

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Identification of Renal Parasite and its Blood Urea-Creatinine Profile on the Indonesian Indigenous Pigeons

J. Prastowo¹, A. Sahara¹, C. Marganingsih¹ and B. Ariyadi²

¹Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta-55281, Indonesia

²Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta-55281, Indonesia

Abstract: In Indonesia, pigeon is one who is familiar with humans, beside can be consumed, usually maintained for hobby or pleasure. One of the parasitic worms that infect the pigeon's kidneys was *Paratanaisia bragai* (*P. bragai*). That worm can cause pathological changes in the renal pigeons. Evaluation of renal function can be done through checking of serum creatinine and urea. This study was conducted to identify the renal parasite and its blood urea-creatinine profile on the Indonesian indigenous pigeons. Fifty eight Indonesian indigenous pigeons were identified for renal parasite. Then all of the collected blood was evaluated for urea and creatinine levels. The difference values of urea and creatinine between infected and uninfected renal pigeon were analyzed by t-test. Fifty one percent (30/58) of them were infected by renal parasite. Identification of renal parasite showed the *P. bragai*. The creatinine levels in infected pigeons (2.650 ± 1.7228) mg/dL were significantly higher than uninfected pigeons (1.732 ± 1.2944) mg/dL. The blood urea levels showed non significant difference between the infected pigeons (7.7700 ± 3.09775) mg/dL and the uninfected pigeons (6.4364 ± 3.60415) mg/dL. The increase of urea and creatinine levels might be not correlated with the number of worms, since the Pearson correlation test showed no significant results. These results will be better if accompanied by histopathology of kidney, uric acid levels and other blood components.

Key words: Pigeons, urea, creatinine, *P. bragai*

INTRODUCTION

Pigeon population in Indonesia until now difficult to quantify and has various type. Traditionally, the pigeon is usually only given in the form of corn meal, brown rice, leftover food and sometimes remain food of family. As a result of improper maintenance and feeding pigeons, it causes often roam to feed themselves. Feeding, cage conditions and poor sanitation, will lead to decreased body immunity of pigeons that causes susceptible to disease. Another factor is the outbreak of a disease carried by other animals, contact with the intermediate host and contamination of food easily cause infection (Sahara *et al.*, 2013).

Trematodes that have been reported in the kidneys of birds are *Renicolidae* family (Munyer and Holloway, 1990), under family of *Eucoylyidae* (Byrd and Denton, 1950). *Eucoylyidae* families who never reported frequently attacked pigeons is a *Paratanaisia bragai* (Luppi *et al.*, 2007), these worms can also invade turkey and quail (Pinto *et al.*, 2004; Brener *et al.*, 2006). In contrast to diseases caused by viruses or bacteria, parasitic worm infections are generally subclinical and only cause minor damage but it could be fatal or severe. This study has been initiated with a description and histopathology caused by worm on pigeon's kidney (Sahara *et al.*, 2013), with the prevalence of the worms

was quite high in the region of Yogyakarta. This prevalence was varies between 10-30%. Trematodes cases in birds might be associated with feeding habits. In the area where the birds are looking for food snails, such as *Subulina octona*, can act as an intermediate host for *P. bragai*. Snails can be infected when feeding on the watery areas in which there is feces containing *miracidium* (worm larvae are active in nature) that it is parasitic stage of *Paratanaisia*. It then developed into *metacercariae* and snails that contain the parasite stage will be ingested by birds (Menezes *et al.*, 2001). *Paratanaisia bragai* located in the kidneys of pigeon causes damage to the kidney structure. The intensity of pathological changes in the kidney caused by *P. bragai* was widely varied. Santos (1934) reported it found no inflammatory reaction caused by that worm, while according to Pinto *et al.* (2004) it found a piece of worm on the collecting ducts without inflammatory reaction. Kidneys function including filtering the blood, maintaining the pH, water and electrolyte balance and excrete substances that are not needed yet through the urine (Guyton and Hall, 1997). Waste substances of metabolism that are no longer needed by the body are including urea (from the metabolism of amino acids), creatinine (from muscle's creatine), uric acid (from nucleic acids), bilirubin, hormone metabolites and

toxins. Urea is the result of protein metabolism in the body that be excreted by glomerulus. Creatinine is the end result of creatine metabolism in the muscle. These compounds must be removed from the body continuously. Chronic renal impairment will lead to a decrease in glomerular filtration rate (renal filtration function), so that the ability of the kidneys to filter urea and creatinine also decreased, as a result of such substances in the blood are increased in number (Doxey and Milne, 1985). Renal function in mammals is often measured by knowing the rate of urea and creatinine. In birds, urea and creatinine measurement for evaluation of renal function showed various results. This study was conducted to identify the renal parasite and its blood urea-creatinine profile on the Indonesian indigenous pigeons.

MATERIALS AND METHODS

Experimental birds: Fifty eight Indonesian indigenous pigeons were obtained from various regions in the province of Yogyakarta, Indonesia. Blood sampling (± 2 mL) was collected from the heart of pigeons and was put into test tubes containing ethylene diamine tetra acetic acid (EDTA). Birds were euthanized for renal parasite observation. Kidney of pigeons was observed to identify renal parasite. Collected blood was examined for urea and creatinine levels. Birds were handled according to Universitas Gadjah Mada rule for experimental animal.

Identification of renal parasite *P. bragai*: Identification of renal parasite was done according to Lima *et al.* (1951) and Cable (1971), with minor modification. Briefly, pigeons were euthanized by embolization. Kidney

of pigeons were taken and placed in the Petri cup. The kidney was cleaned with water, so that the kidney is clean of blood. Then the kidney was prepared and crushed slowly by adding a little water. Then the worms in the kidney were taken and calculated (Fig. 2a). The collected worms were fixated and placed into the object glass. The object glass containing worms was put into a petri dish containing alcohol fixative acetic (AFA) for overnight. Then the worms were washed with distilled water and transferred into the alcohol series from 30, 50 and 70% for 15 min, respectively. The worms were dipped in the 70% of alcohol-odium solution, transferred into 70% alcohol. Then the worms were put into the Semichon's carmine solution for 1 h. The worms then were sprinkled with HCl-alcohol 0.5-1% so that the color becomes pink worms and transparent. The worms were dehydrated in alcohol series from 80, 95 and 100%, then soaked in xylol for 15 min. The worms were transferred on object glass and covered with deck glass. Morphological observation of the worms was conducted under a stereo microscope at a magnification of four and ten times. Then the worms were counted and observed. The worms were observed of the morphology of worms such as mouth sucker, caecal junction, shape of testicle and vitellaria gland. Measurement of the length and width of the body of worms and its organs were conducted using the program AxioVision LE, Carl Zeiss Microscopy GmbH.

Examination of blood urea and creatinine: Blood sampling (± 2 mL) was collected from the heart of pigeons and was put into test tubes containing ethylene diamine tetra acetic acid (EDTA). Examination of serum urea and creatinine were conducted according to Mitruka and Rawnsley (1977).

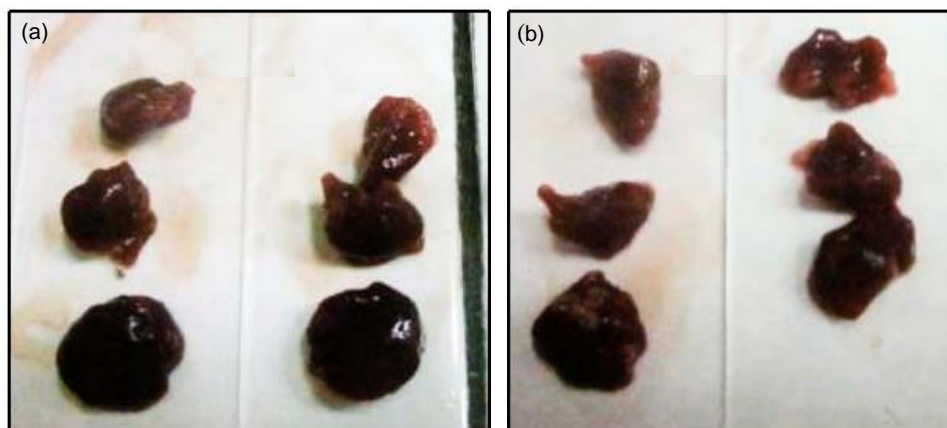


Fig. 1(a-b): Macroscopic observation of the pigeon's kidneys infected and uninfected by worm, (a) shows the pigeon's kidney infected by worms, (b) shows the normal pigeon's kidney (uninfected by worms), (a) shows color of kidney's lobes with severe infection becomes darker and reddish. Incision area becomes wet and fragile compared to the kidney's lobes with no infection. Right posterior lobe and left posterior lobe of infected kidney enlarge

Statistical analysis: The difference values of urea and creatinine between infected and uninfected renal pigeon was examined by t-test. The relationship between the increase of urea and creatinine with the number of worms was performed by Pearson's correlation test. Differences were considered significant at $p < 0.05$.

RESULTS

The obtained worms were varied between 1-727 trematodes in all parts of the left and right kidneys. A large number of worms did not cause a different effect on clinical symptoms in pigeons. In general, the birds still want to eat, drink and have the same conditions with uninfected birds.

Macroscopic observation of the pigeon's kidneys infected and uninfected by worm was shown in Fig. 1. The color of kidney's lobes with severe infection became darker and reddish. Incision area became wet and fragile compared to the kidney's lobes with no infection. Right posterior lobe and left posterior lobe of infected kidney enlarged. Other infected kidneys showed a diverse macroscopic observation. Some kidney did not change compared with non-infected kidney.

The worms infected pigeon's kidney was shown in Fig. 2. Figure 2a showed a macroscopic picture of the worm in the scoured pigeon's kidney. Figure 2b showed microscopic picture of the worm. The anterior part of the worms was narrower than the posterior. The posterior end appeared rounded. Body length of the worms ranged from 1.66-2.8 mm and the width ranged between 0.31-0.69 mm. Mouth sucker was well developed and continue to the pharynx. The size of the mouth sucker ranged from 0.10-0.25 mm. Caeca had two branches, posteriorly extended to the apex forming cyclocoel. Testes was located side by side and looked lobulated. The size of right testicular ranged from 0.13-0.27 x 0.15-0.19 mm. The size of left testicular ranged from 0.14-0.20 x 0.15-0.20 mm. Vitellaria glands extended from the anterior body to the third of the posterior body, its length ranged from 0.71-1.80 mm. The uterus contained eggs was located between the testes toward the posterior part of the body then leads back to the anterior body near the ovaries.

The results of urea and creatinine levels in the blood of infected and uninfected pigeon were shown in the Fig. 3. The creatinine levels in infected pigeons (2.650 ± 1.7228) mg/dL were significantly higher than uninfected pigeons (1.732 ± 1.2944) mg/dL. The blood urea levels showed non significant difference in the infected pigeons (7.7700 ± 3.09775) mg/dL with the uninfected pigeons (6.4364 ± 3.60415) mg/dL.

DISCUSSION

We here identify the renal parasite and its blood urea-creatinine profile on the Indonesian indigenous pigeons. Significant findings were: (1) fifty one percent (30/58) of

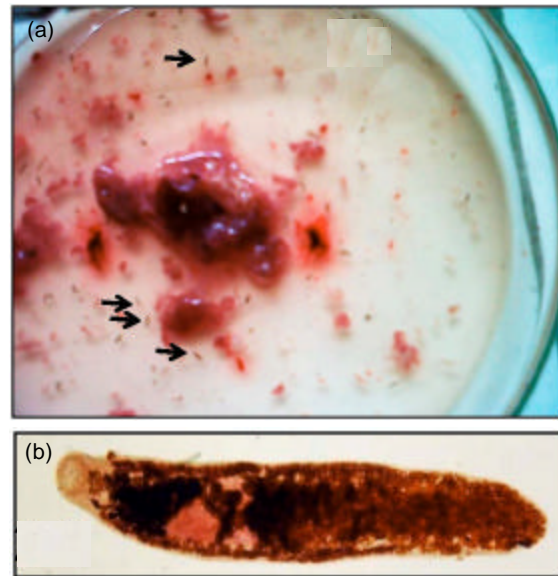


Fig. 2: Worms infect pigeon's kidney, (a) shows a macroscopic picture of the worm in the scoured pigeon's kidney. Arrows indicate the presence of the worms, (b) shows microscopic picture of the worm. The anterior part of the worms is narrower than the posterior. The posterior end appears rounded. Mouth sucker is well developed and continue to the pharynx. Caeca has two branches, posteriorly extend to the apex forming cyclocoel. Testes is located side by side and looks lobulated. The uterus contained eggs is located between the testes toward the posterior part of the body then leads back to the anterior body near the ovaries

Indonesian indigenous pigeons were infected by renal parasite, (2) identification of renal parasite showed the *P. bragai* and (3) the creatinine levels in infected pigeons were significantly higher than uninfected pigeons.

Trematodes found in this study have the characteristics of *Tanaisiinae* subfamily, *Paratanaisia* genus, *bragai* species. According to Gibson *et al.* (2002), trematode that belongs to the *Eucotylidae* family has well-developed mouth sucker, muscular and is located sub-terminal. Stomach sucker is absent or not developed optimally. Caeca is simple shape, undulation and looks smooth. Some caeca unify to form *cyclocoel* in the posterior part of the body and some are not. The testicles are located extra-caecal and some are located intra-caecal. Testes sometimes appear to overlap with the caeca. It looks smooth and irregularly shaped or lobulated, tandem, or diagonal and is located in the medial or slightly towards the posterior. Vitellaria channel is not up to the posterior of the body. In addition, the worms were found in the kidney as well as in the ureter.

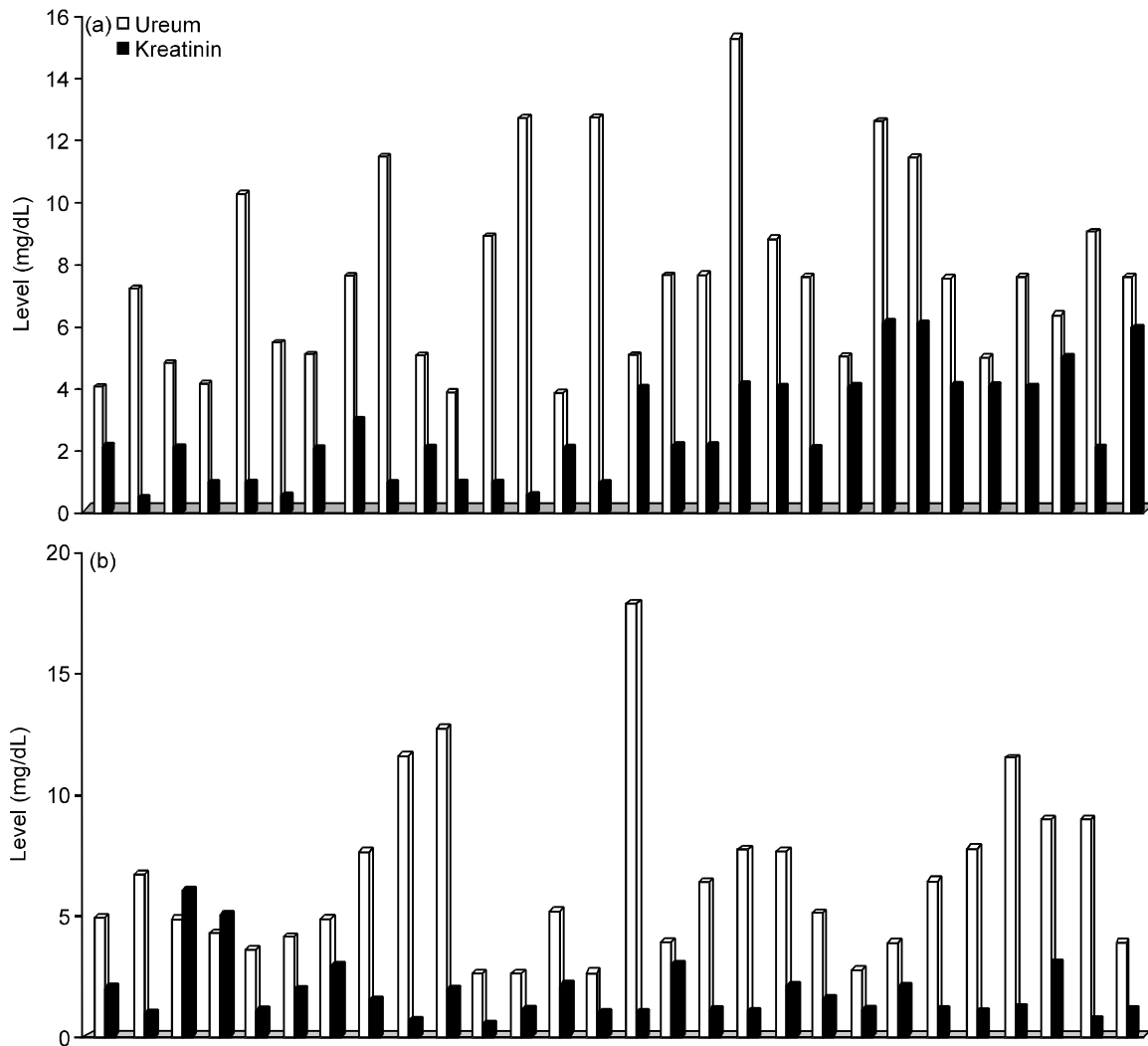


Fig. 3: Results of urea and creatinine levels in the blood of infected and uninfected pigeon, (a) shows the urea and creatinine levels in the blood of infected pigeon, (b) shows the urea and creatinine levels in the blood of uninfected pigeon. The creatinine levels in infected pigeons (2.650 ± 1.7228) mg/dL are significantly higher than uninfected pigeons (1.732 ± 1.2944) mg/dL. The blood urea levels show no significantly difference between the infected pigeons (7.7700 ± 3.09775) mg/dL and the uninfected pigeons (6.4364 ± 3.60415) mg/dL

The results of blood urea levels showed non significant difference in the infected pigeons (7.7700 ± 3.09775) mg/dL with the uninfected group (6.4364 ± 3.60415) mg/dL. According to Mitruka and Rawnsley (1977), normal urea levels in pigeons is 4.6 to 6.8 mg/dL. In this study, on uninfected birds, a total of 34.4% of birds had increased rates of urea (10/29 birds), whereas in infected birds had increased rates of urea of 50% (15/30 birds).

In this study, urea levels showed non significant difference between infected group and uninfected group. This is because the level is low and varies; moreover, some birds produced uric acid. The urea increase in blood may take a while, do not always indicate kidney

failure. This could be agreed as kidney damage if followed by another screening urinalysis. Some studies reported the increase of urea had no relationship with pathologic condition (Fowler, 1986; Ngo and Assimos, 2007). According to Lumeij (1987), birds in dehydration, the urea rate could increase up to 6.5-15.3 fold compared to the normal condition. Additionally, urea level of birds can increased with blood sampling within 12 h. In pathological conditions due to inflammation of the bird's kidneys, urea levels can significantly increased by giving antibiotics such as Gentamycin (Khan *et al.*, 2008).

The creatinine levels in infected pigeons (2.650 ± 1.7228) mg/dL were significantly higher than uninfected pigeons

(1.732±1.2944) mg/dL. According to Mitruka and Rawnsley (1977), the normal creatinine levels in pigeons are 0.8-1.8 mg/dL. Creatinine is the end product of the metabolism of creatine phosphate in the muscle. Creatine is synthesized in the liver and found in almost all skeletal muscles in the form of creatine phosphate. In the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP), creatine phosphate is converted into creatine by kinase creatine enzyme. Along with the use of energy, a small amount of creatine can be irreversibly changed to the creatinine filtrated by glomerular and excreted to the urine. Increase of blood creatinine is influenced several things, including kidney failure that cause function of nephrons decreases. This condition causes its level decreases in urine but increases in plasma (Frandsen *et al.*, 2009). Daily concentration and excretion total of creatinine remains constant despite food changes. Therefore, creatinine can be found in blood and urine with a little concentration in normal conditions.

In this study, the increase of creatinine levels showed a decrease in glomerular filtration rate due to the accumulation of worms in the renal tubules causing inflammatory reactions. Creatinine excretion showed a decrease in glomerular filtration rate showing any disturbance of kidney function. Serum creatinine is a specific indicator for renal function. The creatinine levels double increases indicating a half decrease of kidney function. Statistical analysis showed that there is no significant relationship between the number of worms and urea as well as creatinine level. The lack of correlation between the number of worms and urea-creatinine depends on the degree of pathological changes in the kidney. The presence of worms in the kidney causes diverse pathological response (Pinto *et al.*, 2004).

Conclusions: Fifty one percent of Indonesian indigenous pigeons were infected by renal parasite of *P. bragai*. The creatinine levels in infected pigeons were significantly higher than uninfected pigeons. The blood urea levels showed no significantly difference between the infected pigeons and the uninfected pigeons. The increase of urea and creatinine levels might be not correlated with the number of worms. These results will be better if accompanied by histopathology of kidney, uric acid levels and other blood components.

REFERENCES

Brener, B., R. Tortelly, R.C. Menezes, M.L. Pereira and R.M. Pinto, 2006. Prevalence and pathology of the nematode *Heterakis gallinarum*, the trematode *Paratanaisia bragai* and the Protozoan *Histomonas meleagridis* in turkey, *Melleagris gallopavo*. Mem. Inst. Oswaldo Cruz., 6: 77-81.

Byrd, E.E. and J.F. Denton, 1950. The helminth parasites of birds. I. a review of the trematode genus *Tanaisia* Skrjabin, 1924. Am. Midl. Nat., 43: 32-57.

Cable, R.M., 1971. Parthenogenesis in parasitic helminths, Soc. Integ. Comp. Biol., 9: 1-4.

Doxey, D.L. and E.M. Milne, 1985. Canine diabetes mellitus: a retrospective survey, J. Small Anim. Pract., 26: 1-5.

Fowler, M.E., 1986. Zoo and wild animal medicine. Saude Company, Philadelphia.

Frandsen, R.D., W.L. Wilke and A.D. Fails, 2009. Anatomy and physiology of farm animals, Wiley and Blackwell, Iowa.

Lima, C.M.B.L., F.I.S. Freitas and L.C.S.L. Morais, 1951. Ultrastructural study on the morphological changes to male worms of *Schistosoma mansoni* after *in vitro* exposure to allicin. Rev. Soc., 10: 1-5.

Gibson, D.I., A. Jones and R.A. Bray, 2002. Keys to trematode Nat. Hyst. Mus., 1: 45-50.

Guyton and Hall, 1997. Clinical avian medicine and surgery, WB. Sauder Company, Philadelphia.

Mitruka, B.M. and H.M. Rawnsley, 1977. Clinical biochemical and hematological reference values in normal experimental animals, Masson Publishing, USA.

Menezes, R.C., D.G. Mattos, R. Tortelly, R.M. Pinto and D.C. Gomes, 2001. Trematodes of free range reared Guinea Fowls (*Numida melegris*, Linnaeus 1758) in the State of de Jainero, Brazil, Avian Path., 30: 99-103.

Ngo, T.C. and G.D. Assimios, 2007. Uric acid nephrolithiasis: recent progress and future directions. Rev. Urol., 9: 17-27.

Khan, I., Z.M. Khan, K.M. Saleemi, I. Javed and A. Khan, 2008. Pathological and biochemical effects of intramuscular gentamicin administration in chicken Turk. J. Vet. Anim. Sci., 32: 345-351.

Lumeij, J.T., 1987. Plasma urea creatinine and uric acid concentrations in response to dehydration in racing pigeons *columba livia domestica*. Av. Pathol., 16: 377-82.

Luppi, M.M., A.L. de Melo, R.O.C. Motta, M.C.C. Malta, C.H.S. Gardiner and C.H. Santos, 2007. Granulomatous nephritis in psittacines associated with parasitism by the Trematode *Paratanaisia* spp. Vet. Par., 146: 363-366.

Munyer, P.D. and H.L. Holloway Jr., 1990. *Renicola williamsi* n. sp. (trematoda: digenea: renicolidae) from the South Polar Skua, *Catharacta maccormiki*. Trans. Am. Micros., 109: 98-102.

Pinto, R.M., R.C. Menezes, R. Tortelly and D. Noronha, 2004. First report of a natural helminth infection in the Japanese quail *Coturnix japonica* Temminck and Schlegel (Aves, Phasianidae, Galliformes) in the neotropical region. Rev. Bras. Zool., 22: 1-4.

Sahara, A., J. Prastowo, D.P. Widodo, E.S. Rohayati and S. Widyarini, 2013. Identification and pathological features of trematode in pigeon's kidney. J. Vet., 14: 1-4.

Santos, V., 1934. Monostomose renal das aves domesticas. Rev. Dept. Nac. Prod. Anim. Brazil, 1: 203-215.