UDC 619:616.98:579.842.11:636.4/.5

BIOTECHNOLOGICAL ASPECTS OF AMIXIN® APPLICATION AS AN ANTIVIRAL DRUG FOR TREATMENT OF PIGS AND CHICKEN

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Summary. Veterinary drug Amixin®, active substance dihydrochloride 2,7-bis[2-(diethylamine)ethoxy]fluorene-9-one (AMX), was tested for its antiviral activity with using of the epizootic relevance for Ukraine the infectious agents of Pseudorabies (PR), Teschovirus encephalomyelitis (TEM), classical swine fever (CSF), porcine reproductive and respiratory syndrome pigs (PRRS), 2nd type of porcine circovirus (PCV-2) and parvovirus infections (PPVI), swine (SIV) and avian influenza viruses (AIV). The influence of this drug on agents of the avian mycoplasmosis (Myc) and pasteurellosis (Past) was also learned. AMX action was tested in the concentrations of 0.5–15 mg/ml, expositions for 0.25–12 hours and at the room temperature. Under these conditions, the inactivation of 20–60% (the highest concentrations more likely) the 1000 infectious units (TCID, ELD or $PFU_{50/ml}$, respectively) of all viruses was resulted. The 5000 $ELD_{50/ml}$ of the SIV and AIV viruses were inactivated for 5 hours almost totally. Moderate toxicity of AMX was registered in it doses ≥1 mg/ml (P≤0.01) for tube cultures of PK-15 cell line and Marc-145. At the same time AMX doses ≥0.125 mg/ml inhibited by 20–75% of infective activities of agents of the PR, PRRS, PCV-2, PPVI (n=42, P≤0.01) in cell cultures Marc-145 and PK-15, respectively. The toxicity of the drug for 7–9-days-old embryos chickens began to emerge with a concentration of 1.5 mg/ml (n=18, P≤0.01). Its virostatic effect on SIV, AIV and PR agents was manifested in doses ≥0.25 mg/ml (n=24, P≤0.01). AMX was administrated in a single dose of 540 mg ana partes with sunflower oil and showed no toxicity for suckling piglets (n=4, P≤0.01) and 10-days-old chickens (n=10, P≤0.005). At the same time the antibiotic resistant Myc and Past from the blood of chicken infected by natural mixes of these agents, acquire the sensitivity to commercial food antibiotic after 5-days course of treatment by AMX. These data is interpreted the mechanism of therapeutic and preventive action of AMX through direct antivirus activity. The hypothesis of acquires the antibiotic sensitivity by pathogenic bacteria throughout its bacteriophages inhibition is proposed.

Keywords: Amixin, toxicity, antiviral activity, porcine viruses, avian agents, bacteriophages

Introduction. Antiviral activity of drugs is characterized by its virocid and virostatic activities (Mashkovskyi, 1997). It is very important to conduct of antiviral assessments with use of variants of the infectious agents that are circulated in nozoareals where target drug is planed to use (Drew, 2011; Zhu, Zhou and Tong, 2012). Most actual for Ukrainian pig farming during last 15 years were 'economical' diseases — porcine concurrent virus-bacterial infections with participation of next infectious agents: herpes-, pesti-, arterio-, circo-, tesho-, parvo- and orthomyxoviruses (Buzun, Prokhoryatova and Kolchyk, 2001).

Since 2008, the market of veterinary drugs Ukraine has a new veterinary preparation Amixin® (AMX) with synthetic substance dihydrochloride 2,7-bis[2-(diethylamine)ethoxy]fluorene-9-one. This substance is characterized with immunomodulatory and antiviral properties and produced by LLC 'InterChem' (Odessa) in slightly modified form of the well-known medical drug Amixin® IC of the same manufacturer (interchem.com.ua/en/drugs/amiksin®-Ic). Since 1990th Amixin® IC is widely used in the medical protocols to prevention and treatment of flu, herpetic, chlamydial, arboviral and other human infectious diseases (Ershov et al., 1998; Demchenko et al., 2000). Like to its American analogue — tyloron — there are huge data on the mechanism of therapeutic and preventive action of AMX substance in cells and humans and mammals (Andronati, Litvinova and Golovenko, 1999; Lyakhov and Litvinova, 2008; Zholobak et al., 2012). However, only few results of a veterinary application of this drug we have in pig breeding and poultry — unfortunately without of data on its antiviral activity against the respective porcine and poultry pathogens.

In the current paper presents the summarized results of the study cyto- and ovotoxicity and virocid and virostatic activities of veterinary AMX to determine its mechanism of action in the possible chemoprevention/treatment of Pseudorabies (PR), Teschovirus encephalomyelitis (TEM), classical swine fever (CSF), porcine reproductive and respiratory syndrome pigs (PRRS), 2nd type of porcine circovirus (PCV-2) and parvovirus infections (PPVI), swine (SIV) and avian influenza viruses (AIV). The influence of this drug on actual for Ukrainian poultry pathogens — avian mycoplasmosis (Myc) and pasteurellosis (Past) agents was learned also.

Material and Methods. ***Viruses*** — Porcine Herpesvirus I, Pseudorabies virus (PRV), strain 'Donbas-1082' (Д-1082), with infectious activity 7.0–7.5 lg rabbit-lethal doses ($LD_{50/ml}$), or 6.0–7.0 lg tissue-cytopathic infectious doses ($TCID_{50/ml}$); this agent was isolated from porcine brain in herd of small holding during PR epizootic outbreak in Chervonoarmiys'k Region of Donbas near porcine mega-complex 'Agroinvest' at 2011 and was adapted to PK-15 cell line (was used on level of 7th passage). — Teshovirus (TV), strain 'Butcha', with infectious activity 8.0–9.5 lg porcine-lethal doses ($LD_{50/ml}$), or 6.5–7.0 lg $TCID_{50/ml}$; this agent was isolated from porcine brain in herd of Poltavian back-yard holding during panzootic outbreak of TEM in 2002 and was passaged on guinea pigs and domestic pigs by per-oral route and adapted to different porcine cell lines (was used on level of 24th passage PK-15 cells). — Pestivirus, classical swine fever virus (CSFV), vaccine strain 'C', with infectious activity 4.0–4.5 lg rabbit-fever doses ($RFD_{50/ml}$), or 5.0–5.5 lg plaque-forming doses ($PFU_{50/ml}$); this agent was isolated from porcine blood in herd of Kharkiv small commercial holding on 4th day after regular vaccination at 2010 with standard vaccine manufactured on State Kherson Bioplant and was passaged on rabbits by intravenous route and adapted to PK-15 cell line (was used on level of 6th passage in this cells). — Arteriovirus, Porcine reproductive-respiratory syndrome virus (PRRSV), strain 'Vody Donbasu-08' (WD-08), with infectious activity 5.0–6.5 lg $TCID_{50/ml}$ in porcine alveolar macrophages (PAM) and 2.5–3.0 lg $TCID_{50/ml}$ in MARC-145 cell line; this agent was isolated from porcine lien in herd of Gorlovka' small commercial holding (Donbas) during PRRS outbreak at 2008 and was passaged by consecutive reproduction in suckling piglets and PAM at beginning and then adapted to MARC-145 cell line (was used on level of 9th passage in this cells). — Circovirus, Porcine circovirus type 2 (PCV-2), strain 'Ingulets'kiy-1024' (I-1024), with infectious activity 5.0–7.0 lg $PFU_{50/ml}$ in PAM and PK-15 cell line; this agent was isolated from porcine intestine lymphatic nodules in herd of Kherson farmer holding 'Dulya' in enzootic area for porcine circoviral mix-infections at 2009 and was passaged by consecutive reproduction in suckling piglets and PAM at beginning and then adapted to PK-15 cell line (was used on level of 9th passage in this cells). — Parvovirus, Porcine parvovirus (PPV), strain 'H-1', with infectious activity 5.0–7.0 lg $TCID_{50/ml}$ in PK-15 cell line; this agent was isolated from kidney of porcine embryo in herd of Sumy farmer holding in enzootic area for porcine virus-bacterial mix-infections at 2011, and was passaged by consecutive reproduction and adaptation to different porcine cell lines (was used on level of 4th passage in PK-15 cell line). — Orthomyxoviruses, swine (SIV) and avian (AIV) influenza virus, strain A/swine/Skadovs'k/01/11 (H2N?) and strain A/chick/Sivas/03/07 (H5N1), respectively. Both have infectious activity 5.0–7.0 lg chick embryo lethal doses ($ELD_{50/ml}$); SIV was isolated from lung-lien suspension of pig during epizootic outbreak in herd of pig-breeding holding (Kherson region) at 2012; AIV was isolated from brain of sick chicken during panzootic outbreak of avian flu in back-yard holding (Crimea region of Ukraine) at 2007; both agents was passaged by consecutive reproduction and adaptation to 7–9-days-old chicken embryos (was used as extra-embryonic fluids of 12th passage of the AIV and 7th passage of the SIV).

Natural Consortia (Con) of *Pasteurella multocida* type A, *Mycoplasma gallisepticum* and more then 2 species of unidentified bacteria and bacteriophages, which isolated from blood of chicken of the commercial flock in farmer holding of Kharkiv region at 2015 by traditional bacteriological methods. The consortia used in two forms: isolates 'Con A' — contain microorganisms (including the bacteriophages) which are insensitive to mix of gentamicine-tylosine on test-disks (1 mg/ml every); it was isolated from blood of 10-days-old infected chickens (n=5) before treatment with AMX; isolates 'Con B' — contain microorganisms (the bacteriophages are absent) which are sensitive to mix of gentamicine-tylosine on test-disks (1 mg/ml every); it was isolated from blood of 15-days-old infected chickens (n=5) after treatment with AMX (triple watering, once per-day every day, dose 15 mg/ml).

All of these agents are deposed, stored and maintenance in the Collection of microorganisms of NSC 'IECVM' (Kharkiv).

Systems for viruses' maintenance and titration — Cell cultures — PK-15 cell line (secondary culture of embryonic kidneys pigs) received from the Collection of cell cultures of NSC 'IECVM' and used at 3–7 passages after thawing, grown under standard operating procedures (SOP) NSC 'IECVM' in test tubes and plastic cultural vessels using a mixture of media № 199 and media Eagle MEM with 10% calf serum (Veterinary Medicine Ltd., Kharkiv). The PK-15 cell line used to work as a 2–4-days-old' monolayer. — MARC-145 cell line (the clone of MA-109 cell line secondary culture of the embryonic kidneys green monkey) received, growth and used similarly to PK-15 cell line with next differences: used at 5–11 passages after thawing, growth medium contain 10% bovine fetal serum (Invitrogen, USA). — Porcine alveolar macrophages culture (PAM) prepared from lungs of 3-month old piglets by SOP NSC 'IECVM' in test tubes and plastic cultural vessels using a mixture of Hanks' media with 15–20% homologous porcine serum (growth media); these cultures used to work as a 4–7-days-old' monolayer.

SPF-eggs received from the Department of studying avian diseases NSC 'IECVM' and used for maintenance of PR virus used 5–7-days-old CE and of CSF virus — 9–10-days-old CE.

Pasteurella lytic bacteriophages isolation. 5 ml of 48 hours culture of Con in meat-peptone broth (MPB) combined with 25 ml of MPB ('Meat Peptone' HI Media Laboratories) and incubated at 37 °C with magnetic stirrer for 4 hours. Followed this, 5 ml of a mid-log phase culture (the 5 hours culture of strain MPB with 5% of horse serum) of the 'L' strain of *P. multocida* type A and horse serum to final concentration 5% were added and the mixture incubated at 37 °C overnight without stirring. The following day top 5 ml of culture was harvested and filtered using a 0.20 μm membrane filter (Millipore, Massachusetts, US) to remove the debris. The filtrate was stored at 4 °C until required.

Work with infectious materials was carried out in boxed areas with BSL-2. Infectious activity of model viruses was determined by titration in relevant biological systems (in cell cultures grown in test tubes or embedded in them glasses or in chicken embryos, 4–5 tubes or embryos on every dilution of virus) by Reed and Munch (1938).

Determination of the *Pasteurella* lytic bacteriophages by routine test dilution (RTD) — The turbidity of an overnight culture of the 'L' strain of *P. multocida* type A in MP-Broth with 5% horse serum was adjusted to McFarland standard of 0.5 (1.5×108 cfu/ml) using isotonic peptone-saline. The surface of a MP-Agar plate then covered with 0.2 ml of this suspension and placed in a 37 °C incubator to dry for twenty minutes. Ten microliters of each dilution dropped onto the surface of the inoculated plates and left to dry for ten minutes. After which the plates incubated at 37 °C and examined for the presence of plaques at 24-hours intervals. The most diluted suspension to produce complete clearing considered as the routine test dilution (RTD).

As the main method of assay of virucidal activity of the AMX we used operating protocol to the study of anti-HIV properties of medical drugs (Erice et al., 1993) in the next modification. The cultural fluids of the PCV-2 and CSFV (1000 PFU_{50} of each virus), PRV, TV, PPV and PRRS (1000 $TCID_{50}$ of each virus) and SIV or AIV (5000 ELD_{50} of each virus) every in volume of 5 ml combined with 5 ml of AMX dilutions on solvent (sterile buffered saline with 250 OD/mg mixture of penicillin-streptomycin, pH 7.4) — experimental batch or with 5 ml of solvent without AMX — mock batch. There next AMX final concentrations were checked (mg/ml): 0.1, 0.5, 1.0, 5.0, 10.0, and 15.0. All batches were incubated under room temperature during 15, 30, 60, and 90 min and under 6 °C during 12 hours additionally. Then all batches were passed throughout Sephadex G-25 (coarse) that was packed in self-made micro-columns (high of gel 10 cm, diameter 0.5 cm, eluent — solvent as above) for each specimen (volume 0.75 ml, marker — hemoglobin, velocity of elution — 25–27 drops per min). The first fraction in volume 1.5–1.7 ml was collected in each case. This procedure was performed to liberate of specimen of experimental batch from AMX. Each specimen in volume 0.1 ml was inoculated in 4–5 tubes with mature monolayer of the respectively cell culture or in allantois of chicken eggs (n=4–5 for each specimen). A recognizing of the AMX effect was performed on results of assay of level inhibition (% of inhibition — as can see at Table 1) of viral activity in experimental batch in compare with mock batch. The presence of viruses in cell cultures was assayed by specific TCID with selective verification in immunoperoxidase method (IPM) for PRV, TV, PPV and PRRS or in IPM only for CSFV and PCV-2. The presence of SIV and AIV agents in embryos was estimated by specific signs of embryonic death and by additional titration of allantois' fluids from each embryo in standard hemagglutination (HA) with chicken erythrocytes and by identification of influenza agents in standard HA-inhibition (IHA) tests.

The three repeats of these studies were conducted with each mentioned agent.

Virostatic activity of AMX was studied after determining of its ovum-toxicity, maximal allowable dose (maximum permissible concentration) and cytotoxicity for estimation rank of doses of the drug that are not toxic for chickens, pigs, embryos and cell cultures. Ovum-toxicity was studied on 10-days-old CE and 14-days-old duck embryos. Cytotoxicity determined by inoculation of 3–4 days-old cell cultures PK-15 and AMC solutions of AMX in concentrations of 0.1–10.0 mg/ml (step of dilution — 2) on cultural medium.

The maximum permissible concentration (MPC) of the drug for poultry was determined at 1-week-old chickens, 3-days-old piglets by oral way with water suspension of AMX at doses of 35, 70, 140, 280 and 560 mg, 4 animals for each dose. The animals were observed on manifestation of clinical criteria of 'chronic toxicity' — including the measuring of average growth velocity within three weeks after AMX introduction (Stefanov, 2002).

Results and discussion. Each of the tested viruses in tested concentrations of the AMX drug demonstrated significant virocid activity (n=73, $P \leq 0.025$). The 12 hours exposition at room temperature of epizootic variant of the PRRS virus as well as vaccine strain of CSF virus without AMX (control) led to its inactivation by the 25% rate. The same inactivation degree is registered in these viruses when they were incubated 12 hours with AMX that taken in concentrations of 0.1–0.5 mg/ml (Table 1). Enhancing of the AMX concentrations

to 1.0–10.0 mg/ml led to these viruses inactivation by on 50–75% at incubation at room temperature for 0.5–1.5 hours even. There complete inactivation of PRRS virus in mixes with the AMX was during of 15 min. — incubation period at three repetitions under room temperature when the drug was used at a concentration of 15 mg/ml. Complete inactivation of CSF virus vaccine was observed under the same conditions, but when the reaction mixture incubating at least 1 hour. The most sensitivity to the AMX was revealed in PR, SIV and AIV viruses: they were fully inactivated already when AMX concentration in the reaction mixture was of 5 and more mg/ml under room temperature during 1 hour to 15 minutes: as more the AMX concentration was, then faster these viruses was inactivated.

Table 1 — Amixin® virocid activity

Amixin® concentration, mg/ml	Inactivation level of the viruses, % (high point demonstrates the hour rate)						
	PRV[c]	TD[b]	PCV-2[a]	PPV[b]	PRRS[b]	CSF[a]	TG[d]
0.0	0^{12}	0^{12}	0^{12}	0^{12}	25^{12}	25^{12}	0^{12}
0.1	0^{12}	0^{12}	0^{12}	0^{12}	25^{12}	25^{12}	0^{12}
0.5	25^{12}	0^{12}	0^{12}	0^{12}	25^{12}	25^{12}	25^{12}
1.0	75^{12}	50^{12}	50^{12}	25^{12}	$50^{1.5}$	75^{12}	$75^{1.5}$
5.0	100^{1}	50^{1}	$75^{1.5}$	$25^{1.5}$	$50^{0.5}$	$75^{1.5}$	100^{1}
10.0	$100^{0.5}$	$50^{0.5}$	100^{1}	$75^{1.5}$	$75^{0.5}$	$75^{1.5}$	$100^{0.5}$
15.0	$100^{0.5}$	$75^{0.5}$	$100^{0.5}$	75^{1}	$100^{0.25}$	100^{1}	$100^{0.25}$

Marks: [a]1000 $PFU_{50/ml}$; [b]1000 $TCID_{50/ml}$; [c]1000 $ELD_{50/ml}$; [d]5000 $ELD_{50/ml}$

The high sensitivity to the drug was also found in the PCV-2 agent of pigs: it completely inactivated during 1 hour exposure in an 'AMX-virus' mixtures with the preparation concentration 10 mg/ml and already for a 30-minute after exposure with AMX in concentration 15 mg/ml. The PPV and TV agents were the least sensitive to the contact inactivation with the AMX: their inactivation by drug at it concentrations of 5–15 mg/ml was not finished for 0.5–1.0 hours (n=24) — only when time of their exposition with the drug was raised to 12–18 hours the inactivation of these agents were inactivated completely (in Table 1 not shown).

Therefore as data above demonstrate, the veterinary drug Amixin® in wide of the concentration range hold significant virucidal contact activity against all of investigated of the modern Ukrainian variants of dangerous porcine and avian viruses — the pathogens of SIV, AIV, PR, PRRS, CSF and PCV-2, and in lower degree this relate to the TV- and PPV- pathogens.

Table 2 — Toxic effect of Amixin® in vitro, in ovo and in vivo

Amixin® concentration, mg/ml	Toxic influence in the biological systems after Amixin® inoculation					
	in vitro			in ovum	in vivo	
	PK-15	Marc-145	PAM	Chicken embryos	3-days-old piglets	3-days-old piglets (adm. with oil)
0	0/4	0/4	0/4	0/4	0/4	0/4
0.1–0.05	0/8	0/8	0/8	0/8	-	-
1.0–5.0	4/4	2/4	2/4	2/4	-	-
10.0	4/4	4/4	4/4	4/4	0/2	-
20.0	-	-	-	-	0/4	-
70.0	-	-	-	-	2/4	0/4
140.0	-	-	-	-	4/4	0/3
280.0	-	-	-	-	2/2	0/3
560.0	-	-	-	-	4/4	0/7

Table 2 summarizes the experimental data of toxicity of AMX investigation in cell cultures PK-15, MARC-145 and PAM, chicken embryos and neonatal piglet (with the addition of sunflower oil, and without it). The results show that the aqueous solution of the AMX in the concentration range from 0.1 to 0.5 mg/ml did not cause toxic damage cell cultures and embryos. In the concentration range 1–5 mg/ml some toxicity was showed for PK-15 cell culture (by signs of toxic degeneration of monolayer in all test-tubes) and embryos (delay of embryos development that are not accompanied by their death).

Processing with by AMX of MARC-145 and PAM cultures demonstrated the increasing of anisotropy of cytoplasm in the half of the used cells. This sign was not observed in control test tubes (without AMX). The dose 10 mg/ml of the AMX was toxic for all of used cultures and embryos, but the AMX was nontoxic for suckling piglets (n=6) in doses to 20 mg/ml. When we checked on piglets and chickens the suspensions of AMX in concentrations 70–560 mg/ml, we were must to use the DMSO for better dissolution of AMX. As result we observed some toxic effect of DMSO (as we believe) for piglets but not for the 10-days-old chicken (in Table 2 not shown).

Anyone from these piglets did not show signs of intoxication in the single AMX dose of 5 ml (concentration 560 mg/ml). In next three week after the watering of the drug, their average growth was 42.3±11.7 g (n=20, P≤0.01), while the control (mixture of PBS with oil) — 37.0±10.3 g (n=20, P≤0.005). It should be noted that in the chicken embryos (n=12 of 17) and embryos of ducks (n=19 of the 20, in Table 2 not shown) that survived the treatment of drug in AMX single dose of 0.2 ml (concentration 560 mg/ml, solvation with DMSO). They demonstrated no signs of teratogenicity of the AMX: all chicks and duckling in the next 3 weeks after hatching (time of observation) had no abnormalities in the development and did not differ from control nestlings (n=5) which were treated by saline in the same dose.

On the basis of determining toxicity as above, AMX virostatic action in vitro and in ovum was studied in the AMX range of concentrations of 0.125–1.5 mg/ml (Table 3). There was found that the concentration of 0.125 mg/ml drug blocks the reproduction of viruses CSF, TV, PCV-2, PPV, SIV and AIV for 24 hours, compared to control (infected cell culture without drug treatment).

Table 3 — Virostatic effects of Amixin®

Amixin® concentration, mg/ml	Time of the virostatic effect of the Amixin® in ovo/in vivo, hours						
	PRV	TV	PCV-2	PPV	PRRS	CSF	TGE
0	N	N	N	N	N	N	N
0.125	N	24	24	24	48	24	24
0.250	12	96	96	24	96	96	48
0.500	48	96	96	24	96	96	96
1.000	120	D	D	D	D	D	96
1.500	120	D	D	D	D	D	96

Marks: N — no inactivation of virus; 12–96 — time of the inactivation; D — degeneration of the monolayer of cells (cytotoxic effect)

So, after the AMX administration in the dose of 0.125 mg/ml the PAM and MARC-145 cell cultures infected with PRRS virus (1000 $TCID_{50/ml}$) in all of four test tubes TCID was observed on 48 hours later than in all mock test tubes (i. e. infected cell culture without drug treatment). The application of the drug at concentrations of 0.25–0.5 mg/ml under the same conditions caused the delay almost of on 4 days the TCID of the viruses PRRS, CSF, PCV-2 and TV (1000 $TCID_{50/ml}$), and the viruses SIV and AIV (5000 $ELD_{50/ml}$), everyone.

At the same time, the drug at a concentration of 0.125 mg/ml did not affect the reproduction of viruses Aujeszky's disease and highly pathogenic avian influenza (table not shown). From concentration of 0.25 mg/ml range as initial, the virostatic properties of the drug is extended to the maximum: we observed delay reproduction of all studied viruses in range from 12 hours (PR virus) and 24 hours (PPV) to period 48 hours (viruses SIV and AIV) and 96 hours (viruses CSF, PRRS and PCV-2). This suggests that the agents of CSF, PRRS and PCV-2 are particularly sensitive to virostatic action veterinary drug Amixin®. In all of three repeats the AMX demonstrated virostatic activity on porcine parvovirus.

The reproduction of the all studied viruses in all biological systems in vitro/in ovum was delayed as

more as the AMX dose was to increase. These directly show on a dose-dependent nature of the AMX action. With high probability, the results as above allow assuming the existence of certain range of the AMX concentrations for the each of the investigated pathogens in which this drug can have the optimal therapeutic effect. Also, it is obviously that the level of the AMX dosage for therapy of animals with u napparent infection and of sick animals may not be the same.

Because we had strong data that the AMX is effective in many cases of bacterial infections in piggery and poultry, the learning of nature of these events was launched. For it 10 chickens of 10-days-old were infected by field isolate of bacterial consortia ' Con A', which by data of preview analysis contain different microorganisms including the *Pasteurella* bacteriophage and had not sensitivity to mix of gentamicine-tylosine antibiotics. Five from those chicken were treatment with AMX by triple watering, once per-day every day, dose 15 mg/ml. 15 days apart all chickens was search on presence of antibiotic-resistant bacteria and *Pasteurella* bacteriophage in the blood samples. From chickens, which were not developed with AMX, was isolated the mixture of microorganisms (consortia) which contained *Pasteurella multocida* type A, *Mycoplasma gallisepticum* and more than 2 species of unidentified bacteria. Additionally, investigations allow estimating the presence of the lytic *Pasteurella* bacteriophage in this consortium. The same lytic *Pasteurella* bacteriophage was revealed previously in initial consortium, which used for infection of these chickens. Titer of this bacteriophage in initial and passaged consortium was the same — 10^9 by RTD: this is evidence that studied consortium is stable. The isolated *Pasteurella multocida* type A from untreated flock was insensitive to mix of gentamicine-tylosine on test-disks (1 mg/ml every) like to this bacteria in initial consortium.

At the same time from chicken, which were developed with AMX was isolated analogous consortium contained the same microorganisms with exclusion of the lytic *Pasteurella* bacteriophage. Moreover, there was revealed that isolated *Pasteurella multocida* type A from treated flock had high sensitivity to mix of gentamicine-tylosine on test-disks (1 mg/ml every) that unlike to this bacteria in initial consortium.

Based on the above results, we performed the clinical trials of veterinary drug Amixin® in complex with drugs and measures that was developed in NSC 'IECVM' to control of porcine emergent viral and bacterial reproductive and neonatal mix-infections (PRNI) in industrial pig farming.

Considering the data about AMX virucid and virostatic activity and literature data on interferonogenic activities of it and its analogues (Lyakhov and Litvinova, 2008; Zholobak et al., 2012), we can assume that the therapeutic effect of this drug can be achieved by both direct (contact) antiviral action and by mediation of interferon induction. We believe that 'antibacterial effect' of the Amixin® complex application consist in the elimination of bacteriophages that controlled the sensitivity of its bacteria-host to antibiotics. This in turn allows the infected herds to restore its self-control over the pathogens circulating in Ukraine piggery and poultry farming.

Conclusion. The results of clinical trials confirmed, that the Amixin® demonstrates considerable therapeutic and prophylactic efficacy for control of the modern epizootic situation in Ukrainian piggery. Especially useful is the Amixin® application for sanitation of herds with unapparent infections of reproductive animals with agents of PR, PRRS and with PCV-2. The clinical protocols of the porcine herds rehabilitation from PRNI without slaughtering of infected animals were included in two Guidelines that was approved by State Veterinary Inspection of Ukraine in 2010 and 2015.

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