



EPIZOOTIC AND PATHOGENIC ASPECTS OF OLLULANOSIS INVASION IN PIGS

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ABSTRACT

Ollulanosis in pigs is a poorly understood disease that is quite common on pig farms in some areas; it causes significant damage to health of the animals and adversely affects the economy of animal farms. the influence of worms on the digestibility of the stomach in infected pigs and pathological changes in the liver, kidney, and spleen has been investigated. Ollulanosis infestation causes the development of inflammation in the stomach and other internal organs. Intravital and posthumous diagnosis of ollulanosis is based on a study of gastric mucus. a diagnostic method that enables accurate diagnosis of the intensity and extensiveness of the ollulanosis invasion has been developed.

1. INTRODUCTION

Pigs are one of the main sectors of livestock, it accounts for a significant percentage of the balance sheet of the total meat production in Ukraine. Analysis of the current state of the industry shows that pork production in the country takes place on specialized farms on an industrial basis and farms with traditional technology; pigs are also raised by farmers, on ancillary and individual farms. Important factors that inhibit the growth of livestock productivity and reduces pig numbers are worm infestations, including ollulanosis which occupies one of the most important places as to the size of the inflicted economic damage.

Ollulansare small (0.7-1.5 mm) worms which parasitize in the lumen and in the thickness of the gastric mucosa, ductless glands of the stomach, more rarely in the duodenum. a characteristic feature of the pathogen is that all stages of the parasite develop in the body of the same host. From 2 to 5 eggs are formed in the uterus of mature females. the eggs mature within 24 hours and larvae are hatched at the first stage. Within 3 days the larvae molt twice in the uterine cavity, become invasive and are released by the female into the lumen of the stomach or duodenum. the larvae mature and parasites can find their way into the stomachs of other animals, causing infection. Invasive larvae molt once and reach sexual maturity within 10 days Animals mostly become infected by eating vomit

mass. the disease is widely spread, especially among adult animals. Foci of olluianosis are often observed on traditional technology farms with with low health standards. Hundreds of thousands of parasites can infesta single animal. a certain role in the spread of olluianosis is obviously played by cats, dogs, and rodents [Goncharenko and others 2001]. Invasional so becomes possible though eating stomachs of slaughtered or dead animals.

Olluianosis is pigs is a poorly understood worm disease that tends to be widespread [Zakhrialov and Karchevska 1986; Karchevska 1996; Pozhyvil, Titarenko 1998; Goncharenko 2000]; it causes damage in the form of growth retardation of the young, mass loss in adult pigs, decreased milk production in sows, birth of non-viable offspring, and death of pigs. Thus, the weight of piglets at birth in non infested females was was established to be 103.8 grams higher compared to the offspring from the infested sows; at the age of 30 days this figure increased by 157 g, milk production in sows without worm invasion is by 11.6 kg higher compared to olulan infested animals. Weaker and smaller piglets are born in sows affected by olluianosis, whose death loss can be observed in the first 3-5 days after birth [Volkov, 1983; Volkov, 1985]. Given the facts, the question of distribution of olluianosis, its pathogenic effects on the body of animals and methods of diagnosis is very important and even though it remains poorly understood in Ukraine's conditions.

2. MATERIALS AND METHODS

The material for the study were pigs from the farms with different ownership forms in the central part of Ukraine and Podillya region [Karchevska 2016] . Intotal, 1,877 pigs from 82 farms in Dnipropetrovsk, Zaporizhia, Vinnytsia and Khmelnytskyi regions were examined.

To determine the origin of species, worms were collected from the stomachs of spontaneously infested pigs. Stomachs were selected at meat processing facilities, the former were cut along the lesser curvature, mucus was rinsed with water and scraped into into a test tube. Further mucus was placed in vitro on glass slides; under the microscope control ollas were separated from the mucus with the help of preparation needles.

Then helminthes were covered with cover slip and examined microscopically with low (7X8) and medium (10X40) magnification, measuring was done with an ocular line. While measuring those characteristics of olulans which are relevant for differential diagnosis, we examined and described, namely the length and width of the body, the distance from the tail end to the sexual orifice, number of the tail processes in females, and the structure of spicules in males.

Compression method of gastric mucus examination developed by V. Kazello (1971) was used in our modification to investigate the distribution of ollulanosis. Mucus was selected in graded test tube in the volume of 5.7 ml and examined on glass slides. to improve the compressor method that gives relative ratios of infection intensity, we conducted special research and developed a special coefficient.

Complete helminthological section of the stomach, duodenum, and esophagus was performed for this purpose. Material for research was selected from 15 spontaneously infected animals. Deep scrapings of mucosa from fundic, pyloric, and cardiac areas of the stomach, diverticulum, pylorus, esophageal and duodenal mucosa and mucosa proper were examined by digestion method for the presence of worms.

Simultaneously the number of olulans in 1 ml of mucus sampled from the fundus of the stomach of each animal was counted. Given the total number of parasites for each animal, we calculated the ratio of the total amount of worms against the number found in 1 ml of mucus taken from the fundus of the stomach by compression research and calculated the coefficient. Total infection intensity was subsequently calculated as follows:

$II = C : C1XK$, where:

II -infection intensity;

C - number of olulan specimens in the volume of investigated mucus;

C1 - amount of investigated mucus;

K - coefficient

To compare the effectiveness of our method with the results of complete helminthological section, we performed calculations of the infection intensity by this formula for each animal.

When studying pathological changes of the internal organs of pigs affected by spontaneous ollulanosis material for research (small, 1-2 cm thick pieces of heart, liver, spleen, kidneys, and fundus of the stomach) was selected from animals with infection intensity of 424, 304, 100 specimens in the sample. Material from an animal free of infestation was sampled for reference. the paper relies on conventional methods of histological research. Histological slides made from these organs were examined under a microscope with medium magnification (10X40). To study the effect of olulanson major functional performance of the gastric contents the stomachs of 14 animals were investigated. the sampling took into account the presence of stomach lesions characteristic of chronic hypertrophic gastritis. The study was conducted in terms of total acidity, unbound and bound hydrochloric acid. Acidity of the gastric contents was determined by classic

titration using indicators capable of changing their color in a specific area of pH environment. to this end we used the Töpfer method (1985), in order to separately determine total acidity, unbound and bound hydrochloric acid in two portions. in addition, we determined digestibility of the gastric juice according to Matt.

Mathematical and statistical processing of the research findings was conducted using the I.A. Oiwini's methods of variation statistics.

3. RESULTS

As a result of investigation conducted to identify the olulan species, the morphology of 48 female and 42 male olulans was examined. Comparing our findings with the description of the structure of the four known olulan species, we came to a conclusion that one type of olulans parasitizes both in the Central and Podillya regions of Ukraine: namely, *Ollulanus suis* Kasello, 1972.

As the findings of complete helminthological section revealed, olulans were found in all the investigated areas other than the esophagus. the largest number of olulans was localized in the fundus of the stomach, the percentage of olulans identified here was $88,5 \pm 1,10\%$ of the total. Much less was found in the contents of the organ ($6,25 \pm 2,40\%$), in 13 cases they were found in the cardia of the stomach ($3,93 \pm 1,03\%$), in 7 cases in the pyloric part ($0,88 \pm 0,22\%$), in 3 cases in the glandless part (0.05%) in 1 case in the diverticulum area (0.06%), pylorus zone (0.02%), duodenum (0.02%) and in 9 cases in the gastric mucosa proper, where the number of detected olulans was 0.26%.

As a result of the conducted experiment, we obtained coefficient equal to 12.4. This is the ratio of the total number of olulans found in the animals by complete helminthological section of the stomach, duodenum, and esophagus (with digestion of mucosa) to the number of worms found in 1 ml of mucus taken from the fundus of the stomach using compressor examination. After the infection intensity calculation for each animal according to our formula it appears that the data differ very slightly from the results obtained by complete helminthological section with digestion of scrapings and mucosa of the investigated organs. Statistical analysis of the results indicate that there is no significant difference in determining the infection intensity by both methods ($r > 0,5$). Taken into account the time spent on the study, the advantages of our method are obvious. it is convenient and easy to perform both in practical terms and for the purposes of scientific research.

Investigation concerning the spread of ollulanosis infestations found that in the Central Ukraine helminths were found in the animals of all the investigated areas. of the 70 surveyed farms in this area 49 (70%) proved to be afflicted by ollulanosis. of the 1333 examined 468 pigs ($35,10 \pm 5,82\%$) were infested with olulans. Extensiveness of infestation varied from area to area between 6.25% and 57.14%.

In studying the distribution of ollulanosis invasion in Podillya region, 7 of the 19 surveyed households (36.8%) appeared to be affected by ollulanosis with an average invasion rate of 26, 35%, i.e 117 heads of pigs from the examined 444 were infected with olulans.

Along with studying the spread of ollulanosis in vasionin pigs, we investigated age dynamics. as it turned out, ollulanosis in festation was generally absent in suckling pigs. in 2-4 month old animal sextensiveness of invasion was 7% (infection intensity of 14-148 specimens.), in 5-8 month old animals - 18.4% (11- 406 specimens.) in 9-12- month old animals - 41% (15-816 specimens.), in 1-2 year old animals - 58.17% (37-1014 specimens.) in animals older than 2 years the extensiveness of ollulanosis invasion was 86.15% with intensity of 164 - 6200 specimens). Thus, the higher the age of the pigs, the higher was the factor. the same pattern was observed when determining the invasion intensity.

Microscopically the internal organs of the three infested animals compared with reference group showed that in sows with invasion intensity of 100 specimens, the liver in the sample was enlarged, of soft texture, gastric, mucosa had some mild wrinkles, covered with transparent sticky mucus. the mucous layer was increased by 1.2 times. Pinpoint hemorrhages were observed under the mucosa. When examining histological slides, areas of mild dystrophy were found in the liver, areas of proliferation of reticuloendothelial system cells were observed in the kidneys, severe cell infiltration of the mucosal layer in the stomach slides, growth of young granulation tissue, desquamation and mucous dystrophy of the surface epithelium and epithelium of the gastric pits, and dystrophy of the gastric glands and congestion of the sub mucosal layer proper.

In sows with the invasion intensity of 304 specimens in the sample, the liver was also increased, of soft texture, gastric mucosa was folded, brain-like, the folds increased to 5 mm, with a large quantity of cloudy viscous mucus. Regional lymph nodes increased in volume. Histologically, opaque swelling of the cellular cytoplasm was found in the liver, degenerative changes of the convoluted tubules of the epithelium in the kidneys, necrosis of the columnar

epithelium in the stomach, fragmentation of the gastric pits, necrosis of the fundus glands and mucosa proper.

In sows with invasion intensity of 424 specimens in the sample, enlargement of the liver was revealed, its edges were blunted, the kidneys were gray-brown in color, of soft texture. the mucous membrane of the stomach was wrinkled, brain-like, with pinpoint hemorrhages on the tops of the folds, folds increased to 6.7 mm, with a large quantity of cloudy viscous mucus from the grayish-white loose clots. Histologically degenerative changes in the liver cells were found, degeneration of the epithelium of the convoluted tubules in the kidneys was revealed with the presence of loose protein mass in the lumen of the tubules. the gastric mucosa was sharply increased; the gastric glands and stroma were compressed and were in a state of atrophy.

No changes were found in the heart and spleen of all three animals compared to reference group

Tab. 1. Indicators of acidity and digestibility in gastric contents in olulan infested and non infested pigs

Groups of stomachs with pathological changes of stomach mucosa	Total acidity, titre units	Hydrochloric acid		Digestibility according to Matt, mm	Invasion intensity, number of specimens.
		unbound titre units	bound titre units		
Without visible pathological changes(reference) (n = 5) M±m	52	16	16	2,5	0
	42	12	12	2,3	0
	46	18	14	2,7	0
	44	16	16	2,6	0
	40	14	12	2,8	0
	44,8±9,46	15,2±1,02	14±0,89	2,6±0,36	0
With signs of chronic hypertrophic gastritis (n = 4) M±m p<	18	0	11	1,3	0
	20	0	12	1,6	0
	24	0	14	1,4	0
	17	0	10	1,2	0
	19,8±1,55	0	11,8±0,85	1,3±0,040	0,001
	0,02	0,001	0,1	0,01	0,001
With signs of chronic hypertrophic gastritis in the presence of invasion (n =5) M±m p<	12	0	8	1,0	66
	16	0	9	1,2	44
	10	0	0	0,4	586
	8	0	0	0,2	820
	14	0	6	1,1	112
	14±1,15	0	7,66±0,87	1,1±0,05	74±10,9
0,01	0,001	0,001	0,001	0,001	

As seen from table 1, five of 14 samples of the investigated stomachs were used as reference. These were stomachs without apparent pathological changes and free of olulan invasion. Data obtained as a result of the investigation of these samples were within normal range fluctuations. This confirms the acceptability of our sampling methods for research. the second group consisted of samples from four stomachs having the signs of chronic hypertrophic gastritis and also free from infestation. the third group included five stomachs

with the signs of chronic hypertrophic gastritis in which ollulanosis with the intensiveness of infection from 44 to 820 olulans was found.

The data from table 1 indicate that compared to reference group all the indicators, depending on the depth of the pathological changes, have a tendency towards reduction. In the gastric contents with symptoms of chronic hypertrophic gastritis and stomachs with the signs of chronic hypertrophic gastritis and presence of invasion pathogens, free hydrochloric acid was completely absent. Bound hydrochloric acid, total acidity and digestion in the stomachs with the presence of invasion were significantly lower not only in comparison with the reference group, but also compared with the indicators for non invaded stomachs with the symptoms of chronic gastritis.

In two cases in the samples with the intensiveness of invasion of 586 and 820 olulan specimens (maximum indicators), hydrochloric acid was completely absent, and general acidity indicator and digestability were very low in comparison with the reference group and non invaded stomachs with the signs of chronic gastritis. Statistical analysis of the obtained data showed that only in one case, when comparing indicators of the bound hydrochloric acid, the difference in the results was improbable ($r < 0,1$). In all other cases, the difference in the results was probable ($< 0,001 - 0,02$).

4. DISCUSSION

The results of our studies have shown that ollulanosis invasion is quite common in the surveyed areas and regions of Ukraine. It should be noted that there is no strict interdependence between the extensiveness of invasion and location of farms in particular economic-administrative areas. Although climatic conditions, terrain, nature of vegetation, and soils vary in certain areas, they are unlikely to affect the indicators of the extensiveness of invasion. This is due primarily to the fact that olulans pass their entire lifecycle inside the host, which is their regular environment.

When studying the age related dynamics of ollulanosis invasion, an evident increase in both intensity and extensiveness in relation to the animals age is observed. Our data indicating that these rates increase with the age of the animals are consistent with the results obtained by other authors [Caselli 1971; Volkov, 1984]. In addition, our results are in keeping with the data of F. Volkov (1984), who found olulans starting with 2-month-old pigs. While other authors were able to find them in 1-month-old pigs [Voronkov 1986; Goncharenko 2000]. Arguably, specific factors contributed to this situation.

As for the study of pathological changes during the spontaneous ollulanosis invasion in the first case, when investigating the internal organs of sows with the intensity of infestation of 100 olulan specimens in a mucus sample, we found dystrophy of the liver, interstitial glomerulonephritis, and sub acute catarrhal desquamative gastritis.

When investigating the internal organs of sows with the invasion intensity of 304 specimens in the sample, we found changes characteristic of chronic alternative gastritis, muddy swelling of the liver cells, and initial signs of hypertrophic lymphadenitis.

In the study of the internal organs of sows with the invasion intensity of 424 specimens of olulans in the mucus sample we found dystrophy of the liver and kidneys, glomerulonephritis, chronic catarrhal hypertrophic gastritis with severe gastric epithelial desquamation covering the gastric pits, and signs of hypertrophic hemorrhagic lymphadenitis.

On the basis of the described macro and micro changes in the internal organs it can be assumed that in the animals spontaneously infested with ollulanosis agents, postmortem changes depend on the intensity of infestation: the higher it is, the more changes are produced. This is especially true of gastric mucosa. the development of hypertrophic chronic inflammation of the gastric mucosa is obviously associated not only with the mechanical action of nematodes, but also with the constantly irritable influence of the waste products and decomposition of olulans and various complications of the pathological process of the accompanying micro flora

Inflammation, proliferation of fibrous connective tissue, atrophy of some and hypertrophy of other glands of the gastric mucosa lead to disruption of the secretory activity increasing the quantity of the exported secret, changing both its physical (viscosity, texture, color) and, as it turned out, its biochemical properties.

Since the course of the disease is chronic and waste products of the parasitic helminths protractedly act on the body, it is likely that the changes affect such organs as the regional lymph nodes, liver and kidneys. All this, of course, affects the digestion and absorption of food, as well as overall health of the olulan infected pigs.

In his works Monning (1956) reports that he observed chronic catarrhal gastritis in the infected pigs which fact was confirmed by our research. According to Goncharenko (2001), path morphological changes in spontaneously and experimentally infected animals are dependent on the intensity of infestation: the higher it is, the more significant are postmortem changes, which fact is also confirmed by our research.

The findings of the conducted research as to pathological changes in the gastric mucosa of the olulan infected pigs urged us to continue work in this direction and study the effect of the invasion pathogens in the presence of chronic gastritis on the major functional performance factors of the gastric contents of pigs.

According to our results a complete absence of hydrochloric acid and a significant reduction in total acidity indicates chronic gastritis in progress. This is also evidenced by reduction in gastric activity of the gastric contents. The acidity of the gastric contents is a kind of stimulus for the opening and closing of the sphincter. Evacuation of chyme into the duodenum depends on the concentration of hydrochloric acid in it; with acidity being low, the pylorus sphincter may not close at all, hence development of various dysfunctions of the gastrointestinal tract.

On the basis of the conducted research, we can conclude that chronic catarrhal processes in gastric mucosa with the presence of olulans impact the changes in key functional parameters of the gastric contents in pigs more than chronic inflammation in non infested stomachs and, accordingly, to a deep pathological changes in the stomach and functional capacity of the gastrointestinal tract. Thus, the higher the intensity of ollulanosis invasion, the deeper will be postmortem changes in the stomach and the lower the functional parameters of its contents.

Similar studies in this area have not been conducted by anyone, our findings suggest the possibility of using the acidity indicators and stomach secretions digestible ability to develop intravital diagnostics of ollulanosis.

5. CONCLUSIONS

Ollulanosis in pigs is a fairly common helminthic disease observed in different regions of Ukraine. One type of olulan parasitizes in the central part and Podillya region of Ukraine, namely, *Ollulanus suis* Kasello, 1972. The main area where olulans parasitize in the body is the fundus of the stomach - $88,5 \pm 1,10\%$ of the total number of worms in the stomach, in the cardiac part the figure was $3,93 \pm 1,03\%$, in the pyloric part $0,88 \pm 0,22$, and in the gastric contents - $6,25 \pm 2,40\%$.

To determine the infection intensity of ollulanosis by the compression method, a ratio of 12.4 is proposed which is the total number of olulans found in animals by complete helminthological section of the stomach, duodenum and esophagus to the number of worms found in 1 ml of mucus from the fundus of the stomach during compressor investigation.

49 of the 70 surveyed farms in the Central part of Ukraine (70%) appeared to be affected by olluianosis, and 468 of the 1333 investigated pigs ($35,10 \pm 5,82\%$) were infected with olulans. in the Podillya region 7 of the 19 surveyed households (36.8%) turned out to be affected by olluianosis with an average rate of invasion from 26 to 35%. Extensiveness and infection intensity depend on the age of the animals. These figures were the lowest among 2-4 months old pigs, respectively 7% and 14-148 specimens, the highest in the animals whose age was over 2 years (respectively 86.15% and 64-6200 specimens).

Sub acute catarrhal, desquamative, alternative and chronic catarrhal hypertrophic gastritis and degenerative changes in the liver and kidneys were detected in pigs affected with olluianosis. with olulans present in the gastric contents, unbound hydrochloric acid is completely absent, bound hydrochloric acid content, total acidity, and digestibility of the gastric juice reduced. it has been established that the higher is the infection intensity, the lower are these figures.

REFERENCES

1. Volkov F.A. 1983. Influence of Ollulans on Productivity of Pigs. Syb. Bulletin of Agric. Science, 4: pp.68-72.
2. Volkov F.A. 1984. Season and Age-related Dynamics of Ollulanozis. Diagnosis and Disease Prevention in Animals on Farms and Complexes. Novosibirsk: pp.23-29.
3. Volkov F.A. 1985. Influence of Ollulans on the Growth of Piglets. Syb. Journal of Agric. Science, 4: pp.103-105.
4. Voronkova Z.G. 1986. Epizootological Features of Ollulanosis in Pigs in the North Caucasus Zone. Abstack of 9th All-Union Conference of Helminthological Society. Tbilisi: pp.32-33.
5. Levytska, V., Mushynskyi, A., Hutsol, T.: Detection and Diagnosis of Encephalitozoonosis in Rabbits Under Ukraine-Specific Conditions. Scientific achievements in agricultural engineering, agronomy and veterinary medicine: Polish-Ukrainian cooperation [Electronic resource]: scientific monograph. State Agrarian and Engineering University in Podilya, Agriculture University in Kraków. Vol. I. Kraków: Traicon, 94-107 (2017).
6. Goncharenko V. 2000 Epizootological Aspects of Ollulanosis Invasion in the Polissia Area and Forest-Steppe Ukraine. Bulletin of Bilotserkiv. State Agrar. Univ, Coll. of Research Papers. Bila Tserkva., 14: pp.166-169.
7. Goncharenko V.P. 2001, Ponomar S.I. State of the Cellular Factors of the Immune System of Pigs Afflicted by Ollulanosis. Bulletin of Bilotserkiv. State Agrar. Univ, Coll. of Research Papers. Bila Tserkva., 16: pp.51-58.
8. Goncharenko V.P., Ponomar S.I., Antipov A.A., 2001. Susceptibility of Carnivores and Rodents to Ollulanosis Invasion. Problems of Zooengineering and Veterinary Medicine: Collection of Resarch Papers of Kharkiv Zoovet. Institute -Kharkiv-7 (31): pp.227-228.
9. Caselli 1971. Distribution, Age and Season-related Dynamics of Ollulanosis in Pigs in the Jewish Autonomous Region. Siberian Journal of Agric. Science, 3: pp.82-84.

10. Zakhryalov YA.N., Karchevskaya T.N. 1986. Toward Research of Ollulanosis in the Steppe Pigs in the Southen Area of Ukraine. Materials of X. Ukrainian Conference of Parasitological Society. Odessa, 1: 216.
11. Karchevska T.N. 1996. Ollulanosis in Pigs in Central Ukraine. Extended Abstract of Cand. Sci. Dissertation. 16.00.11. Kharkiv.
12. Karchevska T.N. 2016 Ollulanosis in Pigs – a Topical Problem of Modern Pig Production. Domestic Science at the Turn of Eras: Problems and Prospects. Materials of XXVII All-Ukrainian Scientific-Practical Internet Conference, 27. Pereyaslav-Khmelnytskyi: pp.436-438.
13. Pozhyvil A.I., Titarenko V. 1998. Ollulanosis in Pigs. Vet. Medicine of Ukraine, 4: 17.
14. Monning H. 1956. Veterinary Helminthology and Entomology, London: 232.