EUROPEAN ACADEMIC RESEARCH
Vol. V, Issue 10/ January 2018

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Assessment of Alternative Methods for Diagnosing of Ascariasis in Piglets

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Abstract:

The incidence of ascariasis is high in Albania having adverse affects across the entire levels of the breeding system with far-reaching damage to all pig categories. Ascariasis is rightly assumed to be causing significant damage to pigs until they are bound for slaughter after just 7 months of age with symptoms correlating with high prevalence rate and a heavy parasite load. The losses incurred from Ascaris suum in piglets is compelling us to gear for a series of diagnostic and prophylactic alternatives to be applied in slaughterhouses with the view to boosting the much sought-after profitability. The study was conducted in the country's slaughterhouses involving as many as 162 pigs in which was detected the presence of ascariasis through several alternative diagnostic methods. The coproscopical examination of the samples collected from the slaughter-houses revealed that as many as 124 pigs (76.54% of the total) were infested. Further to this, an examination of the liver clearly showed the presence of the livery milk spots in 49 piglets or 30.24 % of the samples collected and examined. Under examination of the small intestines indicated that the adult parasites were detected in the small intestines of some 78 pigs or an equivalent of 48.14% of the examined samples. During the examination of the lungs as many as 34 piglets or a proportion of 20.98 % of the total samples examined tested positive

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with parasitic pneumonia or with well-established signs of parasitic migration. A closer inspection of nose-leak swabs demonstrated that a total of 26 piglets or roughly 16.04% of the total tested positive with larvae infestation/presence. Coproscopic examination in turn was identified as the most effective and easy-to-use method while liver examination proved to be quite as practical and very useful in terms of the observations occurring in the slaughterhouse. In addition, the examination of intestines was deemed a highly relevant tool in the diagnosis of ascariasis in the slaughterhouse. Whereas the examination of the lungs can serve as a very practical diagnostic tool in the observations done in slaughterhouses, while the examination of the nose swabs turned out to be an alternative method, both in the case of the living piglet and the slaughtered ones in the slaughterhouse.

Key words: Ascariasis, coproscopy, milk spots, intestine, lung, nose swabs piglets.

MATERIALS AND METHODS

The study was conducted across several slaughterhouses in some districts (Lushnje, Fier, Tirana and Shkoder) in which as many as 162 piglets were closely examined between the period 2015-2017. Through the coproscopic examinations of swine faeces it was determined both the parasitic prevalence and lord based upon the epidemiological criteria of geographical variation, sample size and evaluation approaches. Faecal samples were collected directly in the straight intestine which was thoroughly exhausted sample-wise. In this case, the straight intestine was separated from the rest with both ends tied up and deposited into plastic bags in order for it to be transported to the laboratory. For purposes of coproscopical examinations both the qualitative and quantitative methods of sedimentation and floating were employed. The coproscopical examinations were carried out at the laboratory of veterinary parasitology at FVM. Post-mortem examinations at slaughterhouses have been serving as purely alternative

method of study. During the post-mortem control of biological properties of *A.suum* were detected variations in the liver and lungs along with lesions easily noticeable in the migration stage. These observations were done in the organs of the piglets from which a series of samples were collected for the intents of macroscopic, histological and microscopic examinations. As for the biological properties of *A. suum* it should pointed out that livers carrying white spots were identified. In the slaughterhouse the piglets' livers were subjected to the macroscopic examination focusing primarily on the liver milk spots. The livers were carefully examined for hot spots due to the migration of the ascarids larvae. The observations were administered upon the same piglets from which sampling was done for purposes of coproscopical examinations. To distinguish between parasitic spots and spots caused by mold the liver samples were stained with Wright Gimsae and Ziehl Neelsen.

In order to examine closely the small intestines the observations in slaughterhouses were combined with the lab examinations. Initially the intestines were cut into several sections along the ligatures which were in turn cut into 1-2 m long sections. Dispensing with the need to cut open the interior of intestines they were rinsed thoroughly under the tap to be later deposited in 1 L beakers. Right after the rinsing process each section of the intestine was opened with enterotom in which the internal part was examined carefully by putting it onto a glass tray held against a dark backdrop. For purposes of discovering the small nematodes which borrow their way through the intestine mucus were used tensioactive and artificial solutions. In the case of 250 gr - sample were used 6 g. pepsin, 10 cc concentrated HCL, and 600 cc water. The artificial solution was fixed by holding it overnight in a thermostat which was set at 37°C. The solution was likewise used to pin down the parasites. During the microscopic examination of the small intestines there were observed, collected, differentiated and quantified the adult parasites residing in the piglet intestine

already examined. In the case of the adult parasites the paper concentrated on the identification of the species type and their corresponding final counting.

The lungs were also observed in the same piglets. Upon the observation of parasitic pneumonia or in case of suspicions triggered from migrations of larvae to lungs haemorragical lesions and intensive filtrations of eozionphiles around alveoli were detected (from larvae migrating to the bronchial tree). Repeated infections produce haemorrhage, oedema and emphysema. The lungs which proved to be suspicious of affected were sampled partially or in their entirety to be examined in the laboratories. In the laboratories the full lungs were examined through the perfusion method. While the sections/ components were examined in the microscopic swabs (strish) which were acquired from the liquids in the bronchial tree. While in the slaughterhouse swabs were prepared from nose leaks of the same piglets as used in the experiment. The swabs were observed to detect migratory larvae. When larvae were detected they were quantified effectively. A Chi-square test was conducted to investigate the association between the exposure and the outcome (Agresti, 2012). The odds ratio and its 95% confidence limits were calculated to measure the magnitude of the association. A 5% level of significance was used to evaluate significance of association, i.e the p-value was considered significant if it was less than 0.05 . The analyses were conducted using Statulator, an online statistical program (Dhand and Khatkar, 2014).

RESULTS AND DISCUSSION

From the coproscopical examinations were recorded the highest infestation rates by *Ascaris suum* compared to all the diagnostic alternatives used in the study. Of a total of 162 samples examined, 124 of them (or 76.54%) tested positive for *Ascaris suum*. Coproscopy proved quick-to-use and efficient to evaluate

the prevalence of infestation as well as the parasitic load. It can also be applied both in living pigs and slaughtered piglets (carcasses) in slaughterhouses.

Tabele no. 1. The examination results of piglets in slaughterhouses.

Method	No. of examined piglets	Positive no	Positive %
Coprologic examination of piglets in slaughterhouses	162	124	76.54
Presence of hot spots in liver	162	49	30.24
Ascarids in intestines	162	78	48.14
Examination of lungs	162	34	20.98
Examination of nose swabs	162	26	16.04

It was established that as many as 49 piglets or 30.24% of the observed ones were found to be suffering from damage due to migration to the liver. As far as the relationship between macroscopic and histological models was concerned, white and compact milk spots have generally been produced by interstitial eosinophilic hepatitis.

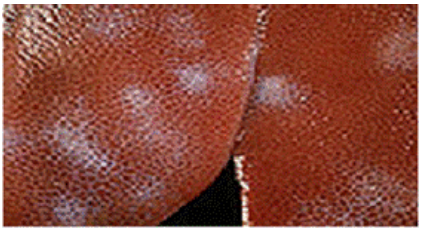

Figure no 1. Milk spots in piglets liver

In the above figure the milk spots have spread all over the whole liver. They are depicted as white areas measuring anywhere from 0.5 to 1 cm in diameter. Larvae migrate to the liver and are capable of causing an inflammatory reaction. This process takes its time and the time in question depends on a number of factors, with the main one being the immunity of piglets. This is subsequently followed by an intensive eosinophil infiltration and collagen production. These lesions are palpably visible in necropsy on the liver surface as white areas and are

commonly referred to as "milk spots". In the absence of re-infections these lesions will begin to regress (retreat) after the larvae migrate out of the liver and the organ will recover completely after 4 to 6 weeks. It could be argued that their presence in necrosis is an indication of the final infestation. In pigs which experience multiple re-infestations during their life cycle, the spots become noticeably fibrotic.

It should be highlighted that in the experimental pigs intestinal examinations were carried out to track and count up the adult ascarids. During the microscopic examinations of the small intestines it was revealed that out of a total of 162 piglets were found to have adult parasites residing in them some 78 piglets or an equivalent of 48.14% of piglets examined. There were also variations in the number of adult parasites quantified in the intestines. The piglets which resulted to have been infested with adult parasites in the intestines was found through post-mortem examination, and consequently the paper concentrated on their categorization.

During the examination of a total of 162 piglets it was revealed that some 34 piglets or a figure equal to 20.98% of the examined piglets tested positive with parasitic pneumonia or with consequences triggered due to parasitic migration. The sample collected for this purpose is considered capable of yielding adequate diagnostic evidence. The damage to the lungs from the migration of migratory larvae of *A. suum* strain can be enough to aggravate the enzymatic pneumonia and to increase the latent infections with the swine flu. The migration of larvae into the lung may also produces hemorrhagic lesions and intensive eosinophilic filtration around the alveolas in which the larvae emigrate on their pathway to the bronchial tree. The repeated infections will trigger the most widespread haemorrhage, oedema and emphysema. The largest hemorrhagic lesions were found in the apical and cardiac lobes of inflamed lungs due to the migratory forms of *Ascaris suum*.

The nose swabs were collected from the piglets examined and the microscopic ones were examined in the stereomicroscope as moist and dry solutions. The *A. suum* migrating larvae were found in these swabs. The technique proved to be quite useful under these conditions since it is well-known that larvae appear in the infested pig's noses on the 7 and 9 days upon infestation. The larvae in the nose and mouth are ingested to go down to the intestines or they are coughed up (sneezed up) leaked out/discharged through the nose to the outer environment. The diagnostic technique proved to be very easy-to-use, extremely efficient and very effective. The reason has to do with the fragility method and the biological properties of *Ascaris suum'*s which for a relatively short period of time can be found in the nose leaks. This coincides with the period when it migrates into the bronchioles and through the mucocilial apparatus it comes straight into the mouth to be swallowed and then to be discharged through the leaks out into the open. The time is rather limited and values will be much smaller. Using the swabs in the nose leaks it was established that 26 piglets or a proportion of 16.04% of the total samples tested positive with the presence of larvae. The advantages of this method are that swab examinations can yield invaluable data in the living piglets (not necessarily slaughtered ones) and positivity along with the parasitic loads appear to be of significant value.

Table no 2. Comparable data for the positivity in piglets dependent upon the methods being used.

Methods	Ascarids in intestine	Hot spots in liver	Coprology examinations	Examinations of lungs	Nose swabs
Coprological examination	77 (-1)	48 (-1)	124	33 (-1)	22 (-4)
Hot spots in liver	41 (-8)	49	44 (-5)	45 (-4)	44 (-5)
Ascarids in intestines	78	47 (-2)	123 (-1)	32 (-2)	20 (-6)
Examination of lungs	32 (-2)	31 (-1)	32 (-2)	34	29 (-5)
Nose swabs	17 (-9)	24 (-2)	19 (-5)	23 (-3)	26

It was only 1 out of a total of 124 piglets which tested negative with adult ascarids in intestines during the coproscopical examination. In this case in point this can be fully accounted for by the fecundity of the parasites, the peculiarities of the parasitic populations while, if probability were to be weighing in, it might happen that, regardless of the large number of eggs laid by a female parasite, it might well be the case that 1 out of 124 samples might have escaped the microscopic observation. Meantime only 1 piglet tested negative for the presence of hot spots in the liver. By estimating the data from the wide-ranging available literature and the research experience to this day this is easily understandable, and it relates closely to the physiological traits of the animal and more specifically to the immunity of piglets.

Method		Post-mortem (white spot)		Total
		Positive	Negative	
Coprological	Positive	41	83	124
	Negative	8	30	38
Total		49	113	162

The odds ratio indicates that the positive animals in coprological examinations has 1.85 times the odds of the presence of white spots in liver than the negative animals. Also, we are 95% confident that the odds ratio in the population would be between 0.78 and 4.40.

Association between the positive results and the outcome was not significant [P-value: 0.158; odds ratio and 95% CI: 1.85 (0.78, 4.40)] (Dhand and Khatkar, 2014).

Only 1 piglet which tested positive during the coproscopic examination tested negative for the presence of larvae during the lung examination. While this number increased to 4 piglets when the examination of the nose swabs was undertaken. The differences are accounted for from the stages of the biological cycle of parasites and the specific traits of the immunity of piglets.

Out of a total of 49 piglets which resulted positive for the presence of hotspots in the liver during the study, 8 piglets did

not display any positive result for ascarids in the intestines, with 5 of them testing negative through the coproscopic examination and 4 others testing positive through the lung examination as well as 5 others testing negative during the examination of nose swabs. The differences proved to be significant in this case especially in the case of ascarids breeding in the intestines and larvae in the noses. We assume that this could be accounted for only by the physiological traits of the piglets, the specific immunity of the piglets which has a direct effect even upon the biological cycle of the ascarids. In the case of individuals possessing strong immunity the intestinal and hepatobronchial migration stages is significantly halted.

Method		Post-mortem (presence of ascarides)		Total
		Positive	Negative	
Coprological	Positive	122	2	124
	Negative	1	37	38
Total		123	39	162

The odds ratio indicates that the positive animals in coprological examinations has 2257.00. times the odds of the presence of ascarides in intestine than the negative animals. There was a significant association between positive coprological results and (P-value: <0.001) with presence of ascarides in intestine than the animals with negative results (odds ratio 95% CI: 198.99, 25599.11).

No positive results were established for the presence of hotspots in liver in the case of 2 piglets which had previously tested positive through the examination of their small intestines. In the case in question this might be fully explained through the traits of immunity in piglets, the fecundity of female parasites and the characteristics of parasitic populations as well as the features of migratory larvae. Meantime only 2 piglets out of a total of 124 which tested positive through the coprological examination turned out to be negative for the presence of ascarids in the intestines. This proved to be a rather complex issue to be explained. It does by no means stand to

reason to assume that a piglet which tests positive through the coproscopy does not display any obvious signs for the presence of the adult parasites in the intestines. In this case there is no justification whatever for the presence of eggs in faeces. By looking at this as a case in isolation this could be easily attributable to the fact of piglets being adult ascarids-free as much as to the presence of prepatente ascarids which are not yet fully matured and fertilized to be laying eggs. Meanwhile 2 piglets which tested positive during the examination of the intestines tested negative for the presence of larvae during the examination of the lungs and that of nose swabs with the number jumping to 6 piglets. The differences could be accounted for through the traits of the parasitic biological cycle and the specific characteristics of immunity in piglets which directly affect the migration of larvae. This indicates that the body reacts forcefully by getting rid of a large number of them. Only a limited number, say, out of a thousand (1-40) could succeed in establishing themselves and becoming mature in the piglets' intestines.

The examination of the lungs proves to be less sensitive when compared with the alternative methods which have been applied and evaluated so far. Two piglets tested negative for the migratory L_3 in the lungs with the latter previously showing signs of adult ascarids in the intestines, 1 piglet testing positive for hotspots in the liver, 2 piglets testing positive during the coproscopic examination as well as 5 piglets testing positive in the examinations of nose swabs. Differences could be accounted for by the biological cycle of the parasite as well as by the specific features of immunity in piglets. The larvae which turn into L_3 in the liver travel in the bloodstream to the lungs descending down through ingestion to the intestines. They encounter a resistance from the body of piglets with their numbers thinning out progressively as illustrated from the values provided by the study. The lose out against the struggle offered by the defense system of the organism with their

numbers diminishing along the way with only a handful of them managing to mature sexually.

Method		Post-mortem (lungs lessions)		Total
		Positive	Negative	
Coprological	Positive	32	92	124
	Negative	2	36	38
Total		34	128	162

There is a significant association between the animals with positive coprological results (P-value: 0.007) and lung lesions, and they have 6.26 times the odds than animals with negative coprological results (odds ratio 95% CI: 1.43, 27.49).

If the examination of the lungs turns out to be less sensitive than the other alternative methods and the comparisons applied and evaluated so far, then the examination of the nose swabs results the least sensitive method in the study. Of the total of 26 piglets which tested positive for L_3 in the nose swabs, 9 others did not turn positive for adult parasites in the intestines, 2 of them did not test positive for hotspots in the liver, 5 of them testing positive in the coprology examination, 3 of them testing negative in the examination of the lungs. The difference in relation to adult parasites in the intestines is due to the long and laborious pathway that larvae have to travel to before arrive as L_3 in the intestines. The numbers of migratory larvae diminish significantly a fair relationship to the pathway travelled. The highest difference in the comparative data was discernible in the intestines. This could be explained in the light of the afore-mentioned deductions coupled with the resistance offered by the intestines no to allow the ascarids to penetrate and fix themselves to the walls of the intestines. There was no significant correlation between the alternative method (P-value: $\geq$0.001) and that of the golden standard. The individuals which have hepatic lesions and clear symptoms of migratory pneumonia do not correlate statistically with the values quantified and analyzed during the examination of the nose swabs. Yet this correlation

is much more unstable for the presence of adult parasites in the intestines.

Method		Post-mortem (results of nasal swabs)		Total
		Positive	Negative	
Coprological	Positive	22	122	124
	Negative	4	14	28
Total		26	136	162

There was an association between coprological results and results of nasal swabs, however it was not significant [P-value: 0.449; odds ratio and 95% CI: 0.63 (0.19, 2.10)]. Logically this correlation should have proven otherwise stable, but the immunity mechanisms in piglets allows for the body to fight off the penetration of the larvae to the mucosa, hence reducing the number of those (1/2) which stand a chance to mature and test positive in the coprological analysis (confidence interval at 95 %).

It could be concluded that the coproscopic examination is proving to be the most effective and easy-to-use method, while the examination of the liver proved practical and quite useful in the observations in the slaughterhouses. Meantime, the examination of the intestines for the presence of adult parasites was rated as being immensely successful for the diagnosis of ascarids in slaughterhouses. While the examination of intestines provided adequate data for the evaluation of the parasite load and the differentiation of ascaride populations which settle in the intestines of pigs. This technique when applied in the diagnostic laboratories is capable of evaluating the prepatente forms of ascarids in the intestines of piglets. Additionally, the examination of the lungs assumes a real significance regarding the observation of piglets in the slaughterhouses, while the examination of the nose swabs proved a simple and effective alternative to the diagnosis of an ascariasis both in the living piglets and the slaughtered ones in the abattoirs.

CONCLUSIONS

The study conducted in the slaughterhouses countrywide comprised a total of 162 piglets where the diagnosis of ascariasis was evaluated simultaneously by applying several alternative diagnostic methods. During the coproscopic examination as many as 124 piglets (equivalent to 76.54%) turned out to be affected. From the examinations done in the slaughterhouse milk spots were established to be present in 49 piglets or 30.24% of the samples surveyed. During the microscopic examination of the small intestines adult parasites were found to be residing in the intestines of 78 piglets or in 48.14% of the piglets examined. During the examination of lungs of piglets it was proven that parasitic pneumonia or clear consequences of parasitic migration were found in 34 piglets or in 20.98% of the samples examined. The examination of the nose swabs revealed that 26 piglets or 16.04% of the total samples tested positive for the presence of larvae. Through the coproscopic examinations it was established that the highest infestation levels of *Ascaris suum* were recorded in piglets against the total diagnostic alternatives as applied in the study. In addition to the lower cost of application, coproscopy was the most successful method used in the diagnosis of ascariasis in living and slaughtered piglets. Coproscopy was identified as a simple and extremely effective method for evaluating the parasitic prevalence and parasitic load. Coproscopy was identified as the most effective and easy-to-apply method while the examination of the liver was practical and very useful for the observations in the slaughterhouse. The examination of the intestines was evaluated as highly relevant to the diagnosis of ascariasis in the slaughterhouse. The examination of the lung can serve as a diagnostic alternative to the observations in slaughterhouses, while the examination of the nose swabs proved to be an alternative method to both living piglets and slaughtered ones the slaughterhouses. The control of liver and

lungs when signs of migrations and pneumonia can be established for the presence of invasive larvae along with intestinal control to evaluate adult ascarids can be applied as quite successful diagnostic techniques in the post-mortem diagnosis of ascariasis in pigs. While in the examination of piglets an analysis of the nose leaks can be administered for the presence of larvae as a highly efficient and cost-effective alternative method in diagnosing and monitoring ascaridiosis.

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