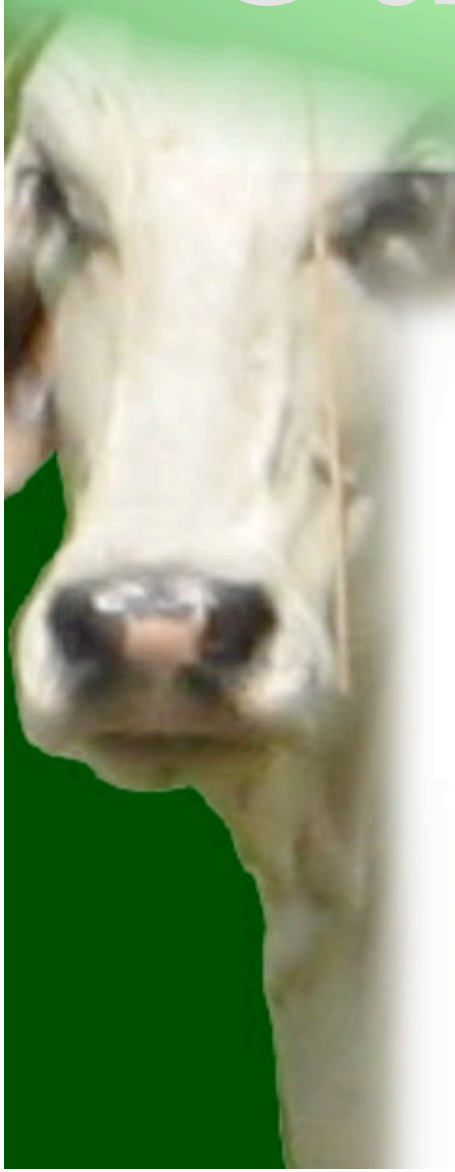


Percentage of Farms with *Cryptosporidium spp.*- Infected Goat, Pig, Chicken and Their Combinations

(n= 332 infected farms)

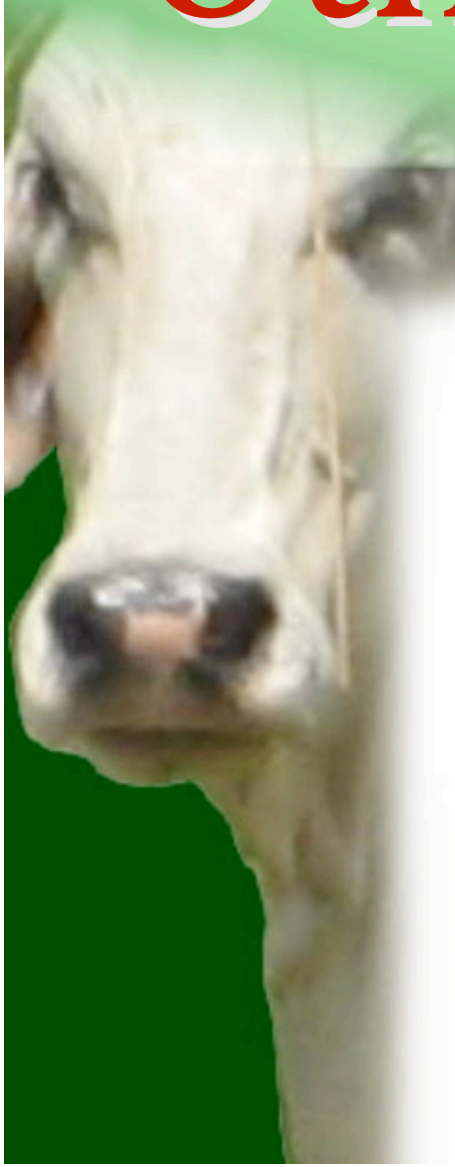


Other findings



- **The pig had the highest prevalence for both *Blastocystis hominis* and *Cryptosporidium* spp**
- **The dog had the lowest prevalence for both protozoa.**
- **Both cattle and buffaloes were infected with *Trypanosoma evansi***
- **Buffaloes having a higher prevalence *T. evansi* than cattle.**
- **PCR detected the pathogenic from the nonpathogenic hemoflagellates (T. theileri)**

Other findings

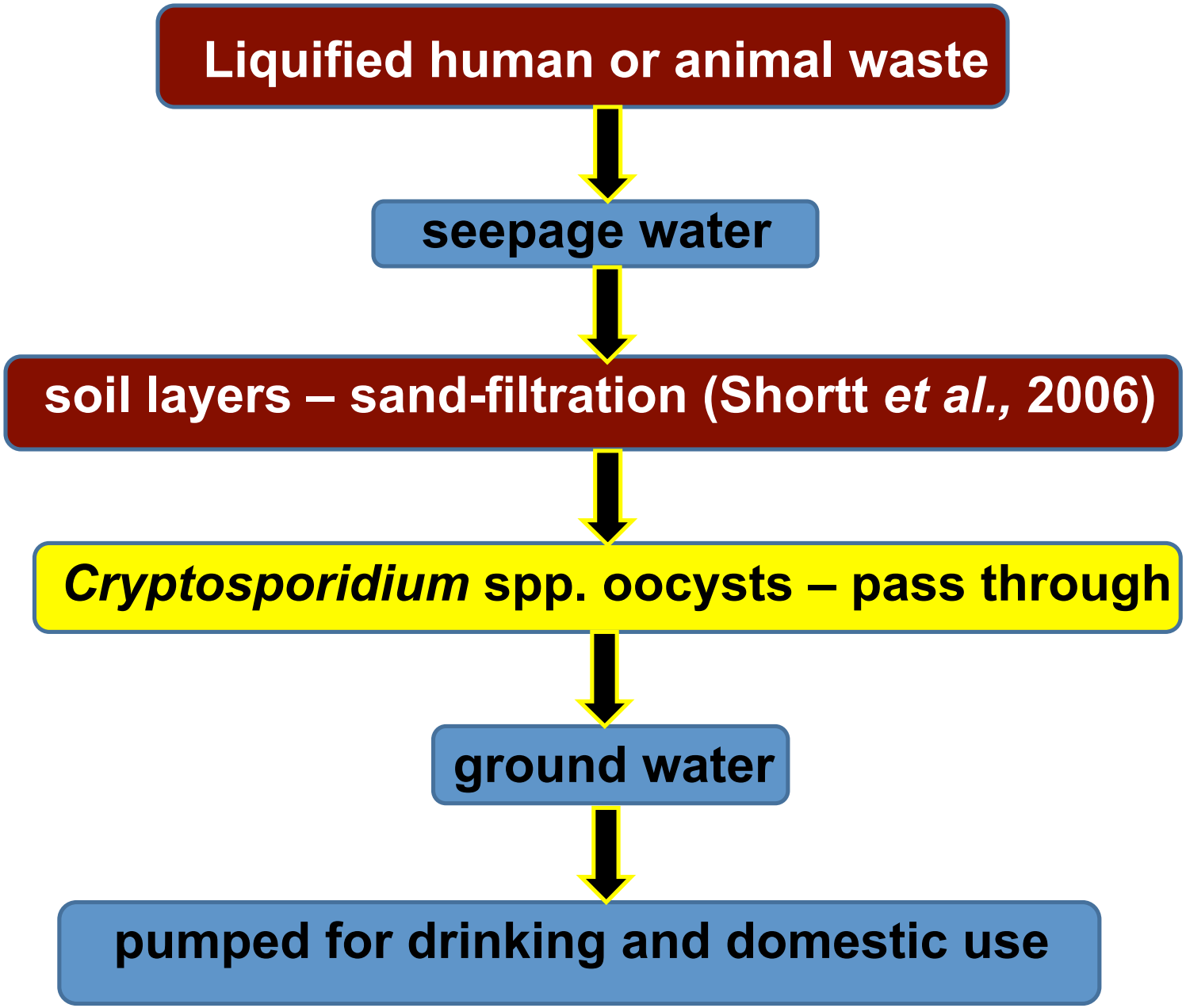


- *Babesia* species were not present in 100 rats, cattle and buffaloes
- *Sarcocystis* spp. oocysts in fecal samples of dogs 10/154 (6.49%) but blood DNA samples of dog owners were negative by PCR
- *Sarcocystis* spp. oocysts in cats- 4/107 (3.74%)
- *Toxoplasma gondii* oocysts in cats- 37/107 (30%) but blood DNA samples of cat owners were negative by PCR

RISK FACTORS IN LIVESTOCK

Among Animal Handlers

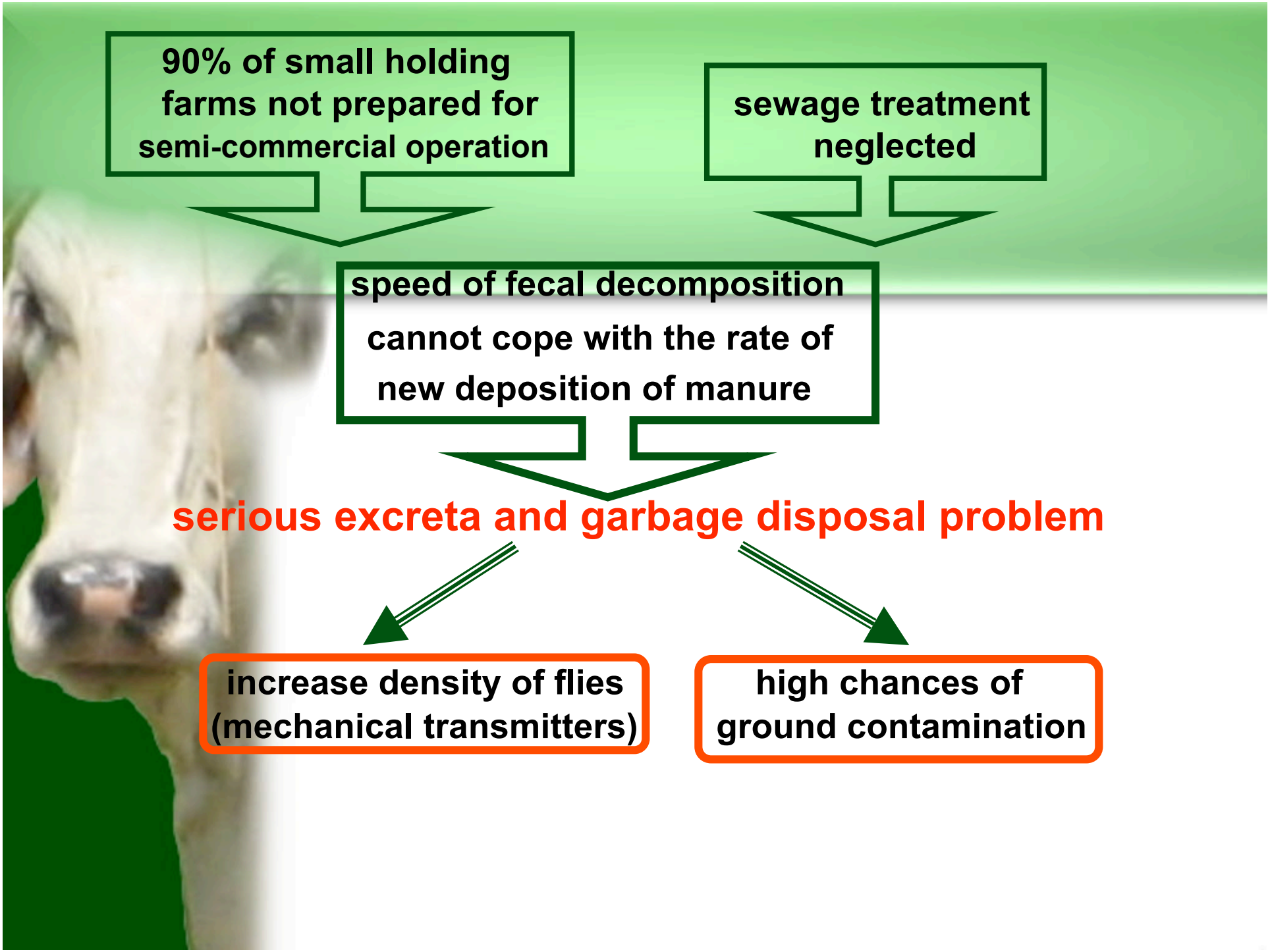
Risk Variable	Odds Ratio	P Value
Characteristic of water source[1]		
a) Good source* but with excreta in the vicinity	1.50	0.052
*public system or protected artesian well		
b) Poor source** and with excreta in the vicinity	2.0	0.014
**shallow well or direct from creeks, rivers, springs		
[1]presence of excreta in the vicinity of drinking water		


Liquified human or animal waste
seepage water
soil layers – sand-filtration (Shortt *et al.*, 2006)
Cryptosporidium spp. oocysts – pass through
ground water
pumped for drinking and domestic use

Among Animal Handlers

Risk Variable	Odds Ratio	P Value
Herd Size		
- Moderate to maximum number[1] of animals in the herd	2.0	0.010
cattle and swamp buffaloes 3 or 4 dams & 2 calves or heifers; goats > 5 to 20 does & 10 to 20 growers; pigs > 3 to 5 sows & > 10 to 20 growers; dogs > 3; chickens > 50 to 100		

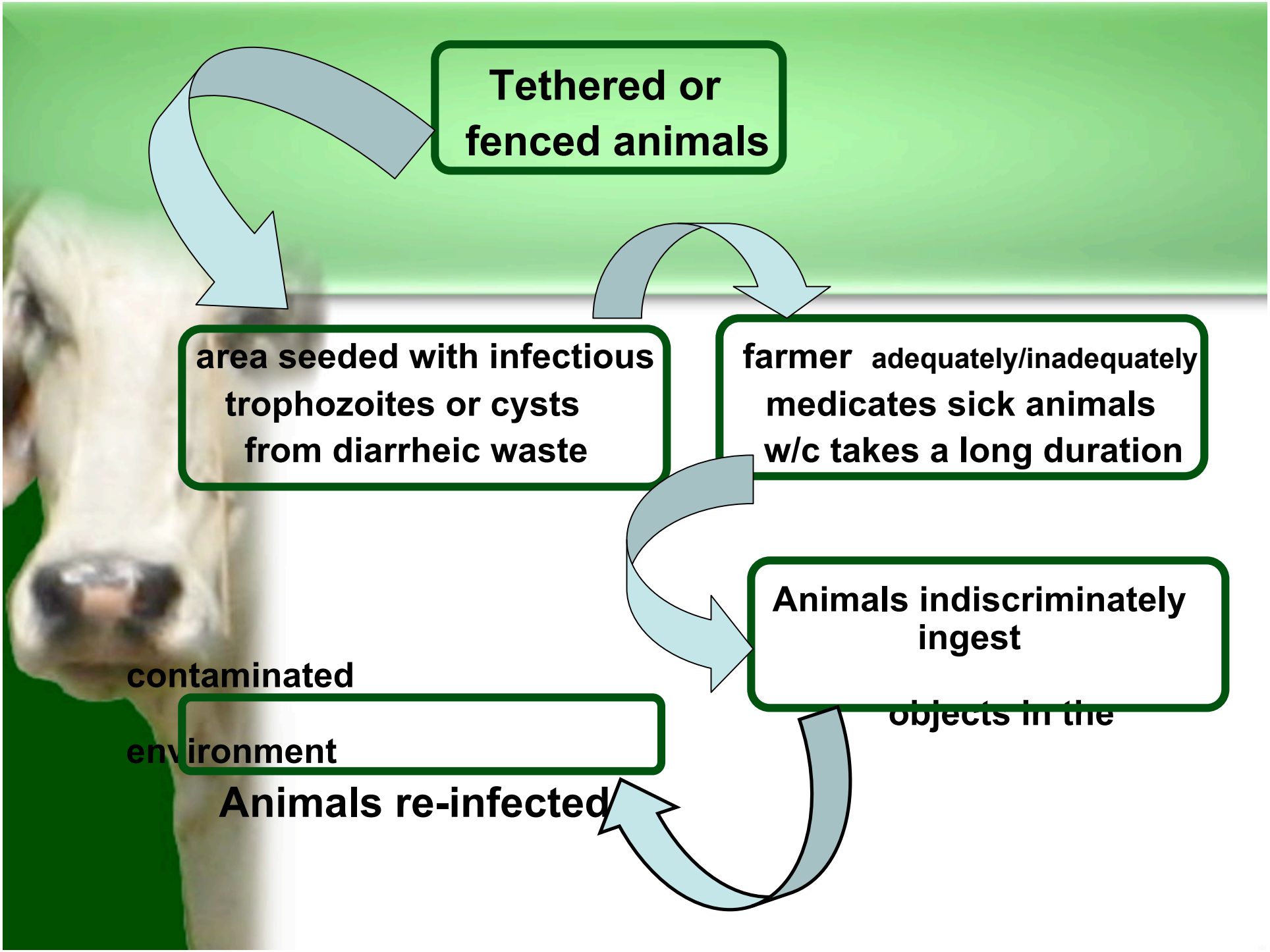
[1]more than minimum standard for a small holding farm


90% of small holding farms not prepared for semi-commercial operation
sewage treatment neglected
speed of fecal decomposition cannot cope with the rate of new deposition of manure
serious excreta and garbage disposal problem
increase density of flies (mechanical transmitters)
high chances of ground contamination

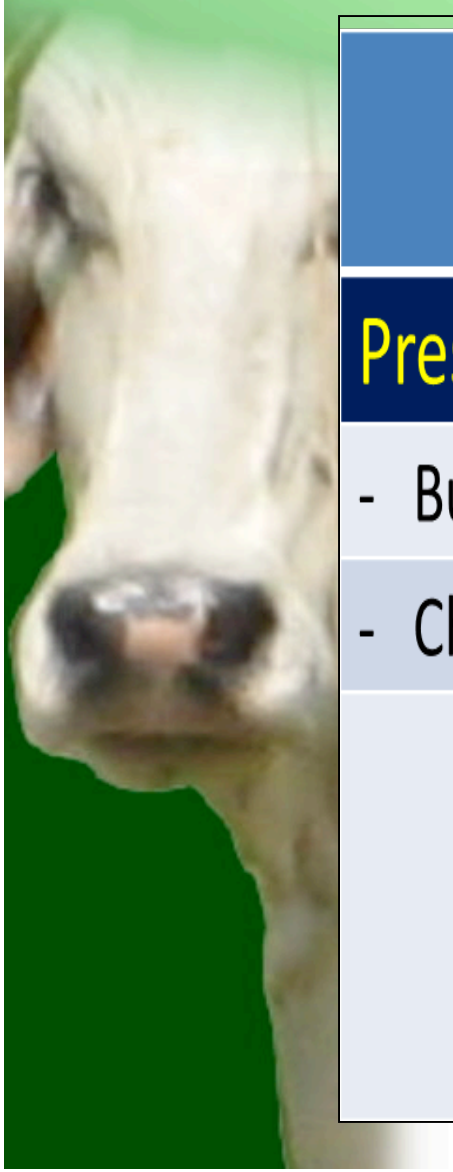
Among livestock

Risk Variable	Odds Ratio	P value
Diarrhea for the past year and manner of medication*		
- Cattle	6.43	0.001
- Goat	2.0	0.049

* proper medication (farmer consulted a credible animal health provider; farmer used either single or combination of antibacterials, anti-protozoals, adsorbents; followed strictly dosage and duration of administration)
* improper medication (farmer did not consult any one; self-prescribed; consulted an animal health provider but failed to follow dosage and duration of the medication)

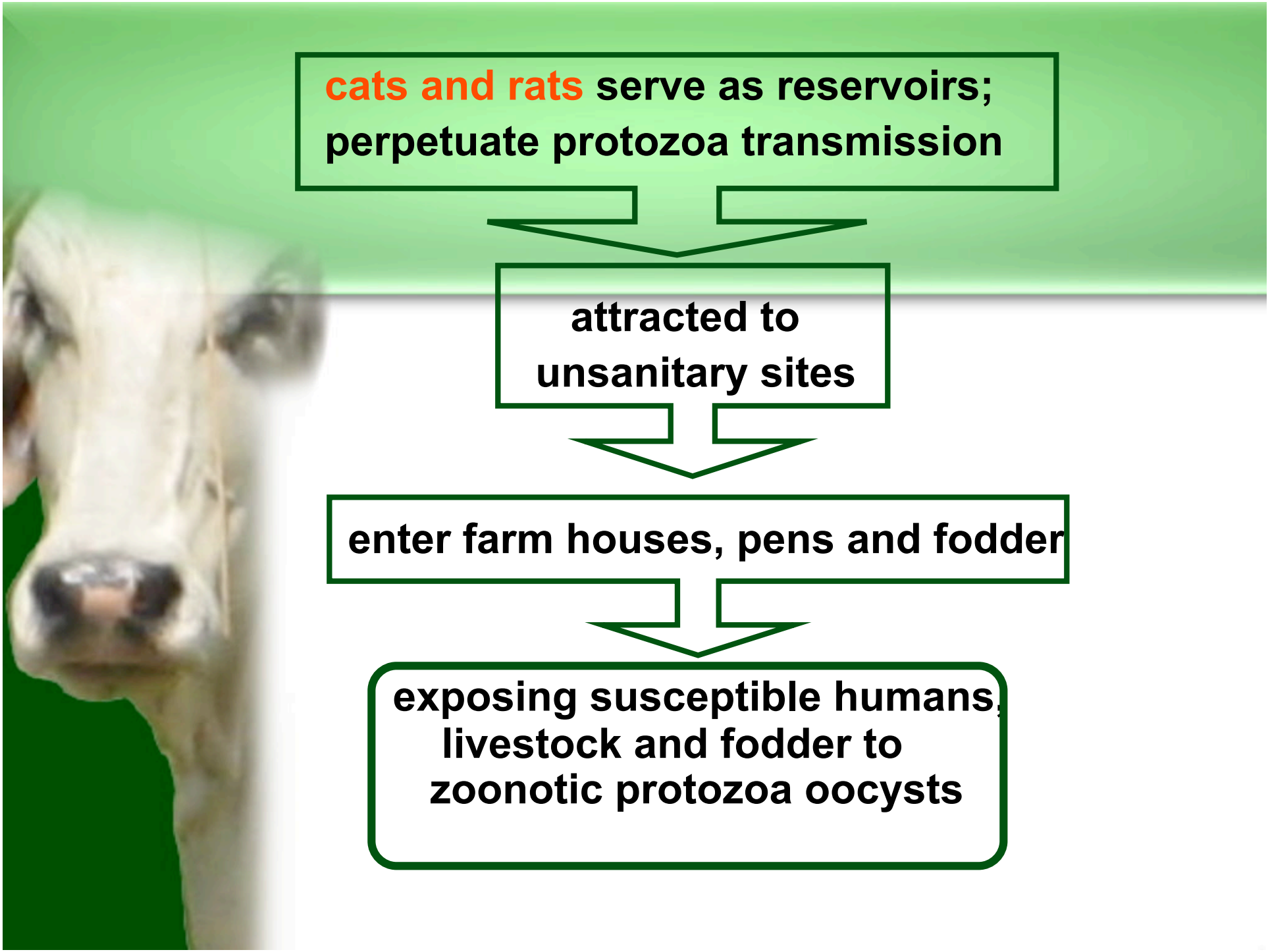

Tethered or fenced animals
area seeded with infectious trophozoites or cysts from diarrheic waste
farmer adequately/inadequately medicates sick animals w/c takes a long duration
Animals indiscriminately ingest
objects in the
contaminated
environment
Animals re-infected

Among livestock



Risk Variable	Odds Ratio	P value
Presence of animal scavengers*		
- Buffalo	2.70	0.005
- Chickens	2.90	0.001

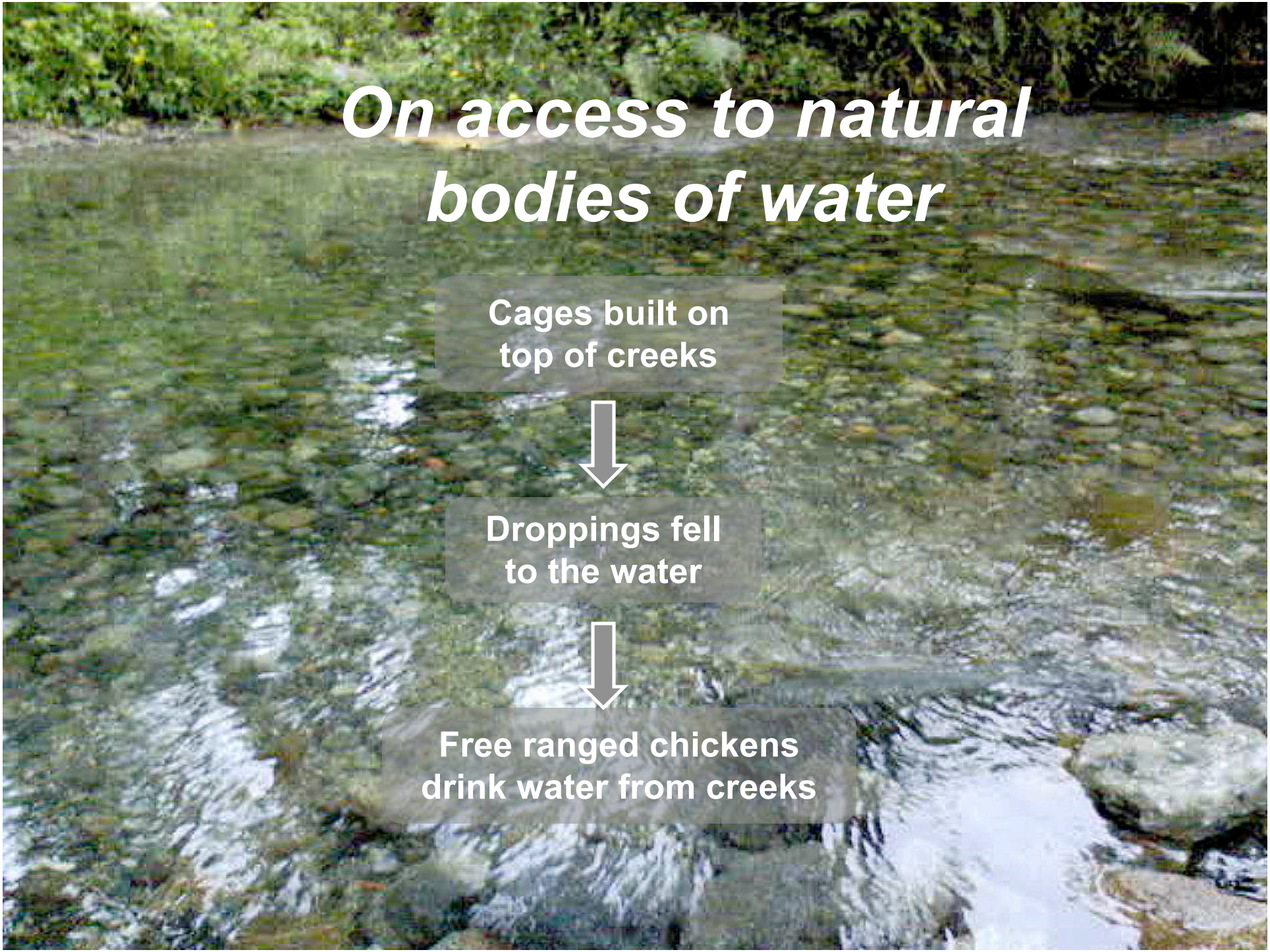
*one or more stray cats reported within the vicinity of the animals or animal fodder or both; one or more rodents reported within the vicinity of the animals or animal fodder or both; both cats and rodents reported within the vicinity of the animals or animal fodder or both


cats and rats serve as reservoirs; perpetuate protozoa transmission
attracted to unsanitary sites
enter farm houses, pens and fodder
exposing susceptible humans, livestock and fodder to zoonotic protozoa oocysts

Among livestock

Risk Variable	Odds Ratio	P value
Access to natural bodies of water*		
- Chickens	3.35	0.01

*allowing farm animals access to creeks, rivers, springs, irrigation canals for the past year in order to do any of the following: bath, drink, wallow, defecate

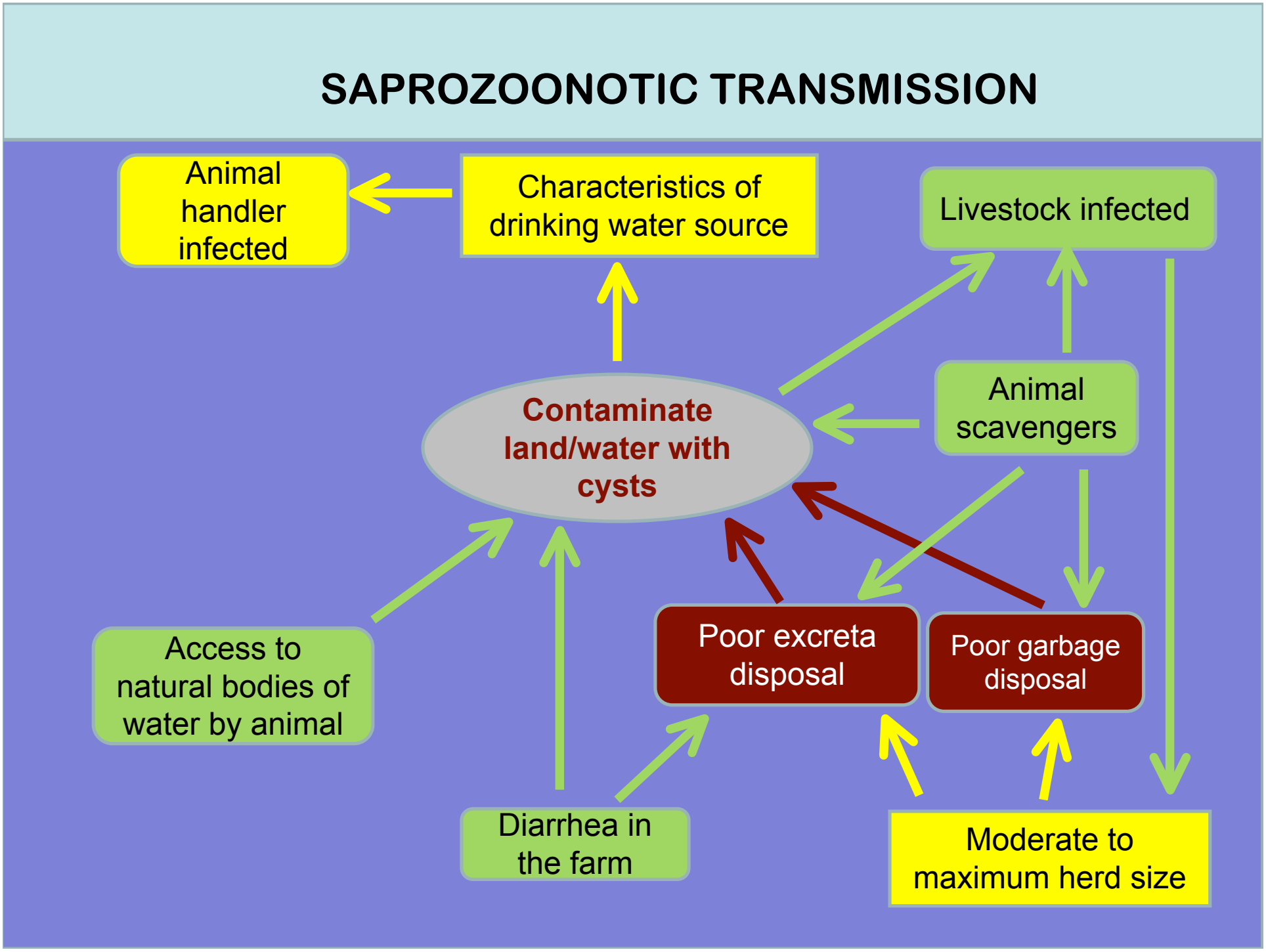

On access to natural bodies of water
Cages built on top of creeks
Droppings fell to the water
Free ranged chickens drink water from creeks

No Risk Factors Associated

Animal Type	Remarks
Pig	▪ Majority not enclosed in pens
	▪ Common, tied to a log
	▪ Ranged freely
Dog	▪ Not restrained within the yard or cage
	▪ Ranged freely

No risk factors associated with infection among pigs and dogs because they were exposed to all kinds of contaminated objects

SAPROZOONOTIC TRANSMISSION



CONCLUSION AND RECOMMENDATION

The study revealed that among the zoonotic protozoa affecting the small holder livestock farmers in Aurora province,

Cryptosporidium parvum had the highest prevalence (20.98% in males and 21.64% in females).

All animal species such as cattle, buffalo, goat, pig, dog, and chicken were infected with *Cryptosporidium spp. and Blastocystis hominis.*

Risk factors significantly associated with the transmission of zoonotic protozoa among animal handlers included characteristics of drinking water source and herd size.

Risk factors associated with the transmission among animals

- **Diarrhea and medication**

  - *cattle and goats*

- **Presence of animal scavengers**

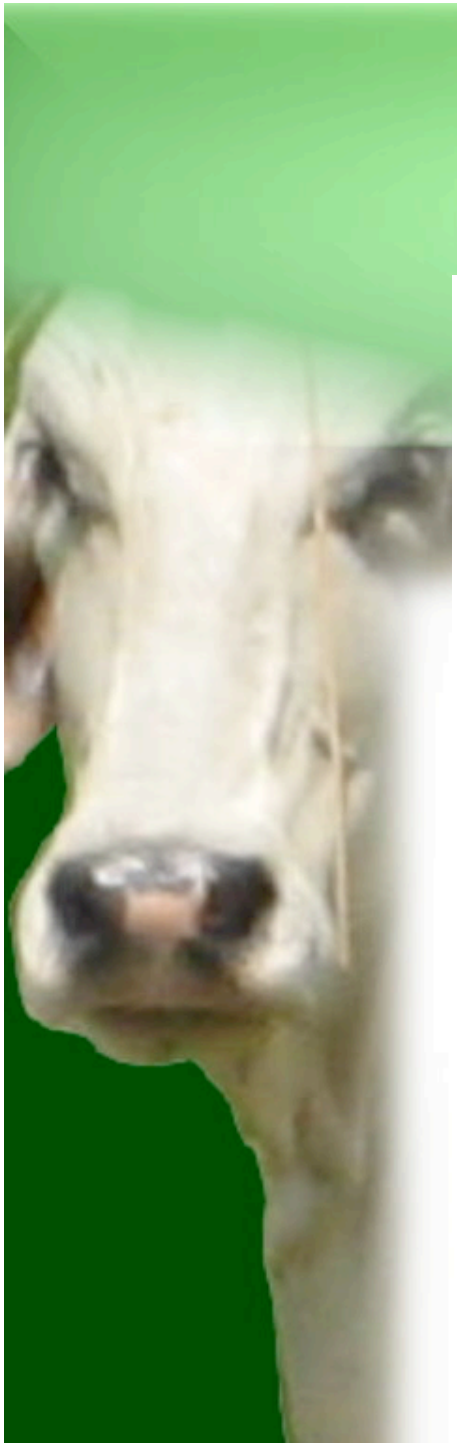
  - *buffaloes and chickens*

- **Access to natural bodies of water**

  - *chickens*

The study recommends the following:

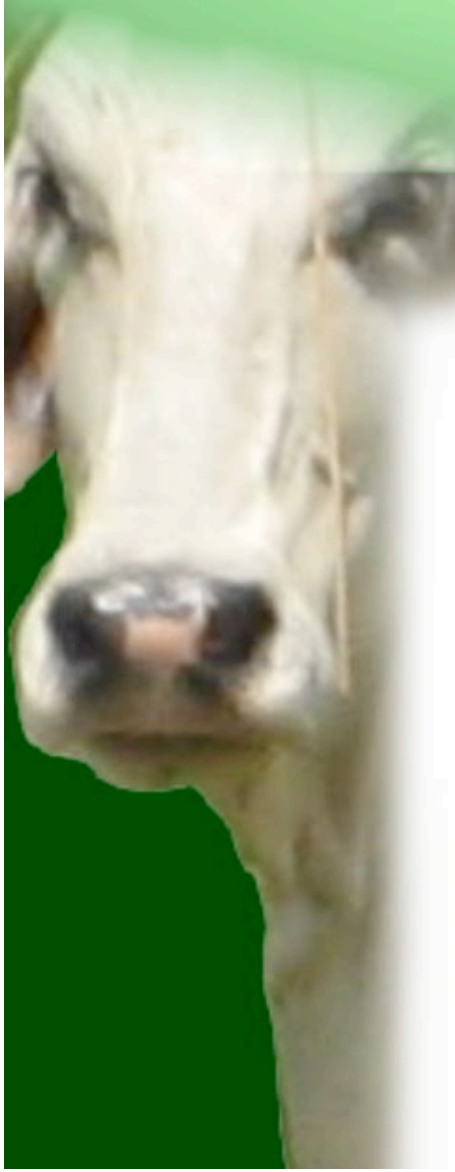
1. promote safe potable drinking water by separating drinking water sources from sewage-contaminated water bodies and protecting water sheds from animal waste contamination through fencing;
2. identification and surveillance of suspected contaminated drinking water sources and natural bodies of water for waterborne zoonotic protozoa cysts;



3. involvement of the local health workers in **educating the farmers** on the harmful consequence of **casual use of unsafe water**;
4. prioritize inclusion of farmers with **large herd sizes** in **government campaign** against zoonotic protozoa infection; and
5. health education on the danger of allowing **dogs and cats access to fodder stores** of farm animals and **defecating in pens** of livestock;

6. promote **environmental sanitation and waste management** to discourage scavengers in the area where the farm animals are located and to prevent contamination of soil and water with oocysts excreted by infected humans and animals.

To prevent transmission from raw milk ingestion:



1. promote hygienic collection of milk from cows, caracows and caprine does by maintaining the animal's udders clean and free from soil or dirt contamination by the milker's hands; and,
2. health education on the protective aspect of drinking pasteurized milk and the danger of drinking raw cow, buffalo or goat milk which could be contaminated by zoonotic enteric oocysts due to poor udder hygiene.

TO GOD BE THE HIGHEST PRAISE

Acknowledgement:

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- Drs. P.T.Rivera, O.Saniel, S. Eduardo, N.Gloriani, L. Leonardo
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- Lakas-Angkan Ministry, Inc.