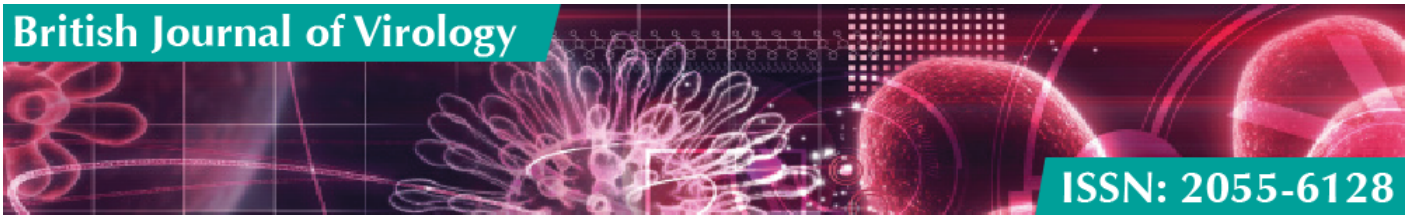

Clinical, pathological and molecular investigation of Peste des petits ruminants virus infection in goats from Turkana County in Kenya

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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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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. An investigation of a suspect PPR disease outbreak was carried in Turkana County in 2011 following which this report describing the clinical. pathological and laboratory finding was made. Major clinical signs observed 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. Histological observations were suggestive of PPRV infection. Peste des petits ruminants virus genome was detected by real-time reverse transcription–polymerase chain reaction (qRT-PCR) from the 17 samples out of 18 samples collected from three goats thus confirming the presence of PPRV in Kenya.

Peste des petit ruminants (PPR) is a highly 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)

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).

This 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 having observed several suspect cases of PPR *(Lomoo,* local name of Peste des petits ruminants) and narrated a history that showed 21 kids, 24 adult goats, one sheep had died from the suspect PPR infection two weeks prior to study visit. Respondents presented cases of suspect PPR to the study team for observation. Laboratory samples that included lung tissues, mesenteric and mediastinal lymph nodes; and colon were collected from carcasses of goats suspected to have died of PPR. Samples for histopathology analysis were preserved in formalin 10% while tissue samples for molecular analysis were transported under ice in cold boxes to University of Nairobi, Department of Veterinary Pathology, Microbiology and Parasitology laboratory and later stored in refrigerated repository at -30°C. Laboratory investigation involved preparation of histology slides for histological examination through the standard paraffin process and stained with Hematoxylin and eosin stain (Luna, 1968).Tissue slides were then examined under light microscope. While molecular analysis entailed analysis of 18 frozen sample tissues which were each separately prepared for extraction of PPR RNA as outlined by RNeasy® mini kit protocol supplied by QIAGEN® (2010). The extracted RNA samples were analyzed in a MicroAmp® Optical 96-well reaction plate by Abiprism® 7500 (Applied Biosystems) thermocycler using a specific qRT-PCR assay called 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).

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.

Cases observed during this study were mainly 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. 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).

Histopathology slides of large intestines and lungs were prepared and examined. The lesions present in the lungs were characteristic of broncho-interstitial pneumonia (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)

Molecular analyses for detection PPR virus RNA was carried out in samples listed in 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.

In summary 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 a confirmed 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 lesions 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.

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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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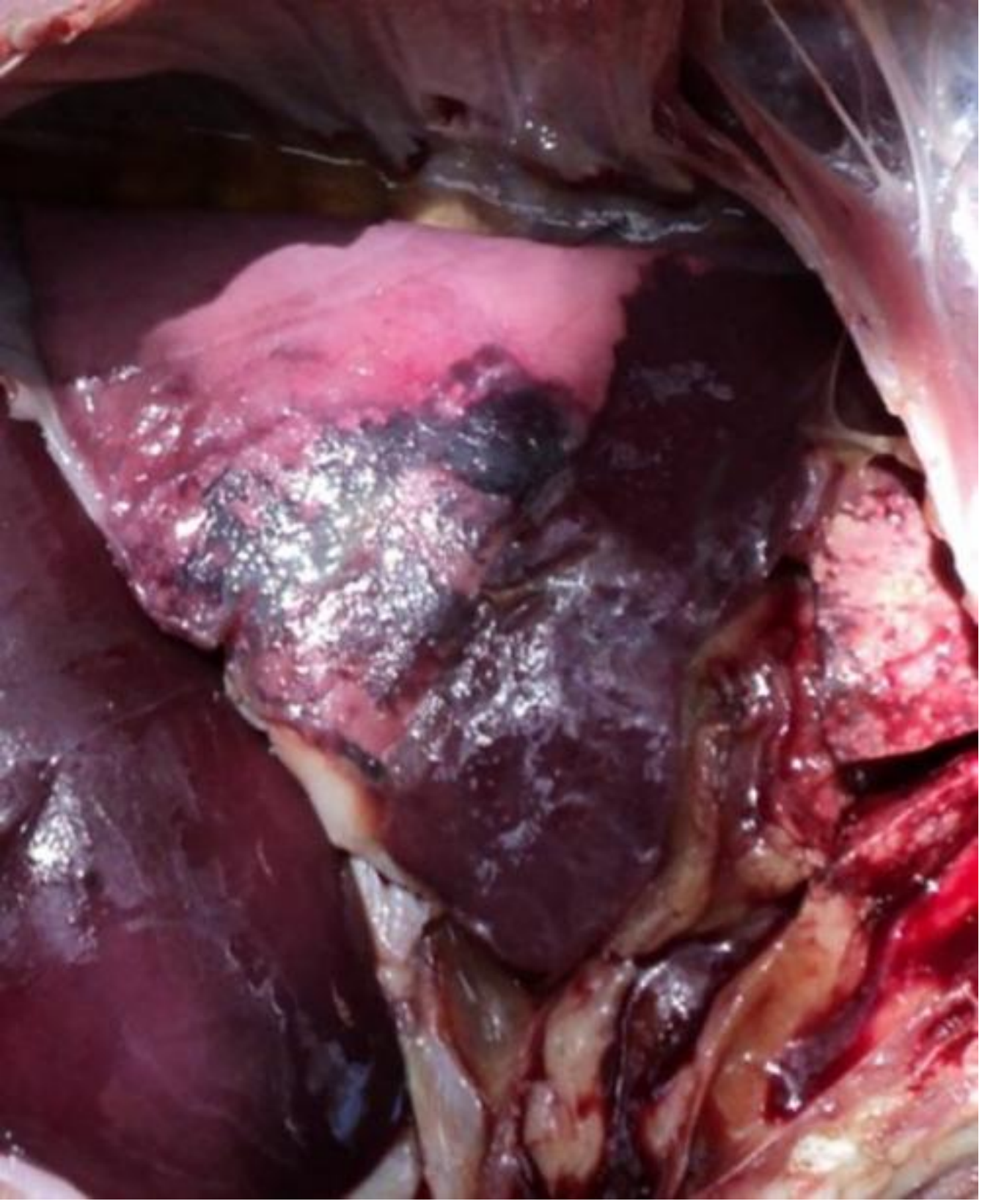

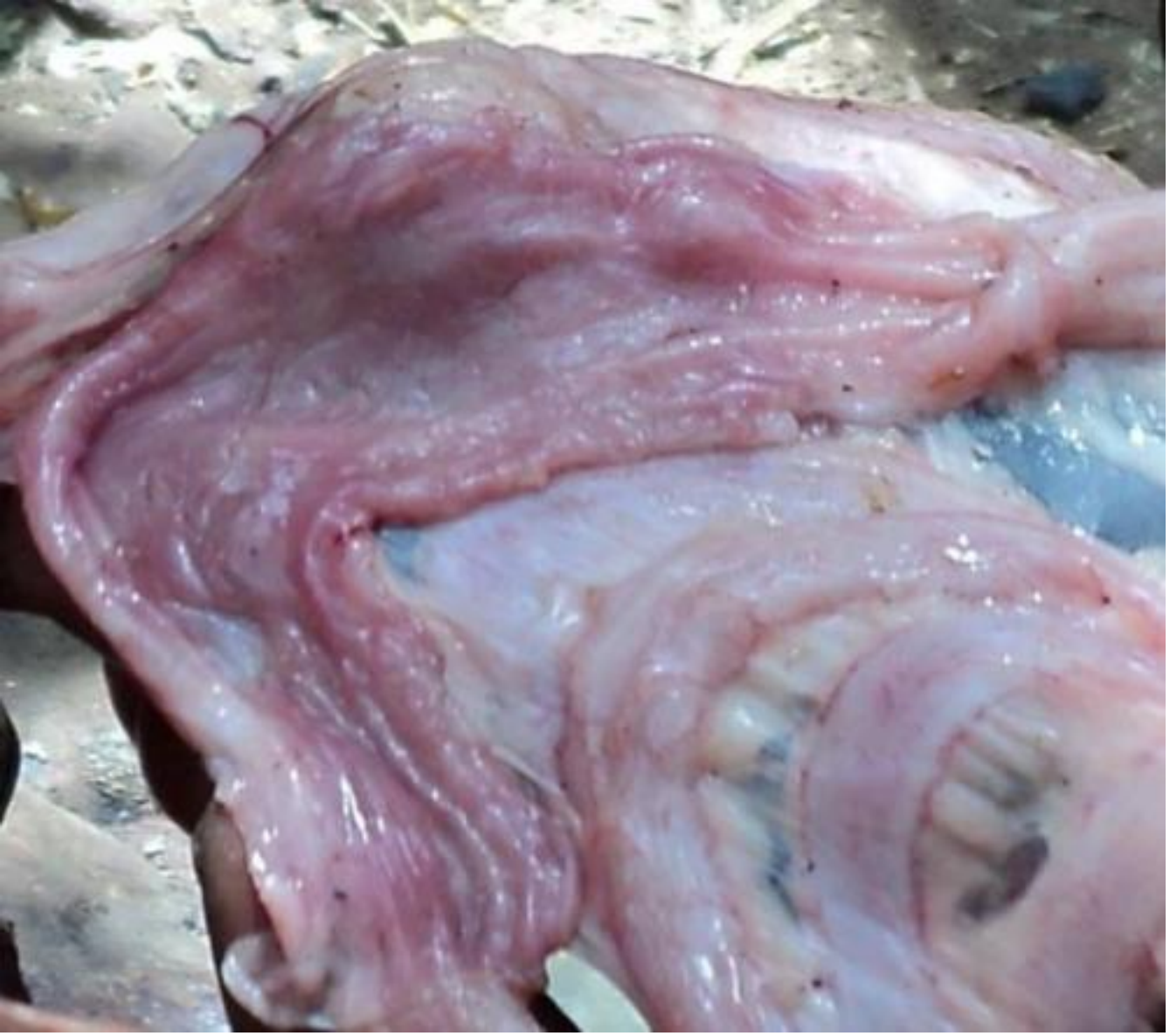

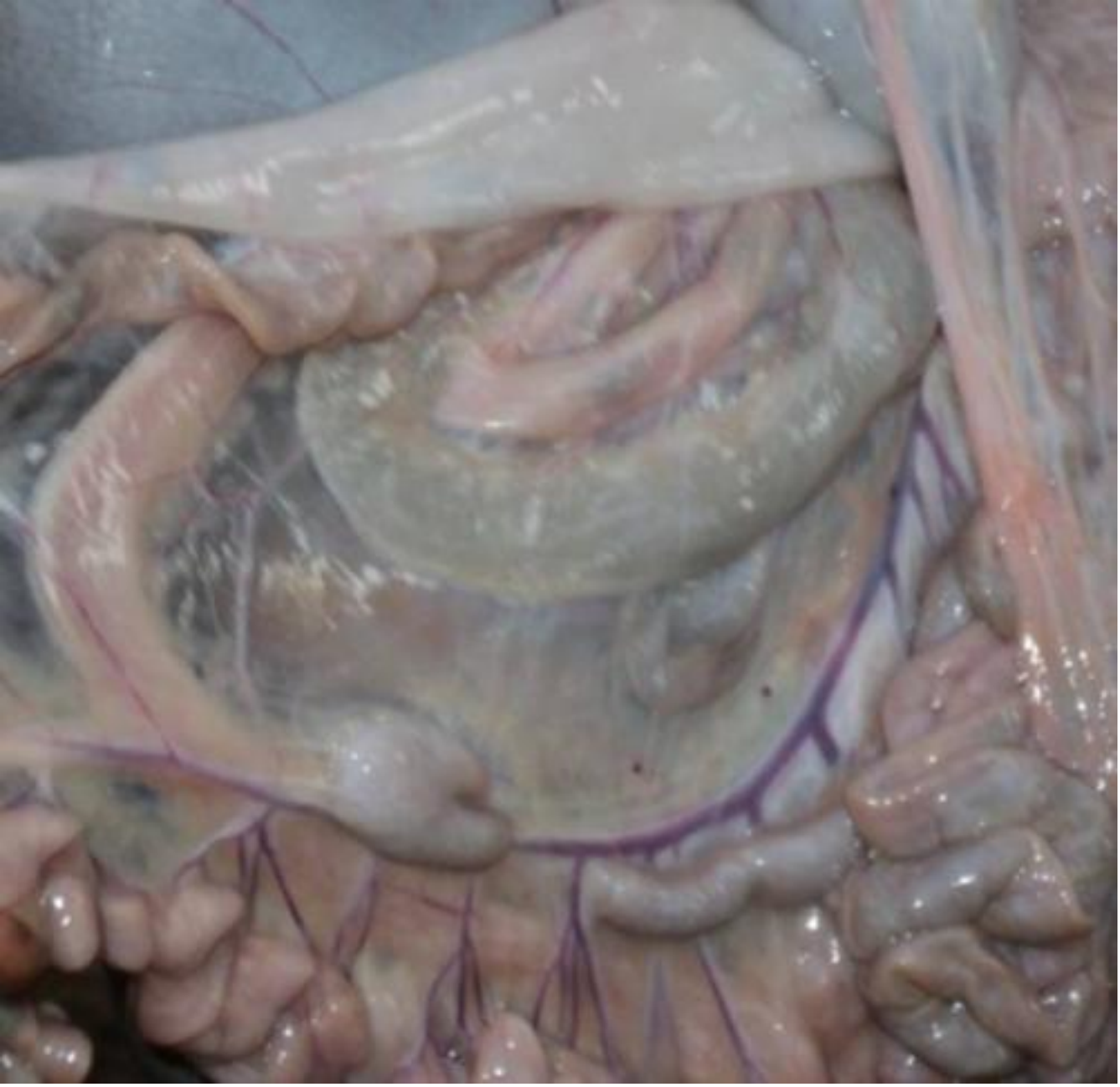

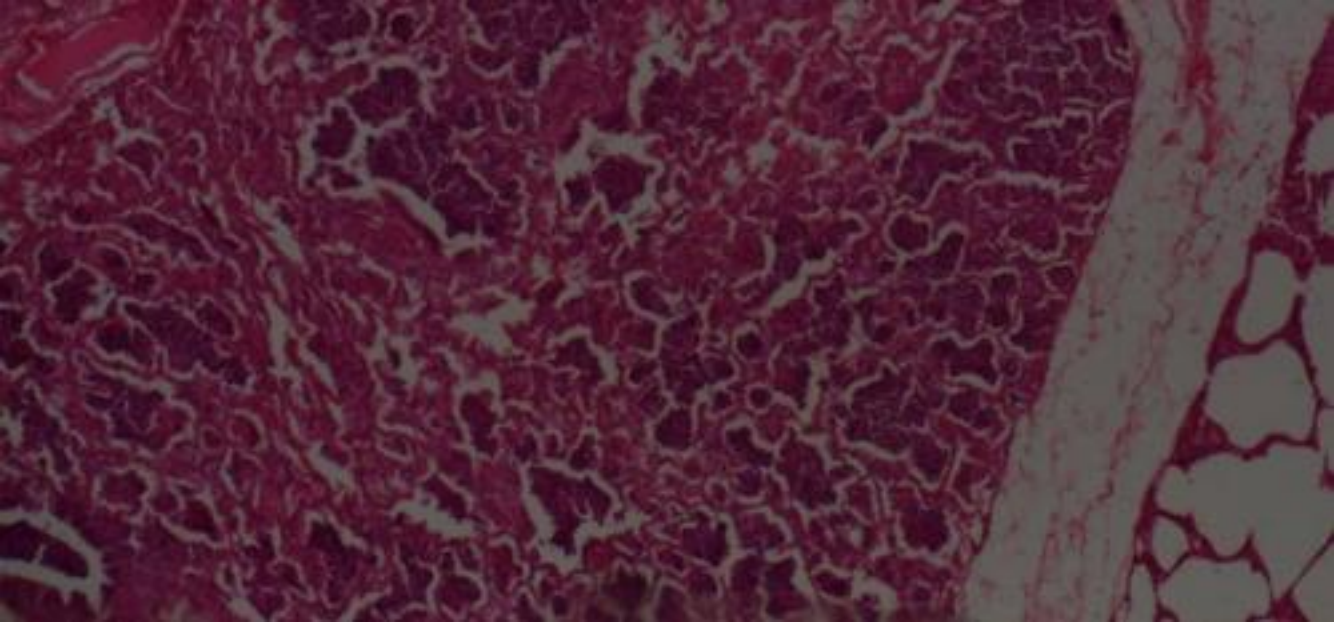

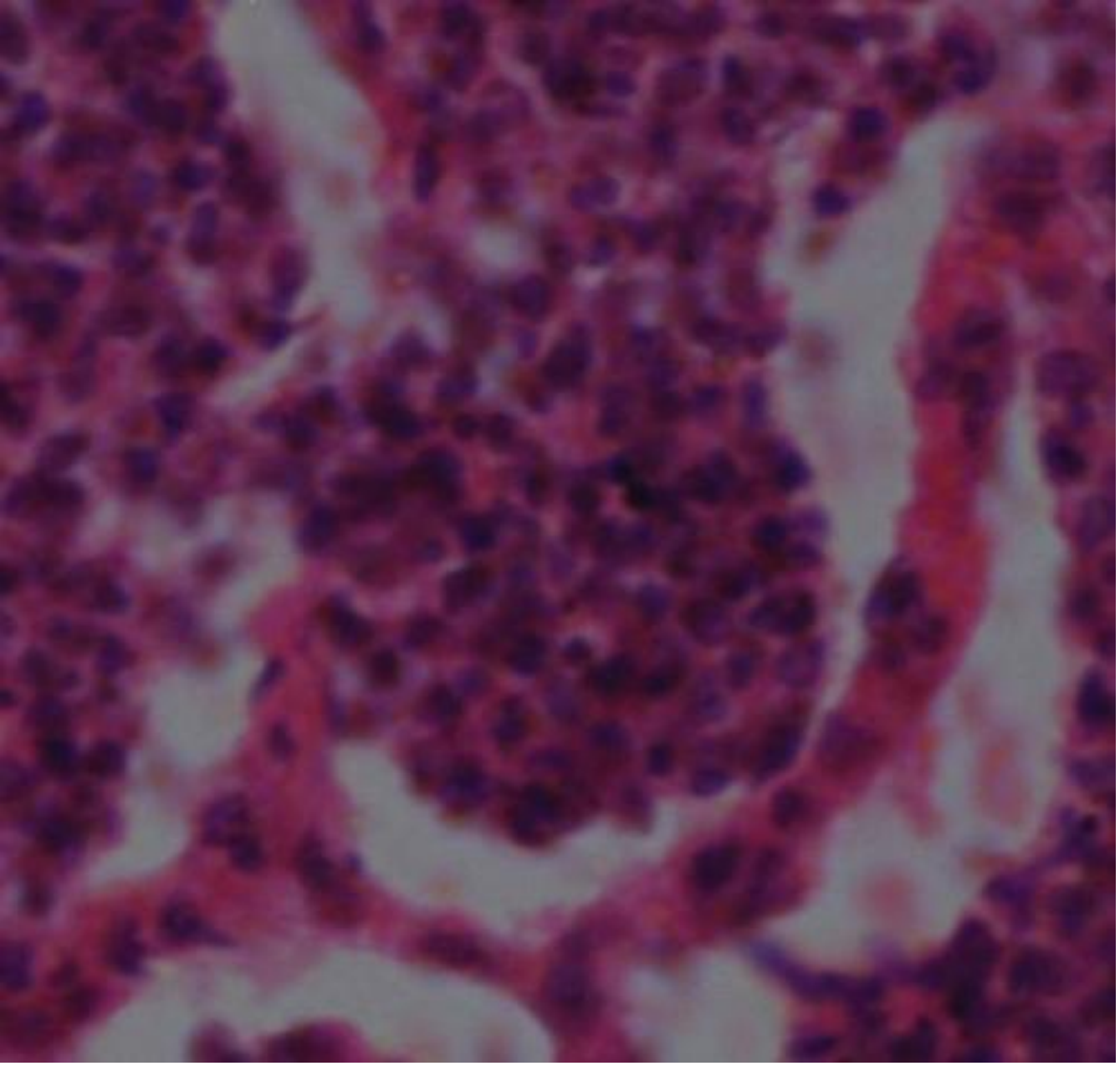

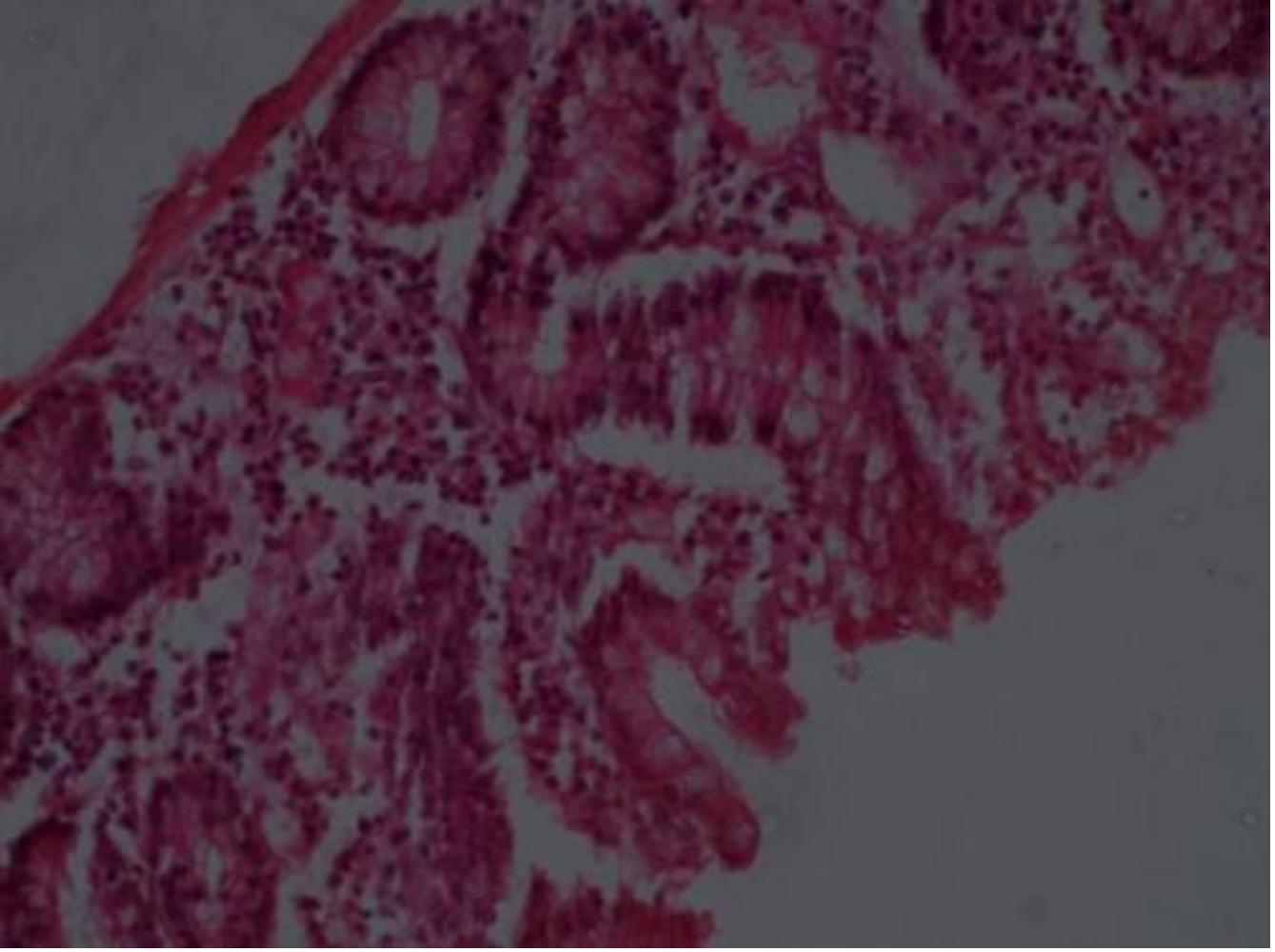

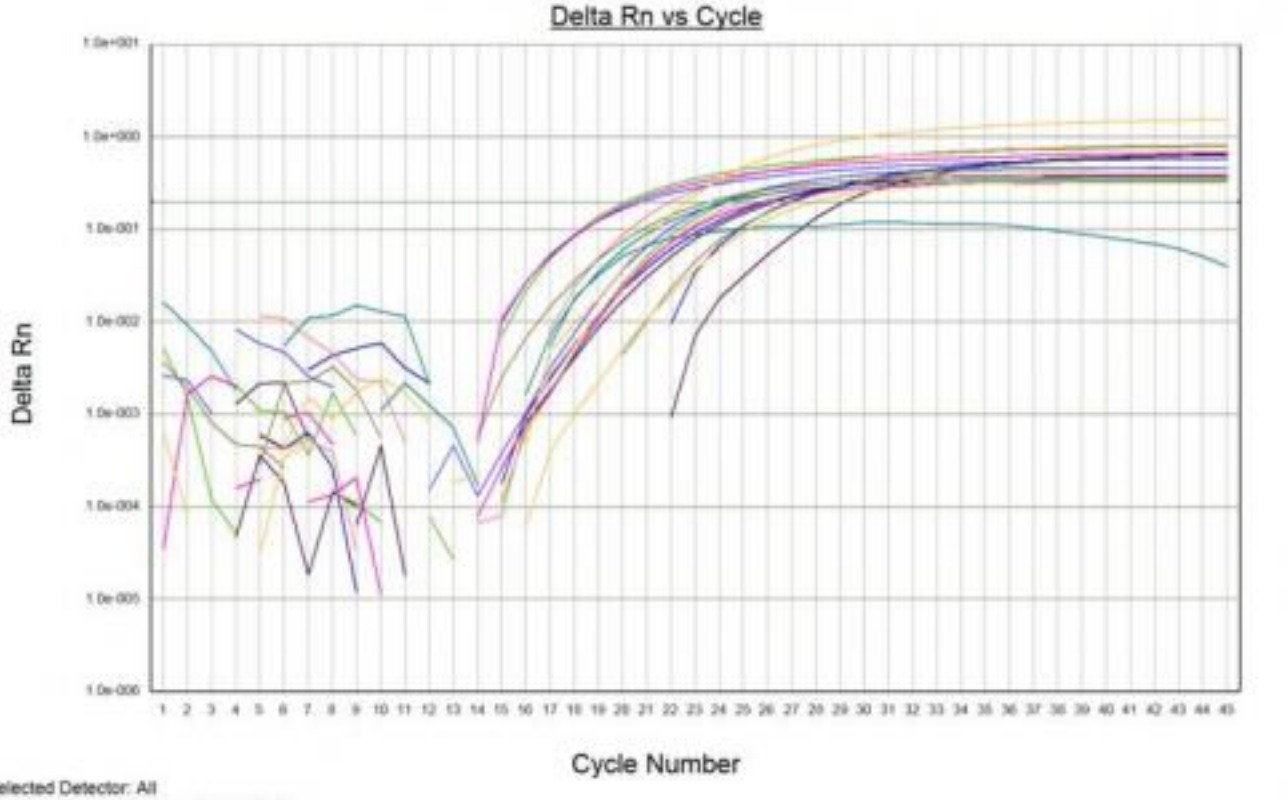

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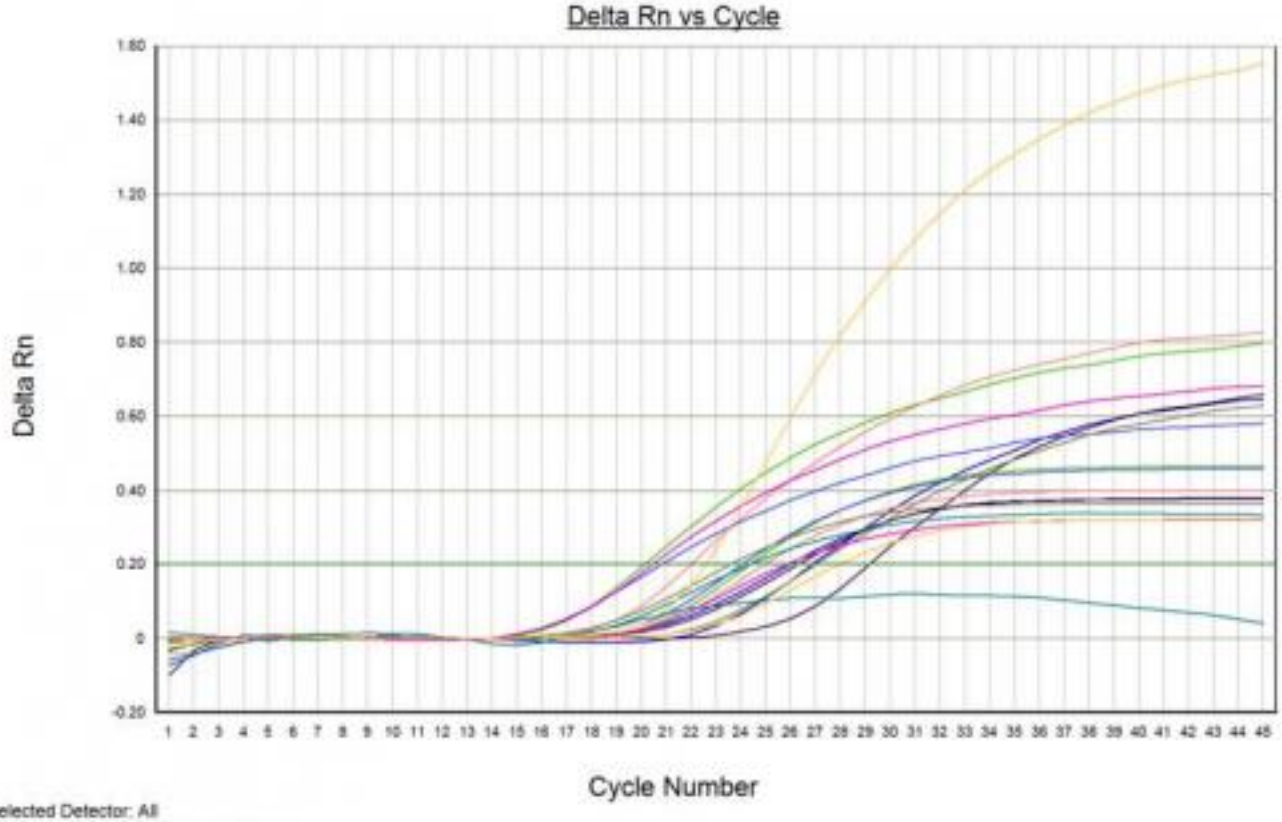

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