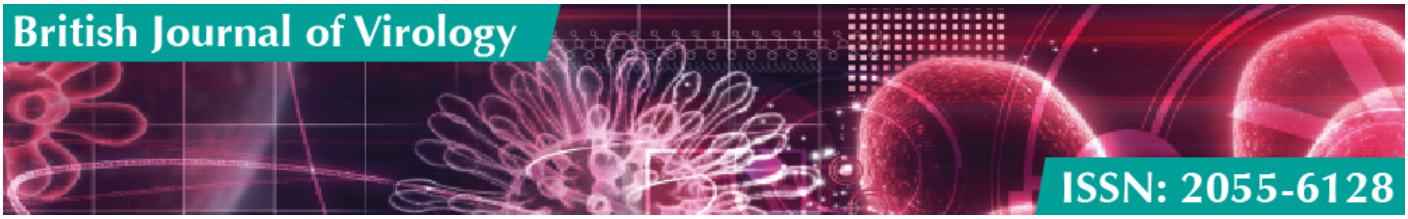

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Clinical, pathological and molecular investigation of Peste des petits ruminants virus infection in goats from Turkana County in Kenya

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Abstract

Peste des petits ruminants, an economically important morbillivirus infection of sheep and goats is widely distributed in Middle East, Central and South Asia, China and Africa. Whereas the PPR antibodies were first reported in Kenyan small stock as early as 1995, the clinical disease was to be observed later in 2006 following dramatic and devastating PPR outbreaks in Turkana County. This study was carried out to detect PPRV and to describe the clinical and pathological presentations in a suspected PPR outbreak in flock of goats in Turkana County in 2011. Clinical and postmortem observations were made on suspect cases and pathological samples collected for histology and RT-PCR assay. Clinical and postmortem signs were typical for of PPR while histological investigations were suggestive of PPRV infection. Major clinical signs were depression, diarrhea, difficult breathing, muco-purulent ocular-nasal discharges with matted eye lids and encrusted nostrils and finally death. Gross lesions included inflammation of apical lung lobe revealing hepatization. Mesenteric lymph nodes were enlarged while colon mucosa was hyperemic. Peste des petits ruminants virus genome was detected by real-time reverse transcription–polymerase chain reaction (qRT-PCR) from the 15 samples out of 18 samples collected from three goats thus confirming the presence of PPRV in Kenya.

Keywords: Peste des petits ruminants virus; Clinical signs; gross pathology; histology; RT-PCR; Turkana; Kenya

Introduction

Peste des petit ruminants (PPR) is a highly contagious, infectious and often fatal disease of sheep, goats and wild small ruminants. The disease occurs in Middle East, Central and South Asia, China and Africa (Barnyard et al. 2010; Munir et al. 2013). Clinically PPR is characterized by the sudden onset of depression, fever, discharges from the eyes and nose, sores in the mouth, disturbed breathing and cough, foul-smelling diarrhea and death within 10 to 12 days post infection (Diallo et al. 2007). Major pathological features are erosive, and ulcerative stomatitis; necrotic tonsillitis; fibrino-haemorrhagic enteritis; and broncho-interstitial pneumonia (Bundza et al. 1988; Troung et al. 2014). PPRV infection has characteristic histopathologic findings that are syncytial cells in affected oral mucosa and lungs, as well as eosinophilic nuclear and cytoplasmic inclusion bodies, especially in the respiratory and alimentary tract epithelia (Kul et al. 2007)

The disease is caused by Peste des petit ruminants virus (PPRV) classified under genus Morbillivirus (Gibbs et al. 1979) in the family Paramyxoviridae. The PPRV genome encodes six structural proteins (nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (H), and polymerase (L)), and two nonstructural proteins (C and V), which are in the order of 3′-N-P/C/V-M-F-H-L-5′ on the genome (Bailey et al. 2005; Hu et al. 2012)

Peste des petit ruminants was first suspected in Kenya in 1992 (FAO, 2008) with further serological reports being made by Wamwayi et al. (1995). Clinical PPR disease in Kenya was first reported in Turkana County of Kenya to (*Office International des Epizooties* (OIE)) in January 2007 (OIE, 2014). Disease assessments carried out in mid-2007 established that the PPR had managed to spread beyond Turkana County into the other arid and semi-arid pastoral Counties in Northern and southern parts of Kenya (FAO, 2009; Kihu et al. 2012).

Despite the spread of PPR in Kenya being acknowledged in various unpublished technical reports, there is no published report on confirmation of PPR virus in Kenya. This study reports on clinical and pathological observations as well as molecular detection of a PPR virus from outbreak in a goat flock in Kakuma division of Turkana County in Kenya.

.**Material and methods**

The study was carried out in Kakuma administrative division of Turkana County in a particular village herd named Lotakaa GPS position (N 03° 38 390; S 034° 50 987). The study respondents demonstrated observing several suspect cases of PPR *(Lomoo,* local name of Peste des petits ruminants) which were presented to study team for clinical observation. A history narrated by the respondents showed that there were 21 kids, 24 adult goats, one sheep that had died from the suspect PPR infection two weeks prior to study visit. Laboratory samples were collected from carcasses of goats suspected to have died of PPR while on observation. The samples collected were lung tissues, mesenteric and mediastinal lymph nodes; and colon and were divided into two separate sets of sample bottles. The first set of samples was prepared for histopathology analysis and had formalin 10% added to each tissue after collection. The second set of tissue sample was transported under ice in cold boxes. All collected samples were transported to University of Nairobi, Department of Veterinary Pathology,

Microbiology and Parasitology laboratory and samples under cold chain stored in refrigerated repository at -30°C while samples in formalin stored at room temperature.

This field study was conducted in manner to ensure quality and integrity of the research. Consent was sought from Directorate of Veterinary Services who granted the permission for collection of field laboratory samples on Peste des petit ruminants vide letter referenced "Ref.Meat/Vol.XIV/42" dated 1st July 2011. Consent was also sought from Turkana herders for voluntary presentation of their small stock for collection of samples which they granted and facilitated the exercise.

Histopathology slides preparation and staining

The formalin fixed tissues for histopathology were prepared into slides for histological examination through the standard paraffin process that dehydrates, clears and infiltrates the tissue with paraffin wax. Embedding was done to allow orientation of the specimen in a block so that it could be easily handled, sectioned and stored. Sectioning was done using a microtome to produce very 5 µm thin sections that were dewaxed, rehydrated and placed on a microscope slide ready for staining. The samples were stained with Hematoxylin and eosin stain (Luna, 1968).Tissue slides were then examined under light microscope.

Viral ribonucleic acid (RNA) isolation from frozen tissues

A total of 18 frozen sample tissues were each separately sliced using a sterile scalpel blade to a required size of 30 mg on a frozen sterile Petri dish. The sliced frozen sample tissue was placed in a clean DNase-RNase free mortar and pestle which was maintained cool in liquid nitrogen. Some liquid nitrogen was poured into the mortar that had the tissues and the tissue disrupted by grinding it with a pestle until it was a very course powder, and further ground into fine powder. The powder was collected into a pre-cooled tube, labeled and stored at -20°C. The process was repeated until all samples were processed. The extraction of RNA was carried out as outlined by RNeasy® mini kit protocol supplied by QIAGEN® (2010). The extracted RNA was collected in microtubes and stored at -20°C a waiting qRT-PCR assay to be carried out.

Real time reverse transcription polymerase chain reaction assay

A total of 18 RNA samples were analyzed using a specific qRT-PCR assay using a TaqVet™ Peste des Petits Ruminants Virus kit which had a set of primers/probes designed from the N-terminus of the N-gene in the Mix PPRV based on a protocol detailed in a report by *Laboratoire Service International* (LSI) (2011), supplier of the assay kit. The forward primer matched positions 483 > 508 in the (5-AGAGTTCAATATGTTRTTAGCCTCCAT-3); the TaqMan® probe positions 551 > 576 (FAM-5-CACCGGAYACKGCAGCTGACTCAGAA-3- MGB) and the reverse primer was located at positions 603 < 624 (5-TTCCCCARTCACTCTYCTTTGT-3) (Batten et al. 2011). The kit also had a set of nucleotides for internal positive control (IPC) forward and reverse primers and IPC probe TaqMan® labeled in VIC-MGB. The external positive control (EPC) in the kit was ready to use solution constituted of extracted PPRV Nucleic Acid labeled EPC PPRV. DNase/RNase free water, extracted as a sample, was used as a negative control sample labeled (NCS). A negative result for the target "PPRV" indicated that no contamination occurred during sample extraction and amplification process. PPRV mix was used as a negative control labeled (NC). A negative result indicated that no contamination occurred during the preparation..

A MicroAmp® Optical 96-well reaction plate with an adhesive cover was prepared noting that the qRT-PCR was to be performed in each one of the wells in the plate. The mix PPRV was briefly vortexed and centrifuged; then 20 µl of the Mix PPRV was added into each well of the Optical 96-well plate used for the assay. In each well of the plate used for the assay, 5 µl of the sample (RNA) or NCS or NC or EPC PPRV was added. The plate was covered with an adhesive cover and put in Abiprism® 7500 (Applied Biosystems) thermocycler. The qRT-PCR run had three steps.

Step 1 : 45°C – 10 min – Repeat : 1

Step 2 : 95°C – 10 min – Repeat : 1

Step 3 : 95°C – 15 s ; 60°C – 1 min – Repeats : 45

The qRT PCR results were interpreted taking into account the threshold level which was the point of reading and analysis in real-time PCR. A signal that was detected above the threshold was considered a real signal that can be used to define the threshold cycle (Ct) for a sample; thus Ct was the fractional PCR cycle number at which the reporter fluorescence (Rn) was greater than the threshold. This outcome was represented graphically with ΔRn values plotted against the cycle number; ΔRn being the increment of fluorescent signal at each point in time. Ct was thus plotted as the point of intersection between the sample amplification curve and the threshold line. Two graphs were produced: a log plot and a linear plot. Samples with Ct less than 45 with PPRV detector were considered to have presence of the virus genome of Peste des petits ruminants while samples with Ct greater than 45 were considered to be absent of the virus genome of Peste des petits ruminants.

Results

Clinical signs manifestations

The clinical signs were observed from kids of four to six months old. The clinical signs presented included: depression (Figure 1), diarrhea (Figure 2), emaciation, difficult breathing, elevated body temperatures of 41 °C, serous and muco-purulent nasal discharges, and encrusted peri-nasal areas (Figure 3). Serous and muco-purulent ocular discharges matting the eye lids at the inner eye canthus and the hair below the eye was also observed (Figure 3). Death due to this infection was observed in three kids and they were all necropsied for examination.

Gross pathological lesions

All necropsied goats showed pneumonic lesions in the lungs. Inflammation of the apical lung lobe revealed red hepatized and congested area (Figure 4). The large intestines showed moderate to severe hemorrhagic enteritis characterized by hemorrhagic intestinal mucosa (Figure 5). Intestinal blood vessels were congested with blood and the mesenteric lymph nodes were enlarged and swollen (Figures 6).

Histopathological lesions

Histopathology slides of large intestines, lungs, mediastinal and mesenteric lymph nodes were prepared and examined. The lesions present in the lungs were characteristic of broncho-interstitial pneumonia (Figure 7). The lesions observed were alveolar collapse, thickening of the alveolar and interlobular septae. The bronchiolar epithelium was thickened and accumulation of exudates within the bronchioles. The blood vessels in the lungs were congested (Figure 7).

Infiltration of the alveoli by mononuclear cells was evident in addition to multinucleated syncitia in the alveolar epithelium (Figure 8). Lesions in the large intestines revealed congestion of blood vessels, edema and infiltration of lamina propria by inflammatory cells (Figure 9)

Two key lesions were observed in the lymph nodes were depletion of the lymphocytes in both mesenteric and mediastinal lymph nodes and congestion of blood vessels in the mesenteric lymph nodes.

Viral RNA detection in frozen tissue

A total of 18 frozen samples were analysed for PPR virus RNA (Table 1). The samples were in duplicates comprising inocula prepared from original frozen tissues samples and the original frozen samples. All original tissue samples tested, gave positive results for the presence of PPR virus RNA except sample labeled 7F. All the innocula samples tested returned positive results for presence of PPR virus RNA except inocula prepared from mediastinal lymph node from goat three (labeled I9) and mesenteric lymph node labaled I4.

The analysis of Ct values for the original frozen tissue samples shows that the cycle thresholds were in range of 20.46 to 29.17 symbolizing presence of large amounts PPR virus RNA in samples (Table 1 and Figures 10 and 11). Inocula samples of the same tissues had cycle threshold of ranges between 28.37 and 40.56, symbolizing reduction in PPR virus RNA due to multiple handling and thawing during the several laboratory experiments they were used in.

Discussion

Peste des petits ruminants clinical disease has been observed in Kenya since 2006 and its occurrence has considered of major significance due to high morbidity and mortality that occasion serious socio-economic loses

to the affected pastoral communities (FAO, 2012). However the clinical PPR disease outbreaks Kenya have not been described except in unpublished administrative reports (Kihu et al. 2012). This report presents a clinical picture of suspected PPR outbreak in a flock of goats in Turkana. The predominant age group affected with PPR was kids of four to six months. The description of the clinical presentation in the effected and observed kids as well as the post mortem finding and histopathological changes were suggestive of PPR infection which was confirmed by detection of PPR virus genome in 17 tissues samples out of 18 samples presented for qRT-PCR assay.

Conclusion

Though there are unpublished reports of molecular confirmation of PPR in Kenya, none of these reports has been published. Therefore these report presents confirmation of PPR virus in clinical cases observed in Turkana County.

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Authors contribution

S. M Kihu., J. O. Oyugi, E. Lutomia and Gitao C.G. collected the data, created the electronic database and cleaned and processed and analyzed the molecular experiments.

S.M. Kihu. D. N. Karanja and L.C. Bebora conceived study analysis methods .

Kihu S.M., drafted the manuscript assisted by Gitao C.G., Bebora L.C., Njenga M. J., and Wairire G.G.

Gitao C.G., Bebora L.C., Njenga M. J., Wairire G.G., Maingi N. and Wahome R.G. raised funding for the study and assisted its coordination. All authors helped with the interpretation of the results and read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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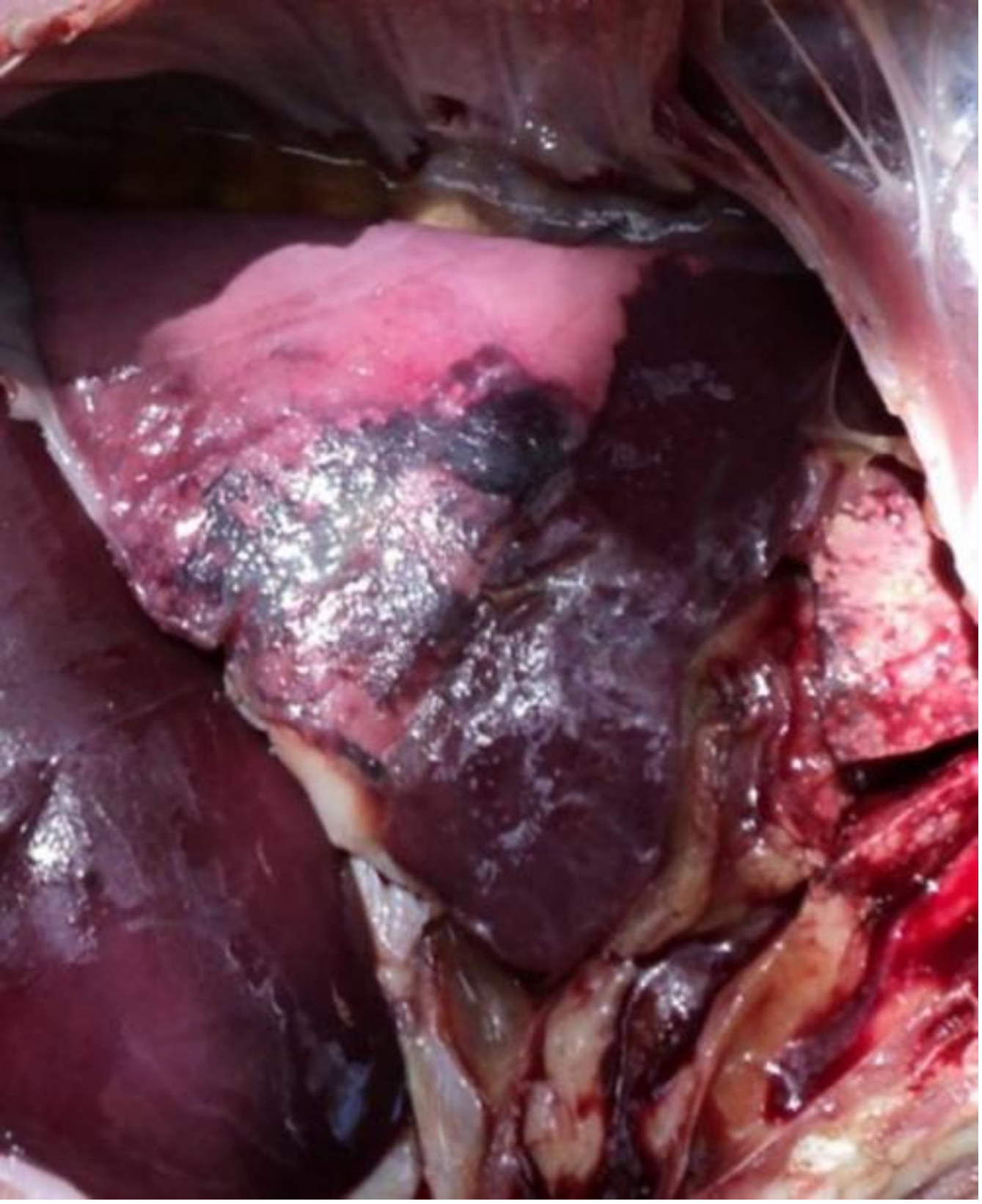
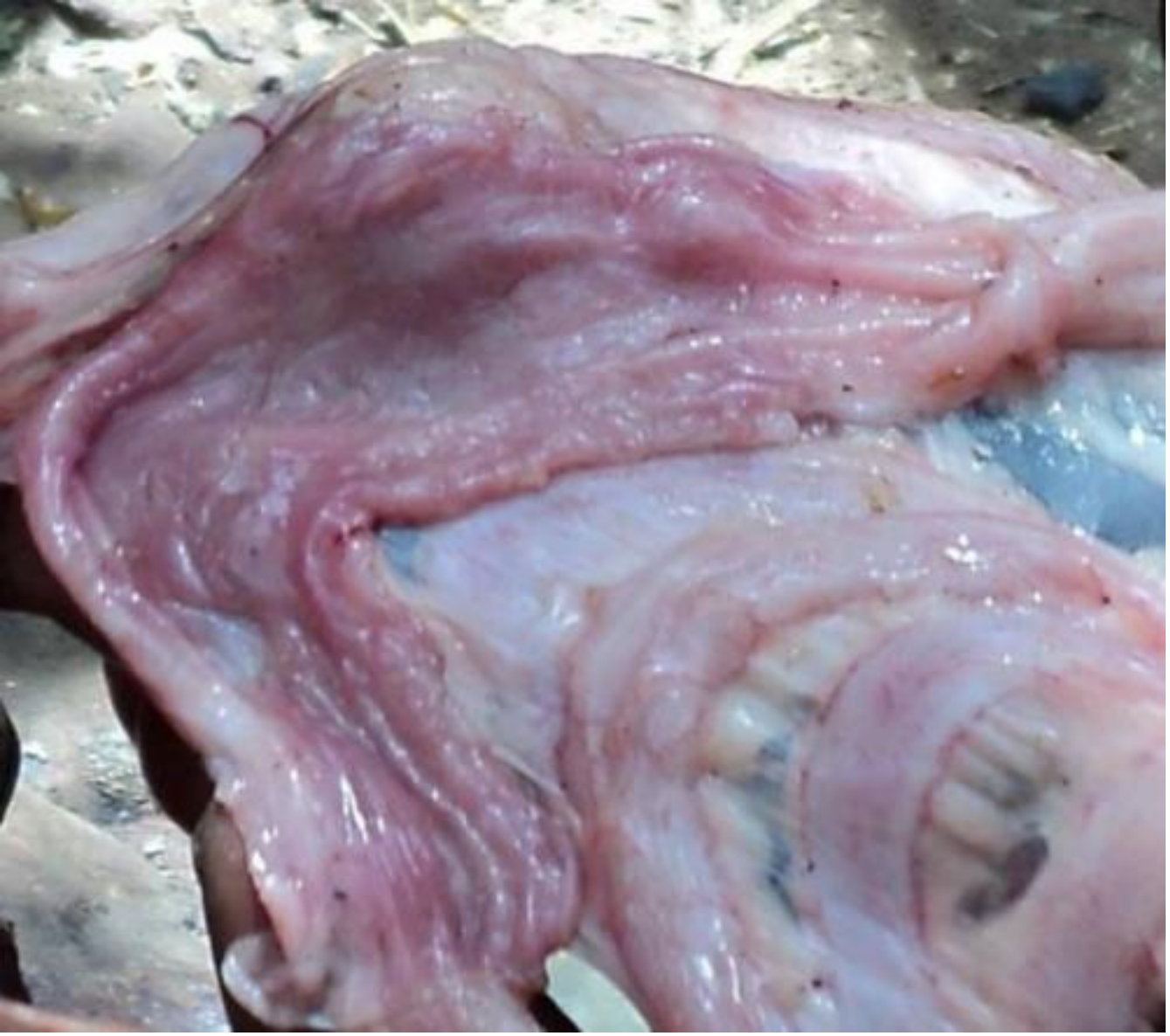
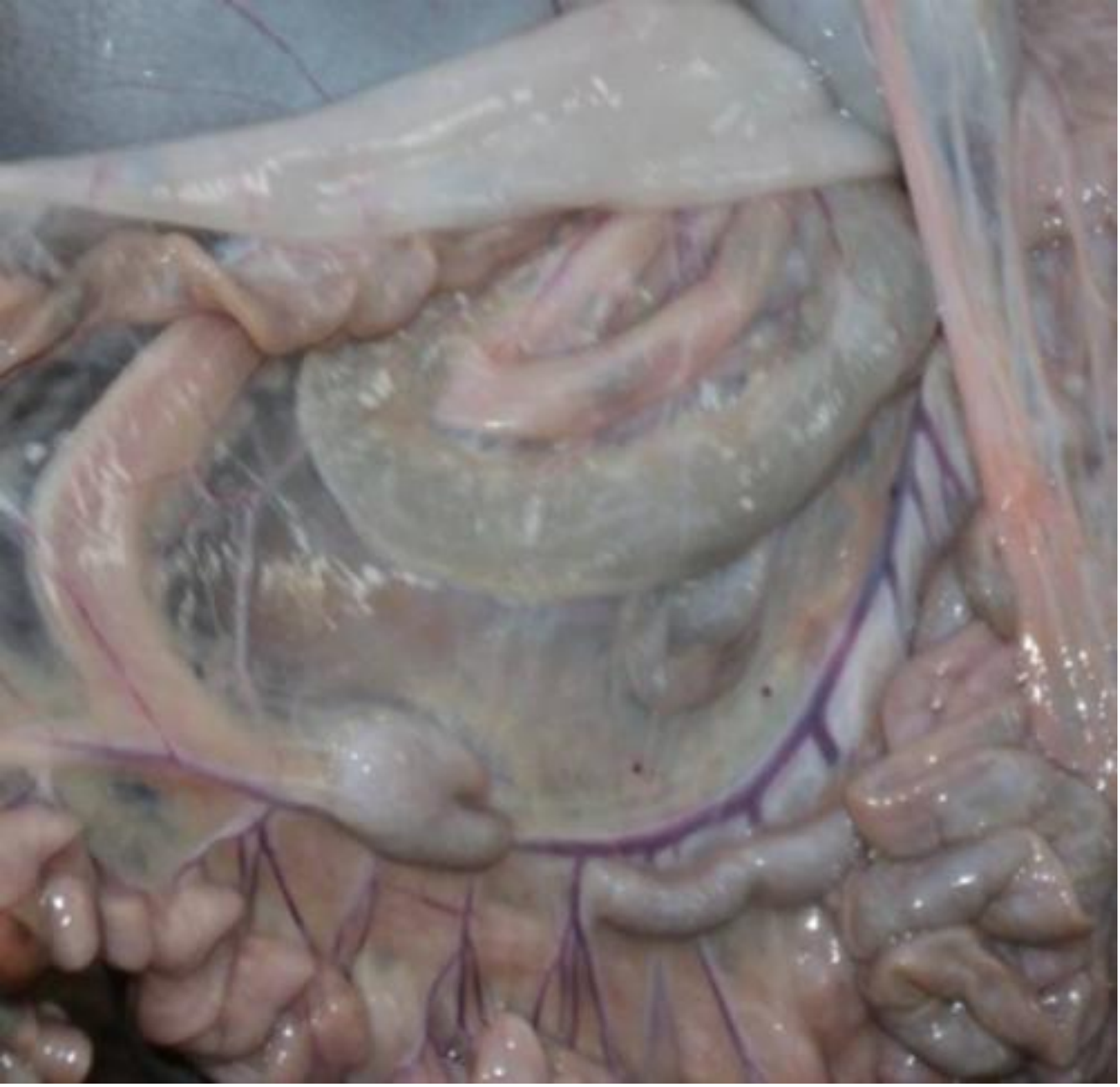
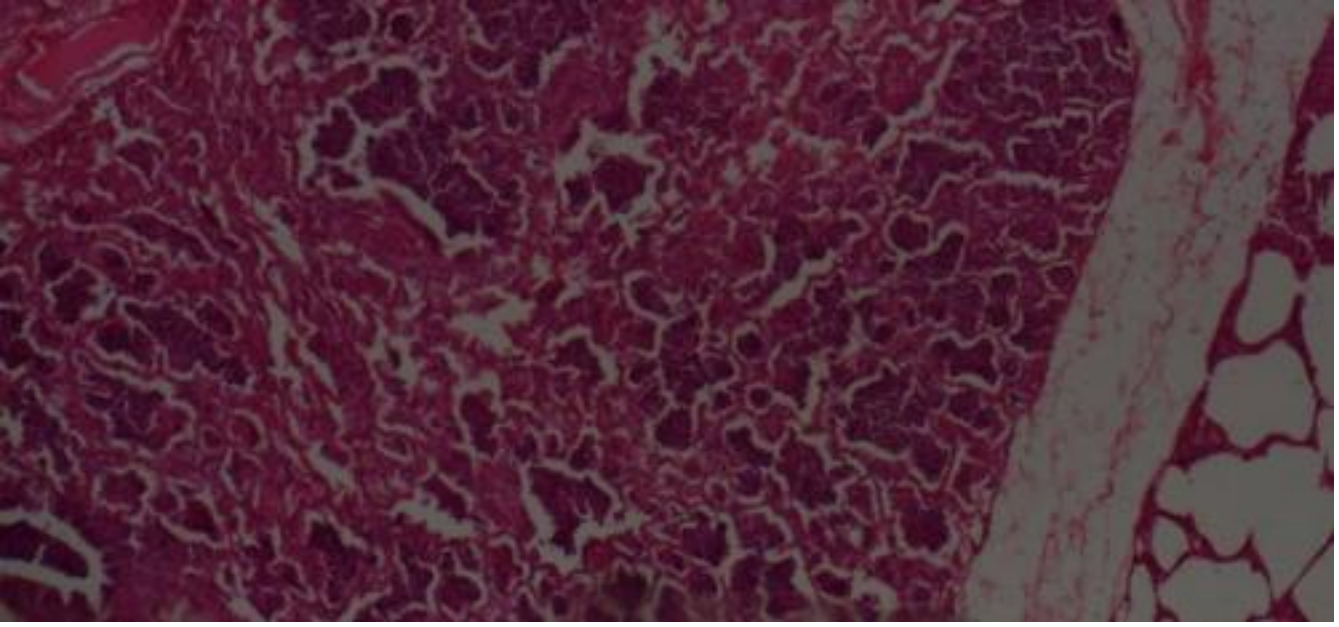
Table 1 Results of qRT-PCR analysis for the frozen tissues

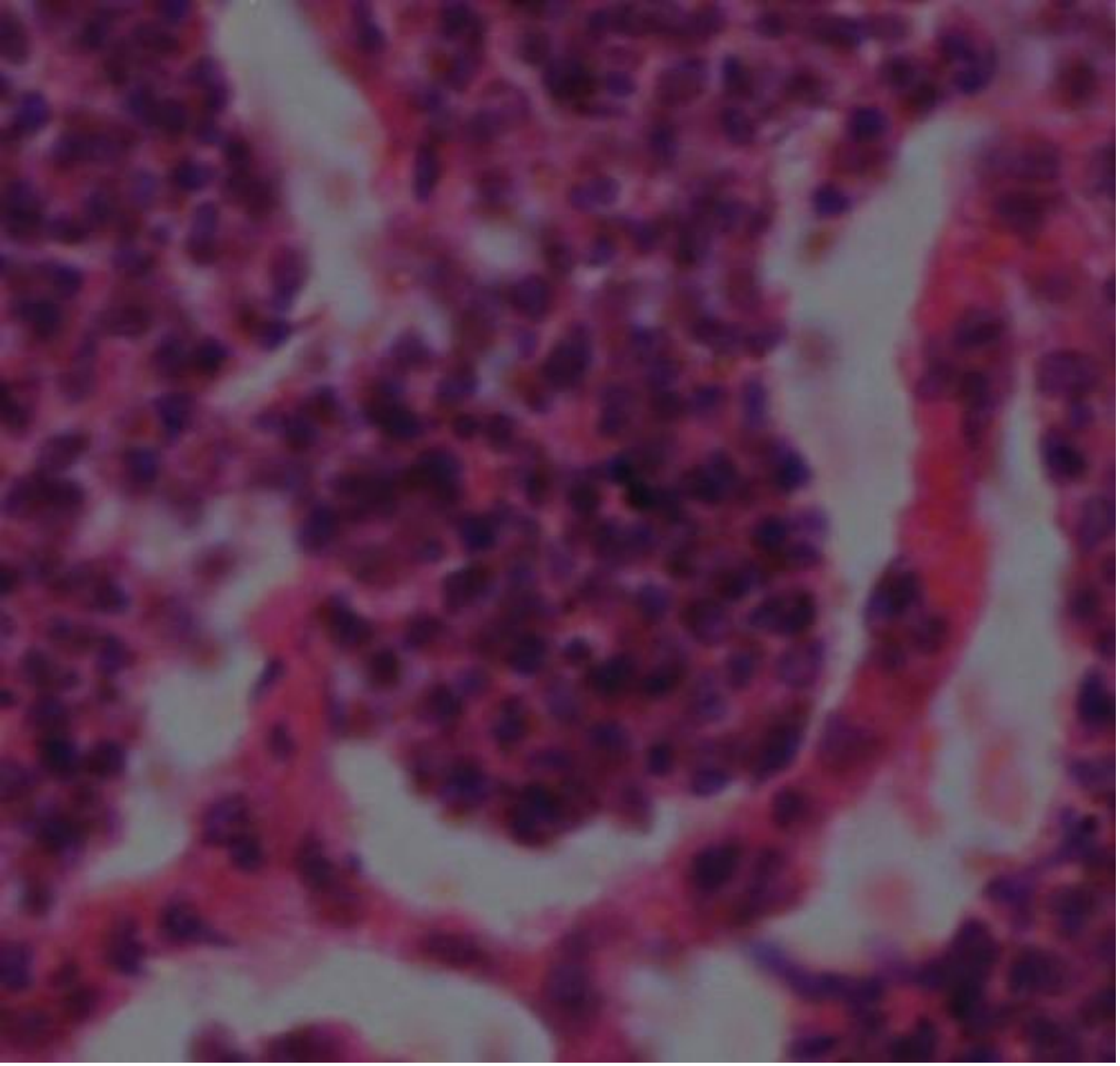
Goat Sampled	Sample Type	Frozen Tissues			
		Tissues Label	Inocula Label	Ct positive samples	Results
Goat 1	Mediastinal lymph node	1F		29.17	Positive
	Lung	2F		26.89	Positive
	Lung		I2	37.95	Positive
	Colon		I3	38.65	Positive
	Mesenteric lymph node	4F		27.04	Positive
	Mesenteric lymph node		I4		Negative
Goat 2	Mediastinal lymph node	5		21.99	Positive
	Mediastinal lymph node		I5	3.28	Positive
	Mesenteric lymph node	6		20.14	Positive
	Mesenteric lymph node		I6	29.29	Positive
	Lung	7F			Negative
	Lung		I7	28.37	Positive
	Colon	8F		20.46	Positive
	Colon		I8	31.28	Positive
Goat 3	Mediastinal lymph node		I9		Negative
	Lung	10F		22.60	Positive
	Lung		I10	40.56	Positive
	Mesenteric lymph node	11F		20.82	Positive

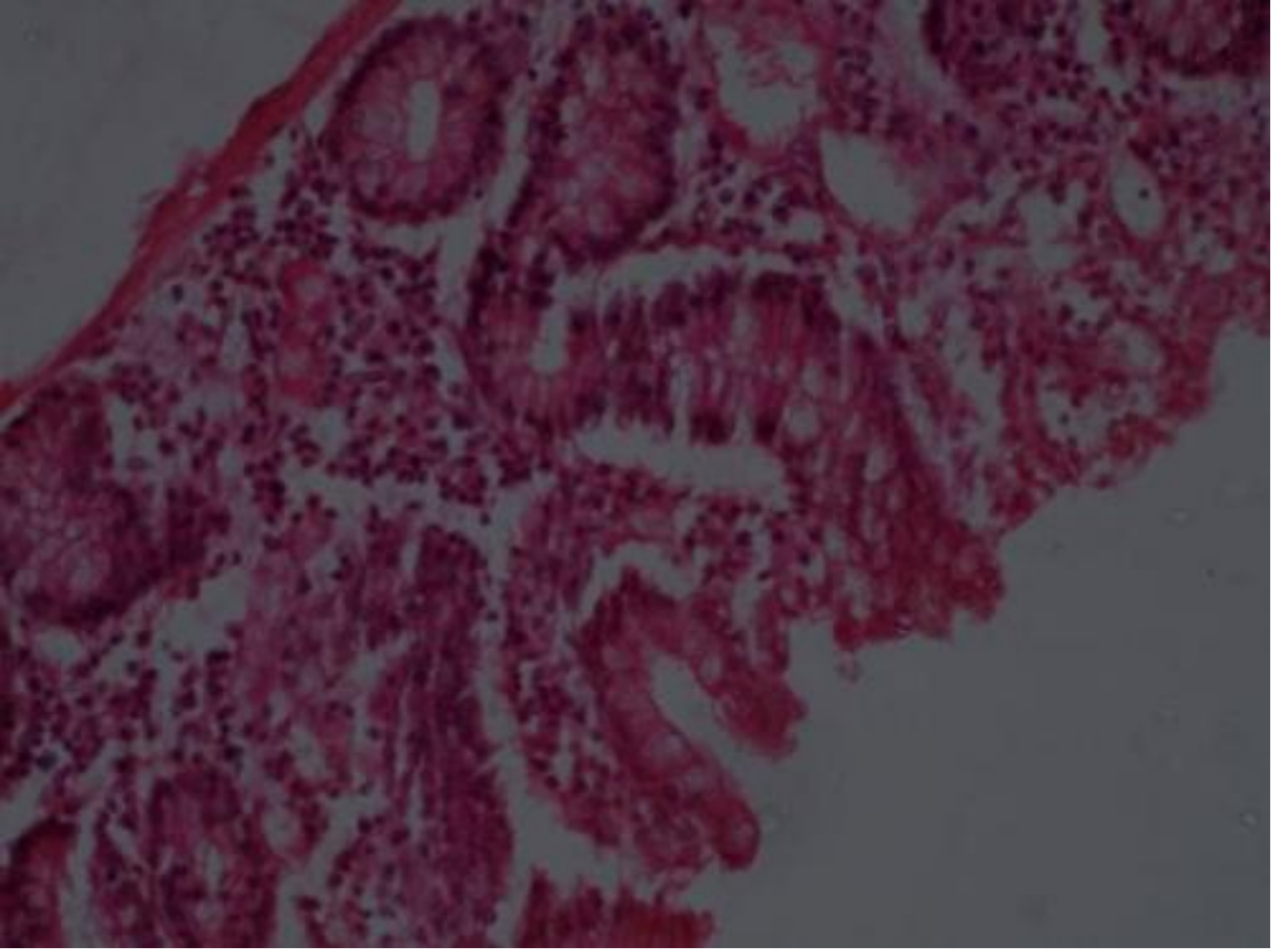


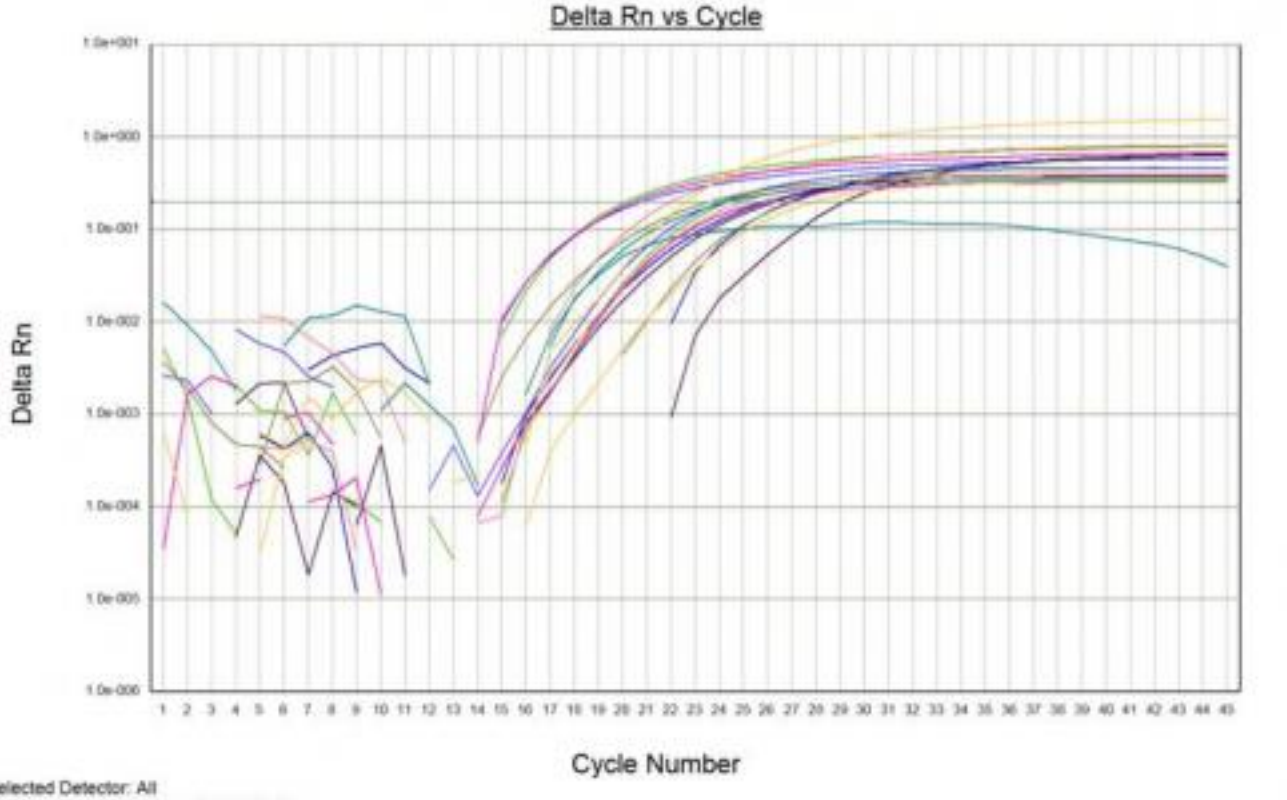












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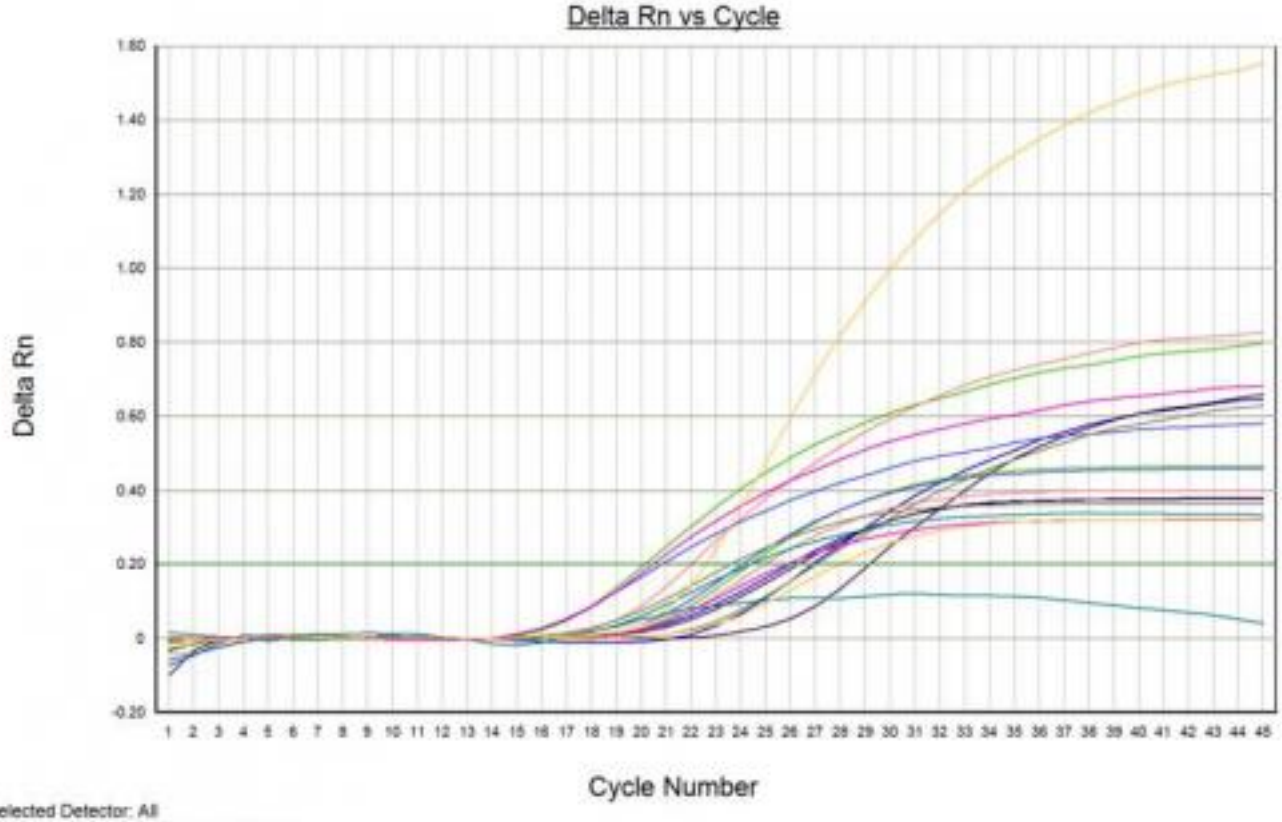

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