University of Novi Sad - Univerzitet u Novom Sadu
Faculty of Agriculture - Poljoprivredni fakultet

CONTEMPORARY AGRICULTURE
SAVREMENA POLJOPRIVREDA

The Serbian Journal of Agricultural Sciences
Srpski časopis za poljoprivredne nauke

NOVI SAD
Vol. 62
No. 1-2
2013.

UDC: 63(497.1)(051)-"540.2"

ISSN: 0350 - 1205

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ISSN: 0350-1205 UDC: 63(497.1)(051)-„540.2“

„CONTEMPORARY AGRICULTURE”
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Publisher / *Izdavač*:
UNIVERSITY of NOVI SAD, FACULTY of AGRICULTURE
UNIVERZITET U NOVOM SADU, POLJOPRIVREDNI FAKULTET

Address of editorship / *Adresa uredništva*:
FACULTY of AGRICULTURE, 21000 Novi Sad, Trg Dositeja Obradovića 8, R. Serbia
POLJOPRIVREDNI FAKULTET, 21000 Novi Sad, Trg Dositeja Obradovića 8, R. Srbija
Phones/*Telefoni*:
++381 21 450-355; ++381 21 6350-711; ++381 21 485-3482; Fax: ++021/459-761.
http://polj.uns.ac.rs/ , E-mail: blagoje.stancic@stocarstvo.edu.rs

Four issues in two volumes per year / *Četiri broja u dva volumena godišnje.*
Cirrculation 200 copies / *Tiraž 200 primeraka.*

Print / *Štampa:* „Atelje Delač“, Iriški put 30, Sremska Kamenica, Serbia.

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UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 599.731.1:314.3

THE INFLUENCE OF CERVIX STIMULATION AND BOAR PRESENCE AT ARTIFICIAL INSEMINATION ON SOWS FERTILITY

IVAN STANČIĆ, IGOR APIĆ, JELENA APIĆ, SLOBODAN KRAGIĆ[1]

SUMMARY: These study compares farrowing rate and litter size in AI sows after cervix stimulation with catheter or fence-line boar contac around insemination. Within firs 7 days after weaning, the total of AI 149 sows were divided into three groups. Group I: cervix stimulation with catheter immediately before and after sperm deposition (n=49), Group II: fence-line boar contact immediately before, within and after insemination (n=50), Group III: not treated, control, sows (n=50). Farrowing rate were significant higher (P<0.05) after cervix stimulation (90%) or stimulation by boar presence (86%), compared with control sows (78%). These value were not significant differ (P>0.05) among the stimulated swos (Group I and II). Performed treatments has no significant effect on litter size. These results indicate that cervix stimulation or boar presence at AI can be useful method to improve sows fertility.

Key words: *cervix,boar presence, stimulation, myometrial activity, fertility, sow.*

INTRODUCTION

During mating and artificial insemination in sows, semen is deposited intra-cervically. From the site of deposition, sperm cells must be distributed over both horns and transported to the tubal end of the horns, ie. utero-tubal junction, which serve as a sperm reservoir (Hunter, 1981). The transport of sperm cells through the uterine horns is believed to be a passive process, in which intrinsic sperm cell motility plays no part (Langendijk et al., 2005). This passive transport is probably driven by the flow of intrauterine fluid containing sperm cells, due to gravitational force, movement of the sow and uterine contractions (Scott, 2000). Inadequate stimulation of the sow during and

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Original scientific paper / *Originalni naučni rad*
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Corresponding author: Ivan Stančić, e-mail: dr.ivan.stancic@gmail.com; phone: +381 21 485-3496.

after insemination result in reduced myometrial contractions (Langendijk et al., 2002) and a poorer sperm cell transport to the oviduct (Langendijk et al., 2003; Stančić et al., 2006). Inadequate sperm transport within the uterus result in decreasing the sows fertility (Langendijk et al., 2005). It has been shown that inadequate stimalation of sow during and immediately after insemination is the most common factor that influence the sows fertility rate (Spronk et al., 1997).

In farm practice, the reproductive performance of artificial inseminated sows is often lower than that achievable with natural breeding (Spronk et al., 1997; Stančić, 2000). It is often result inadequate myometrial stimulation, due to the small dose volume, high dilution rate of native ejaculate, as wel as an inadequate stimulation of the sow by boar presence and absence of mechanical stimulation of the cervix (Langendijk et al., 2003; Beham and Watson, 2005; Kemp et al., 2005; Mezalira et al., 2005; Stančić et al., 2006). An adequate myometrial stimulation is most important in the intrauterine inseminatin technology with reduced volume and spermatozoa number doses (Roseboom et al., 2004; Mezalira et al., 2005; Stančić et al., 2010).

The aim of the present study was to investigate the effects of cervix stimulation by insemination catheter or by presence of boar around the time of sow insemination, on the farrowing rate and litter size.

MATERIALS AND METHODS

The study was conducted during September to November 2011, in an intensive piggery housing about 1,200 sows. Lactation length of herd was average 28 days. Average sows farrowing rate at the farm in 2010. were 76%, and average liveborn piglets per litter were 10.68. Estrus detection of weaned sows involved full boar contact once daily starting on day 2 after weaning. At detection of the first or repeat estrus, sows were inseminated with 4 × 109 sperm cells in 100 mL dose (BTS-extender, Minitübe, Germany). Insemination was repeated 24 hours later if sows still exhibited estrous behavior, using disposable Safe Blue® AI catheters, lubricated and single wraped in protective sheaths, sterilized (Minitübe, Germany). Third estrus inseminations were not allowed. Age of semen at insemination was 4-6 hours to 1 day.

At the time of AI (4-5 days after weaning), experimental sows (2 to 5 parity) were assigned to three groups. Group I: stimulation of cervix by moving the top of the catheter within the cervix, about 1 minute before and 1 minute after sperm deposition (n=49), Group II: fence-line boar contact with sow immediately before, within and about 5 minutes after insemination (n=50) or Group III: insemination without cervix stimulation or boar presence, control group (n=50). Data recorded were farrowing rate after first postlactational insemination and subsequent litter size (liveborn, stillborn and total born piglets).

Obtained date were analyzed by using software package Statistica 10 (StataSoft 2012). Data for litter size were testing by General linear model (GLM) and by LSD test. Farrowing rate was analyzed by test of proportion.

RESULTS AND DISCUSSION

Our results demonstrated that farrowing rate were significant higher (P<0.05) in sows with cervix stimulation, group I, (90%) and in sows stimulated with boar presence, group II (86%), compared with unstimulated, control, sows (78%). These value were not significant differ (P>0.05) among the stimulated swos (Group I and II). Performed stimulation treatments has no significant effect on litter size (Table 1).

Table 1. Farrowing rate and litter size in treated and control sows (average ± SD)

		Treatment groups		
		Stimulation method		Control (Group III)
		Cervix by catheter (Group I)	Boar presence (Group II)	
No. of AI sows		49	50	50
Farrowing rate (%)		90% (44/49)[a]	86% (43/50)[a]	78% (39/50)[b]
Av. litter size (n)	Liveborn	11.98±2.961	11.86±2.562	11.79±2.755
	Stillborn	0.66±0.805	1.25±1.149	0.77±0.777
	Total	12.64±3.170	13.12±2.932	12.56±2.780

[a,b] Within a row, means without common superscripts differ (P<0.05).

Values in parenthesis: No. farrowed/No. inseminated.

The establishment the optimal number of spermatozoa in the utero-tubal junction, caudal istmus and the site of fertilization (ampulo-istmic junction of the oviduct) is the key factor for successful ovulated ova fertilization (Hunter, 1981). After deposition in to the cervix, spermatozoa undergoing the passive transport within the uterine horns to the utero-tubal junction. This passive transport is mainly driven by uterine contractions (Scott, 2000) influenced by dramatically oxytocin concentration increases in the blood of sows, within 2 minutes of the onset of ejaculation by a mature boar (Levis, 2000). During and around mating, several sexual stimuli are involved that may be important for their effect on uterine activity. These stimuli can be divided into sensory stimuli, i.e. tactile, olfactory, visual and auditory stimuli, on the one hand and seminal plasma-related stimuli on the other (Langendijk et al., 2005). The presence of a boar during AI stimulated the estrus signs expression (Kemp et al., 2005) and endogenous release of oxytocin and enhanced uterine contractions (Langendijk et al., 2003; Willenburg et al., 2003; Langendijk et al., 2005; Umesiobi, 2010). Further more, whole boar semen or seminal plasma has been demonstrated to advance the time of ovulation in gilts and sows (Waberski et al., 2006). Namely, the boar ejaculate contains high levels of estrogens (Claus, 1990), which stimulates myometrial contractions (Willenburg et al., 2004) via an estrogen-induced local release of prostaglandin F2α (Claus, 1990; Willenburg et al., 2004). The synchronization of viable spermatozoa presence in oviduct and the time of ovulation is ofextremely importance for successful fertilization. It is plausible that semen-induced cytokines in the uterine lymph undergo counter-current transfer to the ipsilateral ovary and accelerate the final maturation of pre-ovulatory follicles (O'Leary et al., 2004; Waberski et al., 2006). Releasing the endogenous oxytocin and myometrial contraction can also be induced with cervix stimulation by moving catheter within the cervix, immediately before and after AI dose deposition (Fülöp et al., 1992; Steverink et

al., 1998; Grafenau et al., 2005; Stančić et al., 2006). It has been demonstrated (Stančić et al., 2006) that cervix stimulation by catheter immediately before and after insemination, significantly increase farrowing rate (83.3%) and liveborn biglets per litter (10,1), compared with unstimulated sows (Farrowing rate = 71.1%; liveborn piglets = 9.33).

CONCLUSION

Cervix stimulation, immediately before and after sperm deposition or sow stimulation with boar presence immediately around AI, significantly increase farrowing rate (90% and 86%) compared with untreated sows (78%). Subsequent litter sizes were not affected by treatment.

However, according to results of other authors, this method is controversial and the generalized recommendations for use should be made with caution, since the most profound effects occur in sub-fertile farms, groups of sows, seasonal infertility, and with sub-fertile boars. However, in many cases, farrowing rate and litter size are improved.

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UTICAJ STIMULACIJA CERVIKSA I PRISUSTVO NERASTA PRILIKOM VEŠTAČKOG OSEMENJAVANJA NA FERTILITET KRMAČA

IVAN STANČIĆ, IGOR APIĆ, JELENA APIĆ, SLOBODAN KRAGIĆ

Izvod

U radu je izvršeno poređenje vrednosti prašenja i veličine legla kod krmača veštački osemenjenih posle stimulacije cerviksa ili stimulisanih prisustvom nerasta neposredno oko osemenjavanja. Unutar 7 dana posle zaučenja, ukupno 149 krmača je

podeljeno u tri grupe. Grupa I: stimulacija cerviksa kateterom, neposredno pre i posle depozicije sperme (n=49), Grupa II: stimulacija krmača prisustvom nerasta neposredno pre, tokom i posle inseminacije (n=50) i Grupa III: kontrolne, nestimulisane, krmače (n=50). Vrednost prašenja je bila značajno veća ($P<0.05$) posle stimulacije cerviksa (90%) i prisustva nerasta (86%) u poređenju sa nestimulisanim krmačama (78%). Ova vrednost se nije značajno razlikovala ($P>0.05$) između stimulisanih grupa krmača (grupe I i II). Izvedeni tretmani stimulacije nisu značajno uticali na veličinu legla. Ovi rezultati pokazuju da stimulacija cerviksa ili prisustvo nerasta neposredno oko osemenjavanja, mogu biti korisne metode povećanja fertiliteta krmača.

Ključne reči: cerviks, prisustvo nerasta, stimulacija, aktivnost miometriuma, fertilitet, krmača.

Received/ *Primljen*: 02.03.2013.
Accepted / *Prihvaćen*: 26.03.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 8-13, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 599.731.1:581.35

EFFECT OF SEASON AND BOARS BREED ON EJACULATE QUALITY*

BLAGOJE STANČIĆ, ALEKSANDAR BOŽIĆ, IVAN STANČIĆ, SAŠA DRAGIN, IVAN RADOVIĆ, MIHAJLO ERDELJAN[1]

SUMMARY: The effects of various seasons and boar breeds on ejaculate quality parameters were investigated on intensive swine production farms in the Autonomous Province of Vojvodina, Serbia. Significant value differences were noted in the ejaculate volume, the total sperm count and sperm concentration in ejaculates, and the number of poor ejaculates due to hot and cold seasons, as well as boar breed diversity. Therefore, the potential number of insemination doses per ejaculate varies seasonally, as well as according to different boar breeds. These facts should be taken into consideration when planning the intensity of boar reproductive exploitation.

Key words: *season, breed, quolity, ejaculate, boar.*

INTRODUCTION

The fertilisation capacity parameters of native ejaculates primarily determine the number and quality of insemination doses which can be obtained from a boar ejaculate. The most important parameters are the ejaculate volume, the sperm concentration, the total sperm number in ejaculate and sperm progressive motility (Tardif et al., 1999; Stančić et al., 2003; Knox, 2004). The number of insemination doses which can be obtained from a boar ejaculate determines the total number of insemination doses per boar per year. This is the most importance factor for the reproductive exploitation rate of genetically superior boars, which is measured by the number of inseminated sows per boar per year (Stančić et al., 2009). Moreover, the ejaculate quality greatly affects the

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Original scientific paper / *Originalni naučni rad*

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*The paper is a part of the research work on the project "Increasing of boar reproductive efficiancy at farms of AP Vojvodina", file No. 114-451-1311/2012-03, financed by the Provincial Secretariat for Science and Technological Development (AP Vojvodina, Serbia).

fertility of inseminated sows (Stančić et al., 2003; Okere, 2003). Considerable variations of these parameters were noted among different boars depending on the breed, age, sperm collection frequency, and various diseases (Colenbrander et al., 1990; Stančić et al., 2003; Wolf and Smital, 2009).

Seasons and boar breeds greatly affect the variation of native semen quality parameters. The variation of semen quality parameters within various seasons is primarily due to the seasonal variation of the ambient temperature and its great impacts on the physiology of sperm production (Setchell, 1998; Corcuera et al., 2002; Stančić et al., 2003). The value of ejaculate parameters quality also vary according to different boar breeds (Gerfen et al., 1994; Stančić et al., 2003; Smital et al., 2004).

The aim of this paper is to determine the effects of seasons and boar breeds on the main parameters of fertilisation capacity in boar ejaculates, which are used for practical artificial insemination on swine farms in Vojvodina.

MATERIALS AND METHODS

The researches were conducted on large swine farms in the Autonomous Province of Vojvodina, Serbia. The research of the seasonal effects on the ejaculate quality was conducted on one farm. During the period of one year (from March 2012 to February 2013), 4 ejaculates were tested monthly (one ejaculate per week) collected from 5 boars of Large White breed, ranging from 2 to 2.5 years of age. Consequently, 240 ejaculates were tested in total.

The breeds influence on the ejaculate quality were investigated in the pure breeds boars of Large White (n=49), Swedish Landrace (n=39), Dutch Landrace (n=29), Duroc (n=29), Hapshire (n=8), Pietrain (n=6), and in the crossbreds boars of the F_1 generation obtained from different breeds, ranging from 1.5 to 3 years of age.

Immediately after the collection, the volume of each ejaculate was determined (ml) and the ejaculates were transported to the laboratory in air-conditioned boxes for boar semen (Minitüb) at +17^{o}C. The ejaculates were heated up to +37^{o}C in the laboratory.

The following parameters were determined for each ejaculate: (1) the volume (ml), (2) the sperm concentration (x10^6/ml), (3) the total sperm count per ejaculate, and (4) the progressive sperm motility. The sperm concentration, the total sperm count, the number of insemination doses, and the level of required dissolution were determined by the photometer SDM5 (Minitüb, Germany). The progressive sperm motility was determined by a light microscope under the medium power magnification. The ejaculates with the progressive sperm motility <65% were considered as poor using for artificial insemination. The data were processed by *Statistica 10* software.

REZULTS AND DISCUSSION

The mean values of boars semen parameters were within physiological limits: the volume = 246ml, the sperm concentration =235x10^6/ml, the total sperm number per ejaculate = 52x10^9, and the sperm progressive motility = 77%. There were 18% of poor ejaculates in total (Table 1).

Table 1. Boars ejaculate parameters in different seasones

Tabela 1. Parametriejakulata nerastova u različitim sezonama

Parameters / *Parametri*	Months in year / *Meseci u godini*				Total*Ukupno*
	D-J-F	M-A-M	J-J-A	S-O-N	
Ejaculate examin./*Ispitano ejakulata* (n)	60	60	60	60	240
Volume / *Volumen* (ml)	273[a]	265[a]	203[b]	245[c]	246
Conc. / *Konc.* (x 10^6/ml)	265[a]	256[a]	219[b]	200[b]	235
Tot. sperm number *Ukupan br. spermatozoida* (x10^9)	62[a]	56[ab]	52[ab]	40[c]	52
Prog. motility /*Progr. pokret.* (%)	80[a]	80[a]	70[b]	75[a]	77
Bad ejaculates / *Loših ejakulata (%)**	10%	12%	22%	28%	18%

*Smal ejaculate volume (< 120ml), smal total no. sptz. in ejaculate (< 20x10^9), saml progr. motility (< 65%).

**Mali volumen ejakulata (< 120ml), mali ukupan broj sptz. u ejakulatu (< 20x10^9), mala progresivna pokretljivost (< 65%).*

[a, b, c] Values within rows with different superscripts differ ($P < 0.05$).

[a, b, c] *Vrednosti unutar redova sa različitim superskriptima su statistički različite* ($P < 0.05$).

However, the value parameters of the tested ejaculates varied greatly due to seasons. Namely, the mean ejaculate volume in the season of June, July and August, and the season of September, October and November (203ml and 245ml respectively), the sperm concentration (219 and 200x10^6/ ml respectively) and the total sperm number per ejaculate (52 and 4 0x10^9respectively) were statistically significant lower (P<0,01) in comparison with the season of December, January and February (273ml, 265x10^6/ ml, 62x10^9 respectively) and the season of March, April and May (265ml, 256x10^6/ml, 56x10^9 respectively). It was confirmed that a significantly higher number of poor ejaculates ocurred during the warmer seson of the year (22% and 28%) in comparison with the cooler seasons (10% and 12%) (Table 1).

Table 2. Ejaculate parameters in different boar breeds

Tabela 2. Parametri ejakulata nerastova različitih rasa

	Boar breeds / *Rasa nerastova*							Total *Ukupno*
	VJ	ŠL	HL	D	H	P	F_1	
No. Boars/*Broj nerastova*	49	39	29	29	8	6	22	182
Volume / *Volumen* (ml)	253[a]	290[b]	282[b]	190[c]	301[b]	176[c]	246[a]	258
Conc. / *Konc.* (x 10^6/ml)	223[a]	218[a]	210[a]	256[b]	181[c]	222[a]	215[a]	210
Tot. sperm number *Ukupan br. sptz.* (x10^9)	56[a]	63[a]	59[a]	49[b]	54[a]	39[b]	52[b]	56
Prog. motility*Progr. pokret.* (%)	77[a]	76[a]	77[a]	75[a]	81[a]	80[a]	79[a]	77
Bad ejaculates*Loših ejakulata (%)**	21[a]	24[a]	21[a]	15[b]	19[a]	13[b]	12[b]	19

*Smal ejaculate volume (< 120ml), smal total no. sptz. in ejaculate (< $20x10^9$), smal progr. motility (< 65%).

**Mali volumen ejakulata (< 120ml), mali ukupan broj sptz. u ejakulatu (< $20x10^9$), mala progresivna pokretljivost (< 65%).*

a, b, c Values within rows with different superscripts differ ($P < 0.05$).

a, b, c *Vrednosti unutar redova sa različitim superskriptima su statistički različite* ($P < 0.05$).

Based on the statistical significance ($P < 0.05$), the highest ejaculate volumes were noted in the Swedish Landrace (290ml), Dutch Landrace (282ml) and Hapshire (301ml) breeds, whereas the lowest ejaculate volumes were noted in the Pietrain (176ml) and Durok (190ml) breeds. However, the highest sperm concentration in the ejaculate was detected in the Duroc breed (256 x 10^6/ml), and the lowest in the Hapshire (181 x 10^6/ml). Based on the statistical significance ($P < 0.05$), these values differ from the mean value parameters of other tested breeds. The largest number of poor ejaculates was detected in the Large White (21%), Swedish Landrace (24%), and Dutch Landrace (21%) breeds, whereas the smallest number of poor ejaculates was detected in the Hapshire (19%) and Pietrain (13%) breeds, as well as in the crossbreds of F1 generation (12%). The highest and the lowest values differ in a statistically significant manner ($P < 0.05$) (Table 2).

Despite of numerous studies, the exact mechanism of lower fertility capacity in boar sperm during warmer seasons of the year has not been entirely clarified. Nevertheless, the majority of studies indicate that this is due to the effect of increased ambient temperature (Suriyasomboon et al., 2004) and prolonged daily photoperiod (Sancho et al., 2004) in warmer seasons on the process of spermatogenesis and testosterone synthesis. Furthermore, some researches indicate that this could be due to the genetic inheritance passed from wild ancestors to domestic breeds. It is a well-known fact that wild boars demonstrate extreme seasonal sexual activity and provide the best semen quality during mating seasons, which last from late autumn to early winter (Kozdrowski and Dubiel, 2004; Macchi et al., 2010). The results of our researches indicate that the value parameters of sperm fertilisation capacity were significantly higher during cooler periods of the year in comparison with warmer seasons. Various authors have also obtained similar results which confirmed the influence of warm seasons on the semen parameters reduction and ejaculate fertilisation rate decrease in boars (Liao et al., 1996; Kunavongkrit et al., 2005; Ciereszko et al., 2000; Jankevičiute and Žilinskas, 2002; Chukwuemeka et al., 2005). Significant variations of certain parameters in the ejaculate quality due to different boar breeds have been determined by other authors as well (Gerfen et al., 1994; Ciereszko et al., 2000; Jankevičiute and Žilinskas, 2002; Stančić et al., 2003; Smital et al., 2004; Chukwuemeka et al., 2005). The obtained results can enhance the reproductive efficiency in different boar breeds during cooler and warmer periods of the year on large farms in Vojvodina.

CONCLUSION

It has been determined that there are notable variations in value parameters of fertilisation capacity in boar ejaculates due to seasonal temperature and boar breeds. The ejaculate volume, the sperm concentration, the total sperm count and the progressive sperm motility are significantly lower during warmer seasons of the year, and hence

the number of obtained insemination doses per ejaculate in almost twice as small as the number of obtained doses in cooler seasons. The number of insemination doses varies due to boar breeds and the different value parameters of ejaculate quality. These facts should be taken into consideration when planning the intensity of boar reproductive exploitation under production conditions. Therefore, it is possible to significantly reduce the negative effects of warmer seasons on sow fertility.

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UTICAJ SEZONE I RASE NERASTOVA NA KVALITET EJAKULATA

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Izvod

Ispitivan je uticaj različitih godišnjih sezona i rase na parametre kvaliteta ejakulata nerastova, na farmama intenzivne proizvodnje svinja u PA Vojvodini (Srbija). Ustanovljene su značajne razlike u vrednostima volumena ejakulata, ukupnog broja i koncentracije spermatozoida u ejakulatu i u broju loših ejakulata, kako između tople i hladne sezone godine, tako i između pojedinih rasa ispitivanih nerastova. Zbog toga je i moguć broj inseminacionih doza po ejakulatu različit u pojedinim godišnjim sezonama, kao i između pojedinih rasa nerastova. Uve činjenice treba imati u vidu kod planiranja intenziteta reproduktivne eksploatacije nerastova.

Ključne reči : sezona, rasa, kvalitet, ejakulat, nerast.

Received/ *Primljen*: 03.04.2013.
Accepted / *Prihvaćen*: 25.04.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 14-20, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 634.21:159955.3

POMOLOGICAL TRAITS OF NOVI SAD APRICOT CULTIVARS AND SELECTIONS

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*SUMMARY***:** *The paper presents the results of three-year research (2008–2010) on pomological traits of four Novi Sad apricot cultivars (NS–4, NS–6, Novosadska rodna and Novosadska kasnocvetna) and five selections (SK–1, SK–3, SK–5, SK 13a and SK 16a). The research was carried out in an apricot genotypes collection of the Department of Fruit Science, Viticulture, Horticulture and Landscape Architecture of the Faculty of Agriculture in Novi Sad, at the "Rimski Šančevi" locality. The following pomological traits were tested: fruit weight, stone weight, flesh ratio, fruit length, width, thickness and fruit shape index. Those pomological traits were grouped into categories and graded according to the IBPGR descriptor. The pomological traits of Novi Sad apricot cultivars NS-4, NS-6, Novosadska rodna and selections SK-1, SK-3 and SK-13a were also compared to Hungarian best (control), showing better results.*

Key words*: apricot, cultivar, selection, pomological traits.*

INTRODUCTION

The apricot (*Prunus armeniaca* L.) is an important fruit species, having a high biological value due to its fine balance of nutrients, excellent for both fresh consumption and processing into juices, compotes, jams, marmalades, jellies, etc. Apricots can also be used dried or for producing fruit brandies of high quality (Vlahović, 2003). Moreover, sweet kernels can be used as an almond substitute, and bitter kernels for making essential oil for cosmetic products.

Due to the current situation of strong competition in the markets, new apricot cul-

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Original scientific paper / *Originalni naučni rad*

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tivars must be characterised by high fruit quality attributes which satisfy the consumers (Ruiz and Egea, 2008). In Serbia and other European countries, biological traits of certain cultivars and selections are constantly tested in order to select the best ones for commercial production in certain regions. Studies were conducted in France (Moreau–Rio, 2006), Italy (Valentini et al., 2006), Turkey (Dellal and Koc, 2003), Spain (Egea et al., 2006) and Romania (Cociu, 2006) to test introduced and local apricot cultivars of late flowering, with better cropping and fruit quality for future production. The selection and creation of new apricot cultivars from natural population started in 1980 at the Faculty of Agriculture in Novi Sad (Đurić et al., 2005), and in 2004 NS-4, NS-6, Novosadska kasnocvetna and Novosadska rodna were registered as new cultivars.

In Serbia, 24.600 tons of apricots are produced annually. The average yield is 8.5 t/ha, varying from year to year due to the freezing of buds and flowers during low winter temperatures and late spring frosts, or alternative cropping (Keserović et al., 2010). Despite many problems, the apricot production in Serbia does have a great perspective. Over the last years, apricot cultivars NS–4, NS–6, Novosadska rodna and Novosadska kasnocvetna, selected by breeders from Faculty of Agriculture in Novi Sad, have played an important role in setting up new orchards in Serbia (Đurić, 2003; Đurić et al., 2005; Đurić and Keserović, 2007). Furthermore, according to Mratinić et al. (2007), introduced and domestic apricot cultivars, such as Ligeti Orias, Bergeron, Kostjuzenski and Novi Sad cultivars, which are higher-cropping and more resistant, decrease percentage of Hungarian best and Kecskemet apricot in new orchards.

The objective of the research was to compare the pomological traits of Novi Sad cultivars and selections to the cv. Hungarian best in the conditions of Novi Sad locality, and select the ones that had better fruit traits than the standard.

The results could be used to intensify the production of Novi Sad apricot cultivars and selections on other localities with similar ecological conditions.

MATERIAL AND METHODS

The evaluation of pomological traits of Novi Sad apricot cultivars and selections was conducted in an apricot genotypes collection of the Department of Fruit Science, Viticulture, Horticulture and Landscape Architecture, Faculty of Agriculture in Novi Sad, at the"Rimski Šančevi" locality during 2008-2010.

The orchard was planted in 2000, with 5 x 4 m spacing. *Prunus cerasifera* L was used as the rootstock, and *Stanley* plum cultivar as the interstock. The trees had an improved pyramidal shape.

Fruit and stone weight were scale–weighted and expressed in grams. Fruit ratio was expressed as a percentage of total fruit weight/ flesh weight ratio. Fruit dimensions (length, width and thickness) were measured by calliper. Fruit length was determined by measuring the distance from the stem cavity to the apex. Fruit width was determined by measuring the distance from the suture to the dorsal side at the widest part, and thickness by measuring the space between two halves. Based on fruit dimensions, fruit shape index (roundness factor) was calculated by using the following formula: Io = length^2/width x thickness.

The pomological–technological traits of four Novi Sad apricot cultivars (NS–4, NS–6, Novosadska rodna, Novosadska kasnocvetna) and five selections (SK–1, SK–3, SK–5, SK 13a and SK 16a) were studied. Twenty–five fruits of each cultivar and selec-

tion were analysed. The categories of fruit size, shape, suture depth, stem cavity depth, apex shape and fruit attractiveness, as well as stone size and shape were evaluated in accordance with the international IBPGR (International Board for Plant Genetic Resources) apricot descriptor (Guerriero and Watkins, 1984).

The analysis of variance and the F–test for a two–factorial experiment, the A x B model, was used to establish significance of the observed differences (Hadživuković, 1973). For individual cultivar comparisons, the LSD test was used.

RESULTS AND DISCUSSION

Great fluctuations in fruit weight were observed among the genotypes and ranged from 43.90 g (N. kasnocvetna) to 78.23 g (NS–4). Đurić et al. (2005) and Đurić and Keserović (2007) reported similar results for both cultivars. Fruits of Hungarian best were classified by Rahović (2002, 2003) and Milatović et al. (2005) as medium/large (35–50 g).

The pomological traits of the apricot cultivars and selections are shown in the Table 1.

Table 1. Pomological traits of apricot cultivars and selections, "Rimski Šančevi", 2008–2010

Cultivar /selection	Fruit weight (g)	Stone weight (g)	*Flesh ratio* (%)
	Average	Average	Average
NS–4	78.23	2.91	95.96
NS–6	76.13	3.19	95.56
N. rodna	60.12	3.06	94.75
N.kasnocvetna	43.90	2.59	93.98
SK–1	66.28	2.70	95.83
SK–3	61.56	2.50	95.73
SK–5	47.53	2.40	94.95
SK 13a	60.87	2.43	96.00
SK 16a	47.19	2.83	93.62
Hungarian best	55.78	3.05	94.52
Average/*Prosek*	59.76	2.77	95.09
LSD $_{Cultivars}$	3.18	0.21	0.32
$_{0,05}$	4.19	0.28	0.42
0,01			

Compared to the control, Novosadska kasnocvetna cultivar and SK–5 and SK 16a selections have been classified as medium (35–50 g); Novosadska rodna cultivar along with SK–1, SK–3 and SK 13a selections as large (58–70 g); and NS–4 and NS–6 selections as very large (above 70 g). The results obtained for Novosadska rodna are in compliance with the results of Korać et al. (2000), while the results for Hungarian best were better than previously reported by Milatović et al. (2005), Milošević et al. (2010) and Mratinic et al. (2010). Compared to Hungarian best, N. kasnocvetna, SK-5 and SK–16a were of significantly lower weight, while NS–4, NS–6 and Novosadska rodna cultivars, as well as SK–1, SK–3 and SK 13a selections, had higher fruit weight than the control.

Keserović et al. (2005) pointed out that NS–4 cultivar had large fruits and regular cropping.

Stone weight was in a correlation with fruit weight, i.e. as fruit weight was increasing/decreasing, stone weight was also increasing/decreasing. NS–6 had the highest stone weight (3.19 g.), whilst SK–5 (2.40 g) had the smallest stone weight. The observed cultivars and selections can be classified into the following groups: medium stone (SK–3, SK–5 and SK 13a), medium to large stone (NS–4, Novosadska kasnocvetna and Hungarian best, SK–1 and SK 16a) and large stone (NS–6 and Novosadska rodna).

All tested cultivars and selections had a good flesh ratio. SK–13a had the highest flesh ratio (96.00%), and SK 16a selection had the smallest one (93.62%). Compared to Hungarian best, the fruit ratio of Novosadska kasnocvetna cultivar and SK 16a selection was slightly lower or at the standard level, whereas the other cultivars and selections had better fruit ratio than the standard. Stone ratio in fruit weight was low (up to 5.5%, grade 3), ranging from 2.53% (SK 13 a) to 3.34% (NS-6), according to the IBPGR descriptor.

The statistical data analysis showed significant statistical differences among cultivars and selections for the fruit weight, stone weight and fruit ratio.

The highest length (52.9 mm) and width (52.1 mm) were measured on fruits of SK–1 and the highest thickness (51.2 mm) on NS–6. In accordance with fruit weight, the lowest length, width and thickness were measured on fruits of N. kasnocvetna (45.1 mm, 41.4 mm and 41.6 mm, respectively).

The morphometric traits of the apricot cultivars and selections at the "Rimski Šančevi" locality in the period 2008–2010 are shown in the Table 2.

Table 2. Morphometric traits of apricot cultivars and selections, "Rimski Šančevi", 2008–2010

Cultivar /selection	Length (mm)	Width (mm)	Thickness (mm)	Shape index
	Average	Average	Average	Average
NS–4	52.1	46.9	49.7	1.2
NS–6	52.5	49.4	51.2	1.1
N. rodna	48.9	46.3	47.1	1.1
N.kasnocvetna	45.1	41.4	41.6	1.2
SK–1	52.9	52.1	47.1	1.1
SK–3	50.5	49.3	44.9	1.2
SK–5	47.6	47.5	41.9	1.1
SK 13a	50.2	48.7	44.8	1.2
SK 16a	45.8	44.0	41.7	1.2
Hungarian best	50.5	49.6	51.1	1.0
Average/Prosek	49.6	47.5	46.1	1.1
LSD Cultivars0.05	0.91	0.91	0.96	0.017
0.01	1.21	1.20	1.27	0.022

Fruit shape index was calculated based on fruit dimensions, ranging from 1.00 to 1.20. The following fruit shapes were represented: round (shape index below 1.15 – Hungarian best, NS–6, Novosadska rodna, SK–1, SK–5 and SK 16a) and elliptic (shape index above 1.15 – NS–4, Novosadska kasnocvetna cultivars, SK–3 and SK 13a). The statistical data analysis showed significant statistical differences among cultivars and selections for the fruit length, width, thickness and shape index.

Based on the obtained results for fruit size, the tested cultivars and selections were classified into the following groups according to the IBPGR descriptor: small to medium (grade 4 – Novosadska kasnocvetna), medium (grade 5 – SK–5, SK 16a and Hungarian best), medium to large (grade 6 – Novosadska rodna and SK 13 a), large (grade 7 – SK–1 and SK–3) and very large (grade 8 – NS–4 and NS–6). Compared to Hungarian best, one cultivar had smaller, two selections equal, and three cultivars and three selections larger fruits.

High variability was observed for fruit shape, from round to oblong. The cultivars and selections were classified into the following categories, according to the IBPGR descriptor: round (grade 1 – NS–4, NS–6 and Hungarian best), elliptic (grade 3 – Novosadska rodna), ovate (grade 4 – Novosadska kasnocvetna, SK–1, SK–3 and SK–5) and oblong (grade 6 – SK 13a and SK 16a). Fruit shape depends on many characteristics such as stem cavity depth, the suture between two halves and the apex shape. Most cultivars and selections have intermediate or deep stem cavity (grade 6), intermediate suture (grade 5) and rounded apex (grade 3).

Based on stone size, the tested cultivars and selections were classified into the following groups: medium-sized (NS–4, Novosadska kasnocvetna, SK–1, SK–3, SK–5, SK 13a, SK 16a and Hungarian best), and large (NS–6 and Novosadska rodna). Stone shape depends on a cultivar. All the cultivars and selections tested have ovate stones (grade 2).

Fruit attractiveness is a set of traits that have a positive visual effect. It depends on fruit shape and size, as well as fruit colour, i.e. ground and over-skin colour. Two tested cultivars (NS–4, Hungarian best) and two selections (SK–1 and SK 16a) have orange skin (grade 6). The remaining cultivars and selections are light orange colour (grade 5). When it comes to over-colour, it can be found in all cultivars and selections, but in different intensities. Therefore, they can be classified into the following five categories: slight (grade 3: Novosadska rodna and Novosadska kasnocvetna), mottled (grade 4: Hungarian best), intermediate red (grade 5: SK–1, SK–5 and SK 13a), intermediate/mostly red (grade 6: NS–6 and SK–3) and mostly red (grade 7: NS–4 and SK 16a). Đurić and Keserović (2007) and Mratinić et al. (2010) pointed out very attractive cover color of NS 4 which is very appreciated by producers.

CONCLUSIONS

On the basis of triennial research (2008–2010) on pomological traits of apricot cultivars and selections at the "Rimski Šančevi" locality, the following conclusions could be drawn:

1) Novosadska rodna, SK 13a, SK–1, SK–3, NS–4 and NS–6, had larger fruit size and fruit weight than Hungarian best. NS–4 had the highest fruit weight (78.23 g).
2) SK-13a had the highest flesh ratio (96.00%). Better fruit ratio than in Hungarian best was observed for NS–4, NS–6, Novosadska rodna and SK–1, SK–3, SK–5, SK 13a.
3) Apart from the NS 4 and NS 6 cultivars that were already in commercial production, the new apricot selections SK 1, SK 3 and SK 13a, which had medium fruit size (60–66g), high flesh ratio (95–96%) and more intensive over–colour than Hungarian best, have potential for fresh consumption. A further evaluation should be carried out in order to determine suitable localities.

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POMOLOŠKE OSOBINE PLODA NOVOSADSKIH SORTI I SELEKCIJA KAJSIJE

DRAGAN RAHOVIĆ, ZORAN KESEROVIĆ, SLAVICA ČOLIĆ,
IVAN PAVKOV, MILE RADOJČIN

Izvod

U radu su predstavljeni rezultati trogodišnjih (2008–2010) ispitivanja pomoloških osobina ploda četiri novosadske sorte (NS–4, NS–6, Novosadska rodna i Novosadska kasnocvetna) i pet selekcija kajsije (SK–1, SK–3, SK–5, SK 13a i SK 16a). Ispitivanje pomoloških osobina ploda obavljeno je u kolekcionom zasadu kajsije Departmana za voćarstvo, vinogradarstvo, hortikulturu i pejzažnu arhitekturu Poljoprivrednog fakulteta u Novom Sadu na lokalitetu „Rimski Šančevi“. Ispitivane su sledeće pomološke osobine ploda: masa ploda, masa koštice, randman, dužina, širina, debljina i indeks oblika ploda. Pomološke osobine ploda ocenjivane su na osnovu IBPGR deskriptora svrstavanjem u grupe sa pripadajućim ocenama. Pomološke osobine ploda novosadskih sorti i selekcija kajsije upoređivane su sa Mađarskom najboljom (standard). Plodovi novosadskih sorti NS-4, NS-6, Novosadska rodna i selekcija SK-1, SK-3 I SK-13a ispoljili su bolje osobine od Mađarske najbolje.

***Ključne reči**:* kajsija, sorta, selekcija, pomološke osobine.

Received/ *Primljen*: 12.12.2012.
Accepted / *Prihvaćen*: 25.02.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 21-27, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 314.3:581.35

SOWS FERTILITY AFTER OXYTOCIN ADDITION IN SEMEN DOSE OR VULVAR INJECTION TO STIMULATE MYOMETRIAL ACTIVITY AROUND INSEMINATION

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SUMMARY: These study compares farrowing rate and litter size in sows after oxytocin addition in sperm or after vulvar injection immediately before AI. Within firs 7 days after weaning, the total of 150 sows were AI: (a) with sperm doses additioned by 10 IU oxytocine immediately before AI (n=50), (b) after 5 IU vulvar injection prior to AI (n=50), or (c) not treated, control, (n=50). Farrowing rate were significant higher (P<0.05) after vulvar oxytocin injection (88%) or oxytocin addition in AI dose (92%), compared with control sows (78%). These value were not significant differ (P>0.05) after vulvar oxytocin injection or oxytocin addition in AI dose. Treatment with oxytocin has no significant effect on litter size. These results indicate that oxytocin treatment can be useful method to improve sows fertility.

Key words: *oxytocin, addition, sperm, injection, fertility, sow.*

INTRODUCTION

Artificial insemination (AI) is the method used in intensive pig production all ower the world, to improve genetic development faster than natural mating. AI techniques can also lower the risk of spreading reproductive deseases, reduce the number of boars on the farm as well as reducing the number of workers needed during mating. However, in farm practice, the reproductive performance of artificial inseminated sows is often lower than that achievable with natural breeding (Stančić, 2000). It has been shown that semen quality, insemination techniques, optimal AI-timing relative to moment of ovulation as well as inadequate stimalation of sow during and immediately after insemination is the key factors that influence the sows fertility rate (Spronk et al., 1997). An adequate myometrial stimulation is most important in the intrauterine inseminatin technology with reduced volume and spermatozoa number doses (Roseboom et al., 2004; Stančić et al., 2010).

Original scientific paper / *Originalni naučni rad*
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A sufficient number of spermatozoa in the oviductal sperm cell reservoir ie. caudal istmus in the 24-hour period preceding ovulation (Hunter, 1981), is the ultimate factor for successful fertilization (Soede et al., 1995). Any factors that reduces this reservoir may compromise fertility. In the AI, such a reduction in the sperm cell reservoir may result from poor timing of semen deposition relative to time of ovulation (Kemp and Soede, 1996; Stančić and Šahinović, 2001), inadequate stimulation of the sow during and after insemination resulting in reduced myometrial contractions (Langendijk et al., 2002) and a poorer sperm cell transport to the oviduct (Langendijk et al., 2003; Stančić et al., 2006) as wel as by excess semen reflux (backflow) during insemination (Steverink et al., 1998). Inadequate sperm transport within the uterus and consequently reduction the optimal number of sperm population in the caudal istmus result in decreasing the sows fertility. To overcome this problem, several investigators have studied whether farrowing rate and litter size are enhanced by adding: (1) oxytocin to a dose of semen just before insemination, or (2) by injecting oxytocin into the muscle or vulva immediately prior to AI (Peña et al., 1998; Levis, 2000; Gibson et al., 2004; Peláez, et al., 2006).

The aim of the present study was to investigate the effects of oxytocin addition in to the semen dose or injection in to the vulva immediately prior to an intracervical insemination, on the sows farrowing rate and litter size under field conditions in Serbia.

MATERIALS AND METHODS

The study was conducted during September to November 2011, in an intensive piggery housing about 1,200 sows. Lactation length of herd was average 28 days. Average sows farrowing rate at the farm in 2010. were 76%, and average liveborn piglets per litter were 10.68. Estrus detection of weaned sows involved full boar contact once daily starting on day 2 after weaning. At detection of the first or repeat estrus, sows were inseminated with 4 × 109 sperm cells in 100 mL dose (BTS-extender, Minitübe). Insemination was repeated 24 hours later if sows still exhibited estrous behavior, using disposable Safe Blue® AI catheters, lubricated and single wraped in protective sheaths, sterilized (Minitübe, Germany). Third estrus inseminations were not allowed. Age of semen at insemination was 4-6 hours to 1 day.

At the time of AI (4-5 days after weaning), experimental sows (2 to 5 parity) were assigned to three groups: (1) AI doses supplemented with 10 $IUmL^{-1}$ oxyticin (10 IU/mL wather solution, Oxytokel, Kelan N.V. - Belgium), immediately before insemination (n=50), (2) sows injected with 5 $IUmL^{-1}$ oxytocin in the mucosa of the vulvar lips just prior insemination (n=50) or (3) insemination without semen supplementation or injection with oxytocin, control group (n=50). Data recorded were farrowing rate after first postlactational insemination and subsequent litter size (liveborn, stillborn and total born piglets).

Obtained date were analyzed by using software package Statistica 10 (StataSoft 2012). Data for litter size were testing by General linear model (GLM) and by LSD test. Farrowing rate was analyzed by test of proportion.

RESULTS AND DISCUSSION

Our results demonstrated that farrowing rate were significant higher (P<0.05) after vulvar oxytocin injection (88%) or oxytocin addition in AI dose (92%), compared with control sows (78%). These value were not significant differ (P>0.05) after vulvar oxytocin injection or oxytocin addition in AI dose. Treatment with oxytocin has no significant effect on litter size, compared with untreated sows (Table 1).

Table 1. Farrowing rate and litter size in treated and control sows

		Treatment groups		
		Oxytocin addition		Control
		5 IU injection in vulva	10 IU in semen dose	
No. of AI sows		50	50	50
Farrowing rate (%)		88[a] (44/50)	92[a] (46/50)	78[b] (39/50)
Av. litter size (n)	Liveborn	11.52[a] ±2.889	11.41[a] ±2.374	11.79[a] ±2.755
	Stillborn	0.84[a] ±1.140	0.93[a] ±1.296	0.77[a] ±0.777
	Total	12.36[a] ±2.804	12.34[a] ±2.699	12.56[a] ±2.780

[a,b] Within a row, means without common superscripts differ (P<0.05).

Values in parenthesis: No. farrowed/No. inseminated.

Addition of 5 to 10 IUmL^{-1} oxytocin has no effect boars sperm motility or morphology in the semen samples *in vitro* stored at + 18^{o}C for 56 h (Çiftçi, 2005). Farrowing rate were higher (P = .02) if oxytocin was included in the semen for weaned sows bred only once (84.9%) than for repeat sows (63.7%), but litter size was not affected (Gibson et al., 2004). Authors conclude that inclusion of oxytocin in extended semen may benefit sow fertility when breeding management may otherwise result in a smaller sperm cell reservoir in the oviduct.

The farrowing rate was 5.7 percent greater for sows inseminated with oxytocin-treated semen (83%) compared to sows injected with oxytocin (77.3%) immediately before insemination. The litter size was 11.50 pigs for sows inseminated with oxytocin-treated semen and 10.97 pigs for sows injected with oxytocin at the time of insemination (Peña et al., 1998). Hormone (estrogens, oxytocin or prostaglandin F2α) addition to semen increased numbers of fetuses 25 to 30 days after AI (estrogen-7.2; oxytocin-8.4; PG F2α-8.7 and control-5.8). Therefore, in situations of lowered fertility, hormone addition could be a strategy to limit infertility in swine (Willenburg et al., 2003). According to results obtained by other authors, the conclusions from review paper of Levis (2000) are: (1) Adding 4 to 5 IU's of oxytocin to a dose of semen improves farrowing rate and litter size, (2) Use of oxytocin treated semen is more effective in multiparous sows than gilts, (3) During the summer months, oxytocin-treated semen significantly increased farrowing rate and litter size and (4) In most studies, the use of oxytocin at the time of insemination was profitable.

Inseminations performed too early or too late relative to ovulation decrease litter size and especially farrowing rate. This effect can be explained to a large extent by the increase in the percentage of non-fertilized eggs, resulting in partial fertilization or no fertilization at all. In general, insemination between 0 and 24 h before ovulation gives

good fertilization results (Kemp and Soede, 1997). Bath, becouse negative correlations between weaning-to-estrus interval (WEI) and duration of estrus and moment of ovulation after onset of estrus, the sows with short WEI must be inseminated earlier after estrus detection then sows with longer WEI to achieve the optimal farrowing rate and litter size (Vesseur et al., 1994; Weitze et al., 1994; Kemp and Soede, 1996; Stančić and Šahinović, 2001; Timotijević-Koprivica et al., 2001; Stančić and Gagrčin, 2002).

The establishment the optimal number of spermatozoa in the utero-tubal junction, caudal istmus and the site of fertilization (ampulo-istmic junction of the oviduct) is the key factor for successful ovulated ova fertilization (Hunter, 1981). Sperm cells have to be transported from the site of deposition (cervix) to the utero-tubal junction within 15 minutes to 2 hours after deposition in the cervix. These rapid transuterine transport spermatozoa to the utero-tubal junction and oviduct is extremely important for prevent spermatozoa to being phagocytized (killed) by leukocytes (Levis, 2000). This passive transport is mainly driven by uterine contractions (Scott, 2000) influenced by dramatically oxytocin concentration increases in the blood of sows, within 2 minutes of the onset of ejaculation by a mature boar (Levis, 2000). Additionally, the presence of a boar during estrus stimulated the estrus expression (Kemp et al., 2005) and endogenous release of oxytocin and enhanced uterine contractions (Langendijk et al., 2003). Further more, the boar ejaculate contains high levels of estrogens (Claus, 1990), which stimulates myometrial contractions (Willenburg et al., 2004) via an estrogen-induced local release of prostaglandin $F_{2\alpha}$ (Claus, 1990; Willenburg et al., 2004). The synchronization of viable spermatozoa presence in oviduct and the time of ovulation is of extremely importance for successful fertilization. Whole boar semen or seminal plasma has been demonstrated to advance the time of ovulation (Waberski et al., 2000). It is plausible that semen-induced cytokines in the uterine lymph undergo counter-current transfer to the ipsilateral ovary and accelerate the final maturation of pre-ovulatory follicles (Waberski et al., 2006).

According to mentioned facts, lower farrowing rate and litter size, after artificial insemination, my be caused by: (a) inadequate time of insemination related to time of ovulation, (b) lower amount of semen oxytocin and estrogen in insemination dose, due to increase dilution rate of ejaculate, (c) inadequate sow sexual stimulation, due to no full boar contact and act of coitus and (d) semen backflow (reflux) during insemination.

CONCLUSION

Oxytocin addition to semen (10 IU per dose) immediately before AI or vulvar injecting the sow (5 IU) prior to AI, significantly increase farrowing rate (92% and 88%) compared with untreated sows (78%). Subsequent litter sizes were not affected by treatment.

However, according to results of other authors, this method is controversial and the generalized recommendations for use should be made with caution, since the most profound effects occur in sub-fertile farms, groups of sows, seasonal infertility, and with sub-fertile boars. However, in many cases, farrowing rate and litter size are improved.

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FERTILITET KRMAČA POSLE DODAVANJA OKSITOCINA U DOZE SPERME ILI INJEKCIJE U VULVU ZA STIMULACIJU AKTIVNOSTI MIOMETRIUMA KOD INSEMINACIJE

IVAN STANČIĆ, IGOR APIĆ, BLAGOJE STANČIĆ, NENAD STOJANAC

Izvod

U radu je izvršeno upoređivanje vrednosti prašenja i veličine legla u krmača, posle dodavanja oksitocina u spermu ili posle injekcije oksitocina u vulvu, neposredno pre veštačkog osemenjavanja (VO). Unutar prvih 7 dana posle zalučenja, osemenjeno je ukupno 150 krmača i to: (a) inseminacionim dozama u koje je dodato 10 IJ oksitocina (n=50), (b) posle injekcije 5 IJ oksitocina u vulvu, neposredno pre inseminacije (n=50) ili (c) bez navedenih tretmana, controlne krmače (n=50). Vrednost prašenja je bila signifikantno veća ($P<0.05$) kod tretiranih krmača (88% i 92%) u poređenju sa netretiranim, kontrolnim, krmačama (78%). Tretman nije imao značajnog uticaja ($P>0.05$) na veličinu legla kod prašenja. Dobijeni rezultati pokazuju da navedena metoda tretmana sa oksitocinom može povećati fertilitet osemenjenih krmača.

Ključne reči: oksitocin, dodavanje, sperma, injekcija, fertilitet, krmača.

Received / *Primljen*: 14.02.2013.
Accepted / *Prihvaćen*: 02.03.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 28-34, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 595.752.569.73

ACETAMIPRID RESIDUES IN PEPPER AFTER APHIDES CONTROL IN GREENHOUSES

MAGDALENA CARA, VJOLLCA VLADI, JORDAN MERKURI, EJUP COTA, ADELA VRAPI, VOJISLAVA BURSIĆ[1]

SUMMARY: The subject of this study was determination of acetamiprid residues in pepper. The insecticide was used for the control of potato aphides (Macrosiphum euphorbiae, Thomas 1878), in a plastic greenhouse in the district of Durres in 2011. The experiment was laid out with two doses of 20 and 30 g a.i/ha of acetamiprid. The treatments were carried out with minimum and maximum doses recommended. Acetamiprid was found to be effective against aphides in pepper. The samples were taken on the first, third, fifth and seventh day after the last treatment. The Pre Harvest Interval (PHI) of acetamiprid is seven days. On the expiry of PHI the residue level of acetamiprid was lower than Maximum Residue Level (MRL) values. It should be emphasized that the results of the analysis point at the fact that PHI must be observed so as to avoid the increased levels of acetamiprid in fruits of pepper.

Key words: *pepper, Macrosiphum euphorbiae, acetamiprid, residue, PHI, MRL.*

INTRODUCTION

Aphides are an important pest in sweet pepper protected crops (Valério, 2003, 2004, 2005). Hemiptera: Aphididae are very destructive and often dominant pests in sweet pepper greenhouses (Ramakers, 2004). They produce large amounts of honeydew which cause damage on plants and the crop production may be reduced. In sweet pepper protected crops some aphid species can cause direct and indirect damage.

With so many aphid species that can cause damage to sweet pepper there is tradi-

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Original scientific paper/*Originalni naučni rad*

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tional chemical control, that is costly for farmers and has many disadvantages either for public health or for the environment, particularly for its negative effects on beneficial organisms (Zxang, 2000; Ostman, 2001, Barrenechea, et al, 2004). Recently, insecticide neonictinoides have been the fastest growing classes of insecticides in modern crop protection with wide spectrum effect against sucking and certain chewing insect pests (Jeschke and Naumen, 2008). Changes of aphid sensitivity to the insecticides from the group of neonicotinoids have been referred to in literature of cross resistence of the species *Bemisia* tabaci (Homoptera: Aleurodidae) to thiamethoxam and acetamiprid (Elbert and Nauen, 2000), but in the region of the Central and Southeast Europe, a change in the efficacy of products from these insecticides have not been registered, therefore those insecticides are still successfully used (Inđić et al., 2009). These chemical insecticides act agonistically on insect Nicotinic Acetylcholine Receptors (nAChR), blocking the necotinergic neural pathway causing the accumulation of neurotransmitter acetylcholine (Matsuda et al., 2001; Lazić et al., 2008; Tomizawa and Cassida, 2009). They chemical structure resembles the nicotine structure to a great extent, hence the name neonicotinoide (Bursić et al., 2010). There are especially active on Homopteran pest species (Naumen *et al*, 2003).

Because of a realistic risk of the development of resistance to compounds from the group of neonicotinoids, it is recommended for the control of aphides and other harmful insects to avoid alternative application of compounds based on thiamethoxam, acetamiprid, imidacloprid and thiacloprid (Vuković, 2006).

MATERIAL AND METHODS

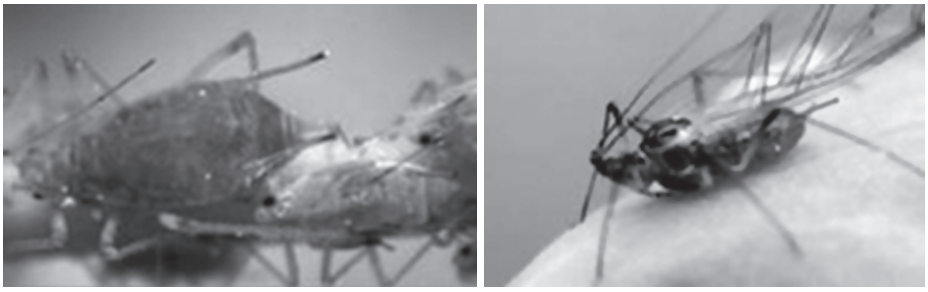
Monitoring aphid species. A greenhouse with sweet pepper was sampled weekly. When the conditions were favorable to the development of aphid populations, the frequency of sampling was intensified. Greenhouses were divided into ten sections and one sweet pepper plant of each section was sampled for monitoring aphid species.

Leaves were randomly collected from two of the three plant levels (superior, medium and inferior). The treatments were carried out with chlorpiriphos, deltamethrin and acetamiprid. Acetamiprid (Mospilan SP 20), Chemical subclass: Chloronicotinyl, International Union for Pure and Applied Chemistry (IUPAC) name: *E-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine.* It was used at the rate of 20 g a.i./ha and 30 g a.i./ha on 15.06.2011. and the samples were taken on the first, third and seventh day after the treatment.

The greenhouse where we conducted the experiments with a surface 1100 m^2, was planted with hybrid pepper. The experiment to assess the level of acetamiprid waste was applied under this scheme: Treatments: 1. Acetamiprid dose 20 g a.i/ha (10 plants), 2. Acetamiprid dose 30 g a.i/ha. (10 plants), 3. Control without treatment (10 plants).

The aphid diagnosis (Photo 1) was made in the Plant Protection Laboratory. The Photo 2a and 2b, show the male and female of *Macrosiphum euphorbiae*.

Photo 1. Moment of sampling for the diagnosis of aphid.



a **b**

Photo 2. Male (a) and female (b); Macrosiphum euphorbiae

In each treatment, 45 leaves of the same physiological age were sampled. The leaves were all sampled from the height of about 1 m above the ground, from 45 different plants at the treatment patch. On each leaf all aphides were counted over the entire leaf surface. At the beginning of this study (zero count) the level of the aphids population was very high which caused an immense damage to the pepper plants.

Gas Chromatography system. Gas Chromatograph with programmed temperature, with electron capture detector (ECD), capillary column and split less injectors. Capillary column of molten silicon, DB-5, with 0,32 mm internal diameter, length 30 m, film thickness of 0,25 μm. Helium carrier gas flow rate: 1 mL/min. Oven temperature program: 170˚C for 1 min, rising to 10ºC/min up to 250ºC, which is held 7 min. Injector temperature: 250ºC, the time of closing of Split: 1 min. Detector temperature: 350ºC. Make-up gas for ECD: nitrogen with 30 mL/min. System calibrated or the calibration curve verified each day. In these conditions, the time ratio of acetamiprid is 7.297 min.

RESULTS

The obtained parameters of the validation of acetamiprid determination by gas chromatography with electron capture detector (GC-ECD) are shown in graph1as well as in figures 3 and 4.

Calibration:

$Y = aX + b$; $a = 2.038266e-006$; $b = -8.793676e-003$; $R^2 = 0.9988559$; $R = 0.9994278$.

Mean RF: 1.917529e-006; RF SD: 1.760782e-007; RF %RSD : 9.182557.

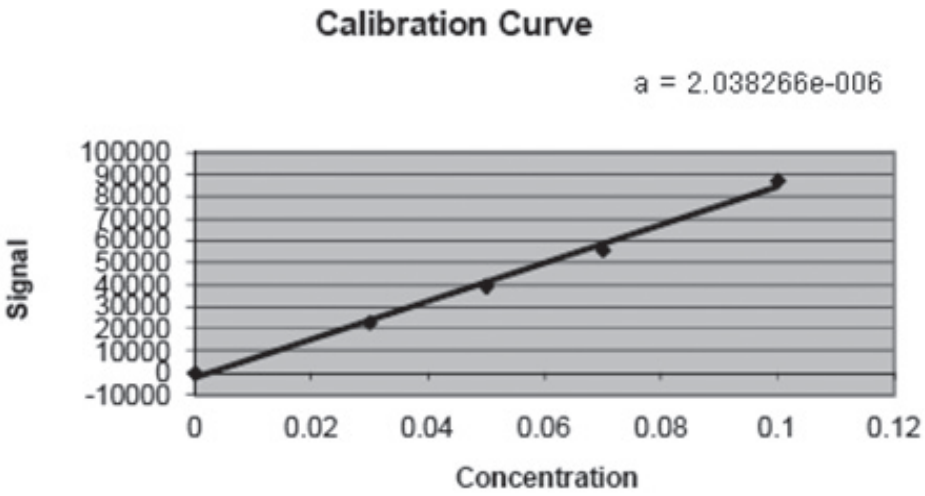


Figure 1. Calibration curve for standard of acetamiprid

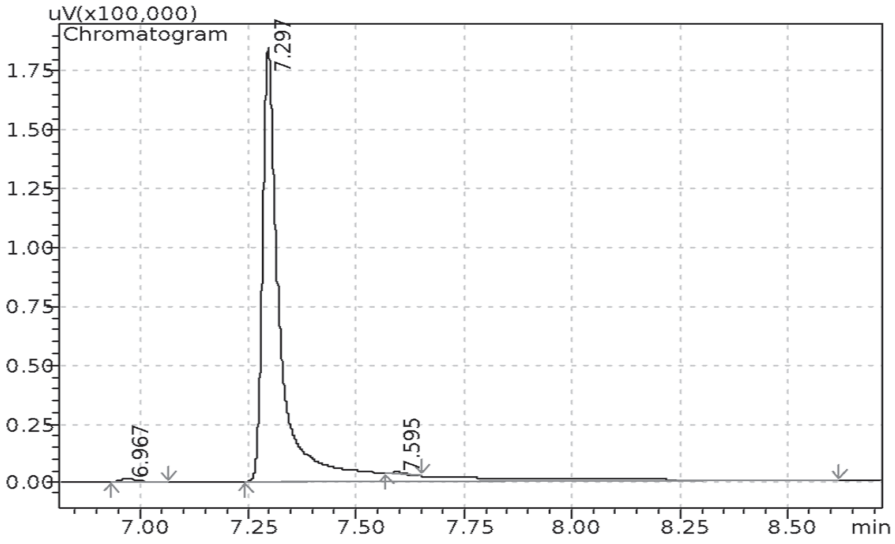


Figure 2. Chromatogram of acetamiprid standard

During the assays, one aphid species was identified *M. euphorbiae.* The occurrence of this population dynamic of the aphid species, much different from the other years, took place when problems in development of sweet pepper plants occurred associated with nutritional and pathogen issues.

Results of analysis method. In accordance with the new regulation Regulation (EC) No 396/20051, European Food Safety Authority 2, 3 European Food Safety Authority (EFSA), Parma, Italy revised the MRLs and it is set that the new MRL of acetamiprid in pepper is 0.3 mg/kg.

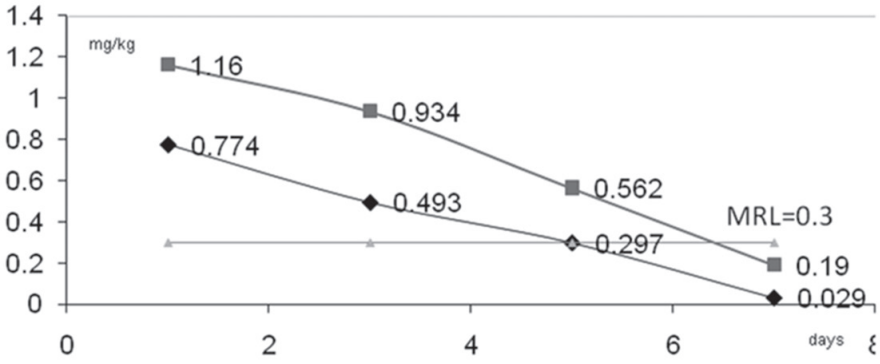


Figure 3. Degradation curve for both doses and MRL

DISCUSSION

The validation parameters of acetamiprid determination GC-ECD were in accordance with the Environmental Protection Agency (Doc. No. 2010-2803) which also suggests the determination of acetamiprid residues by gas chromatography.

The maximum amount of acetamiprid residue is on the first day 0.774 mg/kg and the minimum is on the seventh day 0.029 mg/kg, which is the day of PHI (Pre Harvest Interval). It is noticed that acetamiprid degradation has a good performance on the date of PHI that is the seventh day of acetamiprid for vegetables, it results in values lower than 20 g dose MRL for a.i./ha.

The maximum amount of acetamiprid residue on the first day is 1.16 mg/kg and minimum in the seventh day is 0.19 mg/kg, which is the day PHI. It is noticed that degradation of acetamiprid has a good performance on the date of PHI that is the seventh day of acetamiprid for vegetables, it results in values lower than 30 g dose MRL for a.i./ha. Graph.2.

Lazić et al. (2008) did not detect the residues of this insecticide when analyzing 61 pepper samples for the content of 75 pesticides over the period 2005/2007, which indicates that by good agricultural practice the pesticide residues above the MRLs can be avoided, whereas, on the other hand, it can imply a rapid degradation of this insecticide (Khan, 2010 ili Roberts, 1998).

According to Roberts and Hutson (1999) when acetamiprid was applied as a granular formulation, parent insecticide and its metabolites were below the limit of detection (0.01 mg/kg). Thus our results also indicate that for both doses on the PHI have lower values of residues than the permitted level.

CONCLUSION

Acetamiprid is an appropriate insecticide to treat aphides in pepper cultivated greenhouses. Higher values than MRL are noticed until the fifth day respectively in 0.297 and 0.562 mg/kg. It is not recommended to harvest on the fifth day because it

poses a risk to consumers' health. Lower values than MRL of 0.029 and 0.19 mg/kg were noticed on the seventh day.

It is recommended to harvest after the seventh day on the treatment with acetamiprid. Individual pesticide residue methods are difficult, complicated, and costly methods which require highly skilled personnel and it is better to use the multiresidue method for pesticide analysis.

Acknowledgements: We express our gratitude to Hysni Gjergji from Rade in whose greenhouse our research work was commenced.

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HEMIJSKA KONTROLA VAŠI U ZAŠTIĆENOM PROSTORU I OSTACI ACETAMIPRIDA U PAPRICI

MAGDALENA CARA, VJOLLCA VLADI, JORDAN MERKURI, EJUP COTA, ADELA VRAPI, VOJISLAVA BURSIĆ

Izvod

Predmet istraživanja su mere kontrole krompirove vaši (*Macrosiphum euphorbiae,* Thomas 1878) i metoda dređivanja ostataka insekticida acetamiprida, koji je korišćen za tretiranje paprike. Ovo ispitivanje je sprovedeno u plasteniku u okrugu Drač u 2011. godini. Eksperiment je zasnovan na upotrebi dve doze za tretiranje od 20 i 30 g a.s. acetamiprida / ha. Tretmani su izvedeni sa minimalnom i maksimalnom dozom primene. Utvrđeno je da je acetamiprid efikasan u suzbijanju vaši u paprikama. Uzorci su uzimani prvog, trećeg, petog i sedmog dana po tretiranju. Karenca za acetamiprid je sedam dana. Nakon isteka karence nivo ostataka acetamiprida je bio niži od maksimalno dozvoljenih količina (MDK). Naglašavamo da rezultati analize ukazuju da se mora poštovati propisani period karence, kako bi se izbegao povećan sadržaj ostataka acetamiprida u paprici.

Ključne reči*:* paprika, Macrosiphum euphorbiae, acetamiprid, ostaci, karenca, MDK.

Received / *Primljen*: 15.01.2013.
Accepted / *Prihvaćen*: 01.03.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 35-45, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 663.63:159.944.16:633.15:631.559

EFFECTS OF WATER STRESS ON WATER USE AND YIELD OF MAIZE

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SUMMARY: The study of effects of water stress on yield and water use by maize plants was carried out in the experimental field of the Maize Research Institute at Zemun Polje in the period 2006-2008. Maize sensitivity to water stress was determined using a yield response factor (K_y). The values of K_y were derived from the linear relationship between relative evapotranspiration deficits ($1-ET_a/ET_m$) and relative yield decrease ($1-Y_a/Y_m$). To assess the irrigation effect on maize yield, irrigation water use efficiency (I_{wue}) and evapotranspiration water use efficiency (ET_{wue}) were determined. Values of K_y in the growing season (K_y 0.94) indicate that maize is moderately sensitive to water stress under the climate conditions of Serbia. The amounts of water used on evapotranspiration under irrigation (ET_m) and non-irrigation (ET_a) conditions ranged from 453 to 501 mm, and 257 to 363 mm, respectively. The values of I_{wue} and ET_{wue} varied from 0.020 to 0.036 t ha^{-1}/mm and 0.024 to 0.038 t ha^{-1}/mm, respectively, mostly depending on the favourableness of the year for the maize production and irrigation water applied. K_y, I_{wue} and ET_{wue} can be used as a good basis for maize growers in the region in terms of optimum irrigation water use, for the planning, design and operation of irrigation projects in the region, and also for the improvement the production technology of the crop.

***Key words**: maize, water stress, yield, water use efficiency*

INTRODUCTION

Maize (*Zea mays*, L.) is one of the most important field crops in the world. It is grown on approximately 24% of areas cultivated with cereals (about 155 million ha). Furthermore, the participation of maize in grain production amounted to approximately 30% (i.e. about 609 million tonnes), while the average yield was 4.97 t ha^{-1} in the period

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Original scientific paper / *Originalni naučni rad*

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2006-2008 (FAO Statistical Yearbook, 2008). Maize is even more significant for Serbia. During the same period, maize was cultivated on the 38% of plough field areas, i.e. on 1.23 million ha. The average yield recorded in the period 2006-2008 amounted to 4.4 t ha^{-1}, varying from 3.2 to 5.1 t ha^{-1} (Statistical Yearbook of Serbia, 2008), and correlated, first of all, with the sum and distribution of precipitation.

It is generally considered that maize is resistant to drought and that maize plants use water economically. Nevertheless, maize consumes great amounts of water due to its large vegetative mass, high yields and a long growing season. In the case of water deficiency, maize successfully overcomes drought, but yields less, because plants consume less readily available categories and forms of water from the soil (Bošnjak et al., 2005).

Based on long-term experiments carried out under the conditions of Vojvodina, Bošnjak et al. (2005) point out to maize yields lower by 28.7% as a result of a deficit of readily available soil water with a remark that yield can be lower by 147-159% in extremely arid years in relation to yields recorded under irrigation conditions. Cakir (2004) emphasises that yields amount to 15 t ha^{-1} under irrigation conditions in north-western parts of Turkey, while they amount to 5 t ha^{-1} under rainfed conditions. A striking example of low yields of maize (ranging from 1.22 to 4.63 t ha^{-1}) under rainfed conditions is provided by studies carried out by Vasić et al. (1997) in the arid region of eastern Serbia.

The effect on irrigation on the increase of maize yields depends on weather conditions of the year, primarily on the sum and distribution of precipitation. In dry years, it can be great (Bošnjak and Dobrenov, 1993; Bošnjak and Pejić, 1994), while in wet years, it can be very modest or even it can be omitted (Bošnjak, 1993; Bošnjak and Pejić, 1998; Pejić et al., 2011, Kresović et al., 2012).

The actual evaluation of stress related to the yield due to soil water deficit during the maize growing season can be obtained by the estimation of the yield response factor (K_y) that represents the relationship between a relative yield decrease ($1–Y_a/Y_m$) and a relative evaporation deficit ($1–ET_a/ET_m$). Doorenbos and Kassam (1979) estimate that the average values of K_y is 1.25 during the maize growing season. Vaux and Pruitt (1983) suggest that it is highly important to know not only the K_y values from the literature but also those determined for a particular crop species under specific climatic and soil conditions. This is because K_y may be affected by other factors besides soil water deficiency, namely soil properties, climate (environmental requirements in terms of evapotranspiration), growing season length and inappropriate growing technology.

In order to approach the implementation of any idea on the intensive utilisation of agroecological conditions or the development of new procedures for the irrigation regimes of crops, it is necessary to know precise water needs of plants, i.e. potential evapotranspiration (ETP). Water requirements of maize under agroecological conditions of Serbia vary from 450 to 540 mm (Jeremić and Spasojević, 1968; Bošnjak, 1982; Vasić, 1984; Pejić, 2000).

The estimation of water use efficiency in relation to evapotranspiration (ET_{wue}) can show a more realistic evaluation of irrigation effects, i.e. of the irrigation regime applied in maize crops. Also, the importance of analyizing ET_{wue} is ilustrated by the efforts of numerous studies that consider the total water use for evapotranspiration towards transpiration use as to the productive part of water to plants (Wallace and Batchelor**,** 1977; Howell et al., 1997). The parameter ET_{wue} mostly depends on precipitation amount and distribution and establishes whether the growing period is favorable for plant pro-

duction or not. Wang et al., (1996) pointed out that crop yield depends on the rate of water use and that the factors that increase yield and decrease water used for ET favorably affect the water use efficiency. Howell (2001) indicated that ET_{wue} generally is highest with less irrigation, implying full use of the applied water and perhaps a tendency to promote deeper soil water extraction to make better use of both the stored soil water and the growing-season precipitation.

An even clearer estimation of irrigation effects and the applied irrigation regime can be obtained by the evaluation of irrigation water use efficiency (I_{wue}). If the irrigation regime is not synchronised with water needs of crops, water and physical properties of soil and weather conditions, the effect of irrigation can fail, that is the I_{wue} values can be bellow the optimum. The parameter, I_{wue}, generally tends to increase with a decline in irrigation if that water deficit does not occur at a single growth period (Howell, 2001).

The objective of the study was to estimate the yield response factor (K_y) and on the basis of this factor to analyse a seasonal maize response to water stress and in such a way to obtain additional information that can be useful in the improvement of maize growing practices under climate conditions of Serbia. The established values of ET_{wue} and I_{wue} will be used in analyses of the applied irrigation regime and effects of irrigation on maize yields with the aim to use water more efficiently in irrigation practice. Estimated values of water use on maize evapotranspiration will be compared with previously established water requirements by maize under agroecological conditions of Serbia.

MATERIAL AND METHODS

The trial was carried out on calcareous chernozem in the experimental field of the Maize Research Institute at Zemun Polje during the period 2006-2008. Furthermore, the trial was set up according to the block design and was adapted to sprinkler irrigation. There were two variants in the trial: irrigation with the pre-irrigation soil moisture of 80-85% of field water capacity (FWC) and control (non-irrigated variant). Irrigation was scheduled by monitoring soil moisture levels at 10-20 cm intervals down to 60 cm depth. This was estimated by using a gravimetric method at about 10 day intervals depending upon the weather conditions (Tapanarova**,** 2011).

Maximum evapotranspiration (under irrigation, non limiting conditions) (ET_m) of maize during growing season was calculated using the bioclimatic method that employs hydrophytothermic index (K) with its values 0.11 for May, 0.18 for June, 0.18 for July, 0.18 for August, and 0.11 for September taken from Bošnjak (1982). After determining the ET_m value (1) the actual evapotranspiration (non-irrigated conditions) (ET_a) was calculated on the basis of precipitation data and pre-vegetation soil water reserve using the water balance method (Bošnjak et al., 2012). These values were then used to calculate the soil water deficit in the maize growing season.

$$ET_m = \sum_{i=1}^{n} (K \cdot T) \qquad (1)$$

Where:

ET_m = monthly maximum evapotranspiration for maize (mm)

K = hydrophytothermic index for maize

T = sum of mean daily air temperatures in a given month (^oC)

The yield response factor (K_y), as a parameter of effects of water stress on maize yield decrease during the growing season is calculated using the formula given by Vaux and Pruitt, 1983 (2).

$$\left(1-\frac{Y_a}{Y_m}\right)=K_y\left(1-\frac{ET_a}{ET_m}\right) \quad (2)$$

Where:

Y_a = the actual harvested yield (non-irrigated conditions, t ha^{-1})

Y_m = the maximum harvested yield (under irrigation, non limiting conditions, t ha^{-1})

K_y = the yield response factor

ET_a = the actual evapotranspiration (mm) corresponding to Y_a

ET_m = the maximum evapotranspiration (mm) corresponding to Y_m

$(1–ET_a/ET_m)$ = the relative evapotranspiration deficit

$(1–Y_a/Y_m)$ = the relative yield decrease.

Irrigation water use efficiency (I_{wue}, t ha^{-1}/mm) and evapotranspiration water use efficiency (ET_{wue}, t ha^{-1}/mm) were estimated by expressions given by Bos (1980, 1985), (3, 4):

$$I_{wue}=\frac{Y_m - Y_a}{I} \quad (3)$$

$$ET_{wue}=\frac{Y_m - Y_a}{ET_m - ET_a} \quad (4)$$

Where:

I = the amount of irrigation water applied (mm)

The elementary plot size amounted to 20 m^2 (7.14 m x 2.8 m). The hybrid ZPSC-684 of FAO maturity group 600 with the sowing density of 55,000 plants ha^{-1} was used in the trial. Harvest was done by hand at harvest maturity, while yield (Y) was calculated in t ha^{-1} at 14% moisture. The experimental maize plots received conventional growing technology adjusted to the conditions of irrigation. Statistical processing of data was done by the analysis of variance (ANOVA) and testing the obtained results by the Fisher's LSD test ($P< 0.05$ levels between the means). The relationship between crop yield and water used by evapotranspiration, relative yield decrease and relative crop evapotranspiration deficit for maize growing season were evaluated using regression analysis.

Data on precipitation and air temperatures were recorded in the meteorological station of the Maize Research Institute, Zemun Polje. During the three-year trial period, the average air temperature for the period May-September (20.1°C) was higher, while

the precipitation sum (249.9 mm) was lower than a long-term average (1980-2005) (Table 1). From the aspect of a water supply of maize, 2006 was favourable, 2007 was moderately favourable, while 2008 was unfavourable for the maize production. Amounts of water added by irrigation were correlated with the amount and distribution of precipitations. The irrigation water applied was 100 mm, 155 mm and 280 mm in the irrigation period of 2006, 2007 and 2008, respectively (Table 2).

Table 1. Mean monthly air temperatures (°C) and monthly precipitation sum (mm) during maize growing season

Year	Month										Seasonal average	
	May		June		July		August		September			
	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm
2006	15.8	33.3	18.8	143.6	22.8	27.3	19.6	109.0	18.5	10.8	19.1	324.0
2007	18.8	42.0	22.5	63.0	23.9	18.7	23.7	51.6	15.1	73.0	20.8	248.3
2008	18.3	39.7	22.3	36.3	22.6	46.2	22.8	19.7	16.6	55.4	20.5	177.3
Average 2006/2008	17.6	38.3	21.2	81.0	23.1	30.7	22.0	60.1	16.7	46.4	20.1	249.9
Average 1980/2005	17.2	56.4	20.3	92.3	21.9	61.0	22.1	62.2	17.8	53.8	19.9	325.7

RESULTS AND DISCUSSION

The calculated values of water use on maize in irrigated conditions (ET_m) varying from 453 to 501 mm (Table 2) are in accordance with values previously recorded for the agroecological conditions of Serbia. Jeremić and Spasojević (1968) have determined that water requirements of maize in the Morava region ranged from 418 to 475 mm. Bošnjak (1982) has determined in field plots that water requirements of maize for the conditions of Vojvodina varied from 460 to 520 mm. Škorić and Berić (1994) have determined for the same climate conditions by the calculation over reference evapotranspiration (ETo) and crop coefficients (K_c) that water requirements for normal growth and development of maize amounted to 523 mm. Vasić (1984) has established that water requirements of maize under the Zemun Polje conditions and different methods of irrigation ranged from 451 to 526 mm. The determined values of water use on maize in non-irrigated conditions (ET_a) varied form 257 to 363 mm (Table 2). The calculated values of the deficit in readily available soil water (ET_m - ET_a) ranging from 90 to 233 mm point to the fact that the genetic potential for yield of otherwise very high-yielding maize hybrids will not be fully realized, since the amount of precipitation determines the potential yield levels. Agriculture in Serbia indubitably lacks water as one of the cornerstones of crop production (Vučić**,** 1976).

Effects of irrigation on the maize yield increase in the investigation period amounted to 47.8%, i.e. 4.88 t ha^{-1} (Table 2). Obtained results are in accordance with results gained by Bošnjak and Dobrenov (1993), who have established the average maize yield increase of 45.8% under weather conditions of Vojvodina. Bošnjak and Pejić (1994) have indicated that the average yield increase of maize was 40-44%, with variations over years from modest 6-8% to very high 147-159%.

The relationship between maize yields (t ha^{-1}) and seasonal water crop use (ET, mm) of maize was linear ($r = 0.92$, $P < 0.05$, Fig. 1). This linearity has also been established

by other researchers (Steele et al., 1994, Howell et al., 1995; Istanbulluoglu et al., 2002; Dagdelen et al., 2006; Payero et al,; 2006; Pejić et al., 2011).

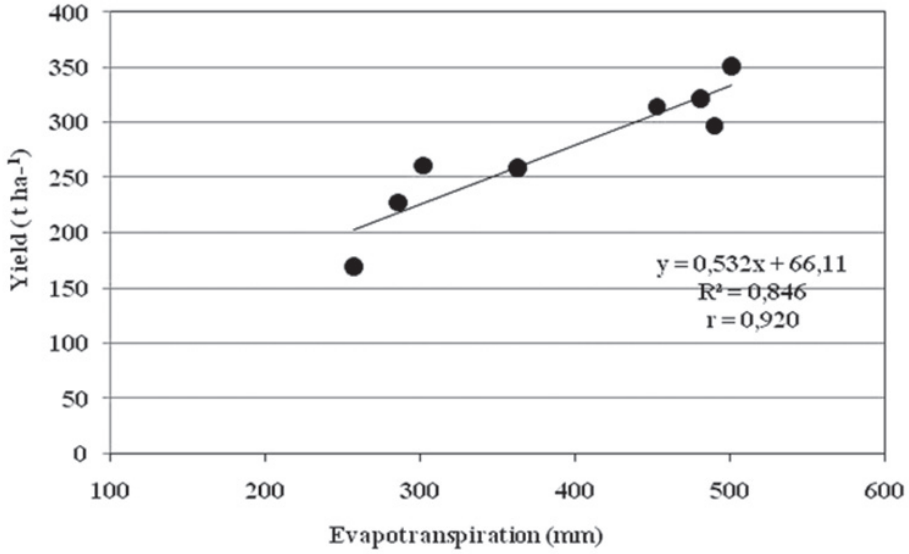

Fig. 1 Relationship between grain yield (Y) and seasonal crop water use (ET) of maize

The value of K_y of 0.94 recorded for the maize growing season (Fig. 2) is lower than values established by other researchers based on results obtained under arid climatic conditions (1.25 - Doorenbos and Kassam, 1979 - FAO publication; 1.47 - Howell et al., 1997 for Bushland in Texas; 1.36 - Cakir, 2004 for arid conditions of Turkey). Calculated values of K_y are in accordance with results obtained under moderate climate. Craciun and Craciun (1999) point out to values for temperate conditions of Romania ranging form 0.66 to 0.86 for hybrids of various maturity groups. Furthermore, Kanber et al. (1990) and Istanbulluoglu et al. (2002) have established values of 0.93 and 0.76 for coastal areas of Turkey. Pejić et al. (2009) point out that the K_y values of 0.65 determined for climate conditions of Vojvodina are the result of weather conditions, first of all, because of precipitation amount and their distribution. They stressed that only three out of 11 investigation years were extremely dry, while remaining eight were without or with an insignificant water deficit. Values of 0.65 in the growing season indicate that maize is moderately sensitive to the soil water deficit under climatic conditions of Vojvodina.

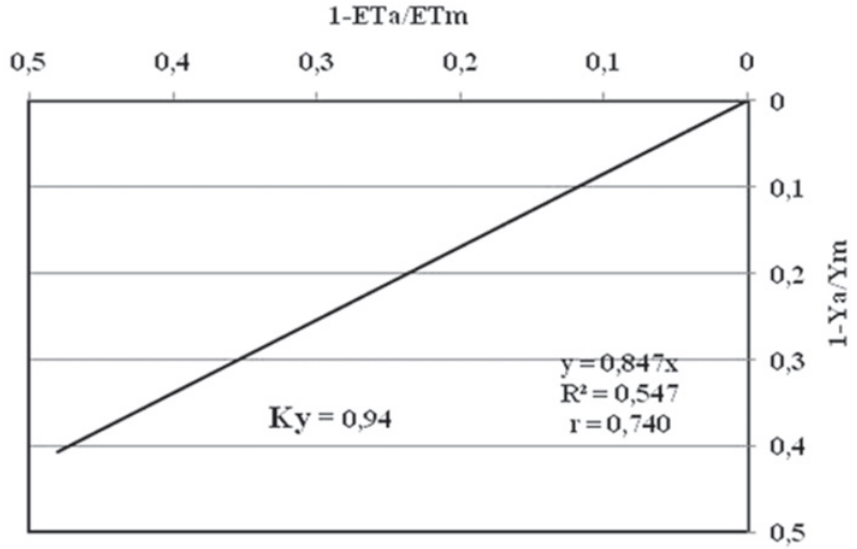

Fig. 2. Maize response to water stress for the total growing season

The best method to describe the role that irrigation has in water use efficiency (WUE) in irrigated agriculture is by expressions given by Bos (1980, 1985). Many researchers have evaluated water use efficiency in different ways (Viets, 1962; Begg and Turner, 1976; Bos, 1980, 1985; Howell, 2001; Pejić, 2010, Pejić, 2011). Consequently, care should be taken when comparing WUE values. Gained results under given agroecological conditions can be compared only in the approximately same temporal distance, because not only genetic potential of yielding was smaller more than 30 years ago (Pejić, 2000), but also growing practices have been significantly modified (Videnović et al., 2007).

Table 2. Water used on maximum (ET_m) and actual (ET_a) evapotranspiration of maize (mm), yield in irrigated (Y_m) and non-irrigated (Y_a) conditions (t ha^{-1}), irrigation water applied (I, mm), yield response factor (K_y), evapotranspiration (ET_{wue}) and irrigation water use efficiency (I_{wue}) (t ha^{-1}/mm)

Year	ET_m	ET_a	$1-ET_a/ET_m$	Y_m	Y_a	I	$1-Y_a/Y_m$	K_y	ET_{wue}	I_{wue}
2006	453	363	0.20	14.59	11.14	100	0.24	1.20	0.038	0.034
2007	501	286	0.43	16.33	10.74	155	0.35	0.81	0.026	0.036
2008	490	257	0.48	14.33	8.73	280	0.39	0..81	0.024	0.020
2006/8	481	302	0.37	15.08*	10.20	178	0.32	0.94	0.029	0.030

Numbers followed by * indicate statistical significance (LSD test P≤ 0.05)

Values of the water use efficiency in relation to evapotranspiration (ET_{wue}) ranged from 0.024 to 0.038 t ha^{-1}/mm (Table 2). The determined values are in accordance with the results gained by Hook (1985) who pointed to the ET_{wue} value of 0,030 t ha^{-1}/mm under

humid conditions of south-eastern regions of the USA and by Pejić (2000) who determined ET_{wue} value of 0.026 t ha^{-1}/mm under conditions of sprinkler irrigation under climatic conditions of Vojvodina. The greatest values of ET_{wue} (0.038 t ha^{-1}/mm) were recorded in 2006, which was favourable for the maize production, during which the lowest amount of water was added by irrigation (100 mm, Table 2). Results are consistent with the statement of Howell (2001) who pointed out that the greater values of ET_{wue} were obtained if smaller amounts of irrigation water was used, because water was then used more efficiently, including precipitation water during the growing season and water reserves from deeper soil layers.

Irrigation water use efficiency (I_{wue}) determined in the period of investigation ranged from 0.020 to 0.036 t ha^{-1}/mm (Table 2). Obtained values are congruent with results of Cassel and Edwards (1985) gained for the conditions of North Caroline (0.003 - 0.036 t ha^{-1}/mm) and Pejić (2000) gained for the conditions of Vojvodina (0.029 - 0.031 t ha^{-1}/mm). Robertson et al. (1980) recorded maximum values of I_{wue} (0.045 t ha^{-1}/mm) for the conditions of Florida noting that the highest maize yields were recorded when I_{wue} ranged from 0.020 to 0.030 t ha^{-1}/mm.

CONCLUSION

Based on results gained on effects of water stress on water use and maize yields under climate conditions of Serbia it can be concluded that the maize yield under rainfed conditions (10.20 t ha^{-1}) was significantly lower than the yield (15.08 t ha^{-1}) recorded under irrigation conditions. Evapotranspiration rate under irrigation conditions (ET_m) ranged from 453 to 501 mm, while they varied from 257 to 363 mm under non-irrigation conditions (ET_a). Irrigation water use efficiency (I_{wue}) ranging from 0.020 to 0.036 t ha^{-1}/mm and evapotranspiration (ET_{wue}) varying from 0.024 to 0.038 t ha^{-1}/mm indicate that the applied irrigation regime in the maize crop was optimal in relation to water and physical properties of soil and biological traits of maize plants. Values of K_y (0.94) in the maize growing season point to the fact that maize is moderately sensitive to water stress under climate conditions of Serbia. The determined values of K_y, I_{wue} and ET_{wue} can be a good basis for maize growers in the region in relation to the optimum irrigation water use, planning, projecting and utilisation of irrigation systems, and also for the improvement the production technology of the crop.

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UTICAJ VODNOG STRESA NA POTROŠNJUVODE I PRINOS KUKURUZA

BORIVOJ PEJIĆ, BRANKA KRESOVIĆ, ANGELINA TAPANAROVA, BOŠKO GAJIĆ, KSENIJA MAČKIĆ

Izvod

Eksperimentalna istraživanja uticaja vodnog stresa na potrošnju vode i prinos kukuruza su obavljena na oglednom polju Instituta za kukuruz Zemun Polje iz Zemuna u periodu od 2006-2008 godine. Osetljivost kukuruza na vodni stres u periodu vegetacije utvrdjena je na osnovu vrednosti koeficijenta opadanja prinosa - K_y. Vrednosti K_y su obračunate iz odnosa relativnog opadanja prinosa ($1–Y_a/Y_m$) i relativnog deficita evapotranspiracije ($1–ET_a/ET_m$). Za ocenu efikasnosti navodnjavanja, odnosno realizovanog zalivnog režima utvrdjen je koeficijent iskorišćenosti vode dodate navodnjavanjem (I_{wue}) i koeficijent iskorišćenosti vode u odnosu na evapotranspiraciju (ET_{wue}). Vrednosti K_y u vegetacionom periodu (K_y 0,94) ukazuju da je kukuruz umereno osetljiv na vodni stres u klimatskim uslovima Srbije. Utrošak vode na evapotranspiraciju u uslovima navodnjavanja (ET_m) kretao se u intervalu od 453-501 mm, a u uslovima bez navodnjavanja (ET_a) u intevalu od 257-363 mm. Vrednosti koeficijenta iskorišćenosti vode dodate navodnjavanjem (I_{wue}) su bile u intervalu 0,020 do 0,036 t ha^{-1}/mm, a koeficijenta iskorišćenosti vode u odnosu na evapotranspiraciju (ET_{wue}) u intervalu 0,024 do 0,038 t ha^{-1}/mm u zavisnosti od povoljnosti godine za proizvodnju kukuruza, odnosno količune vode dodate navodnjavanjem. Utvrdjene vrednosti K_y, I_{wue} and ET_{wue} mogu biti dobra osnova za proizvodjače kukuruza u regionu u pogledu optimalnog korišćenja vode za navodnjavanje, za planiranje, projektovanje i korišćenje zalivnih sistema, a takodje i za unapredjenje tehnologije proizvodnje kukuruza.

Ključne reči: kukuruz, vodni stres, prinos, efikasnost korišćenja vode.

Received / *Primljen*: 04.03.2013.
Accepted / *Prihvaćen*: 18.03.2013.

UDC: 614.449:599.735.5

EPIDEMIOLOGICAL ANALYSIS OF BVDV INFECTION IN CATTLE FARMS OF KHARKOV REGION, UKRAINE*

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SUMMARY: Bovine viral diarrhea is a widespread infection of cattle caused by bovine viral diarrhea virus (BVDV),a member of the Pestivirus genus of the Flaviviridae family. The virus persists in the cattle population by a unique combination of transient and persistent infections. Persistently infected (PI)animals may succumb to mucosal disease, which is characterized by lesions in the gastrointestinal tract and its invariably lethal outcome. This study was focused on identification PI animals in cattle farms of Kharkov region, Ukraine. For this reason 1080 blood samples from three different farms were tested for presence BVDV specific antibody by ELISA and viral genetic materials by real-time RT-PCR. In this study 5 PI animals were detected in two farms. Following phylogenetic analysis in 5'-UTR (245 bp fragment) was used for the genetic typing of revealed BVDV isolates into subgenotypes. The genetic typing indicated that all 4 viruses from second farm were typed as BVDV-1b and all of them were absolutely identical in 5'-UTR. The virus from third farm typed as BVDV-1f.

Key words: *bovine viral diarrhea, real-time RT-PCR, ELISA, genotyping, phylogenetic analysis, cattle.*

Original scientific paper / *Originalni naučni rad*

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*This work is a part of the project supported by Swiss National Science Foundation Project SCOPES (NF number IZ730Z0_128050: Epidemiology of BVD in Ukraine: Development of a control program), from 2011 to 2012.

INTRODUCTION

*Pestiviruses*family displays marked genetic and antigenic diversity (Becheret al., 2003). There are four main species, namely, BVDV-1 and -2, Border disease virus and CSFV in the family. A marked diversity is observed also within the BVD viruses. In particular BVDV-1 viruses are very heterogenous, with at least 13 subgroups, whereas two subgroups are differentiated in the more homogenous BVDV-2 viruses (Jackovaet al., 2008).

BVDV is present in the cattle population worldwide (Nettleton and Entrican,1995). The success of BVDV rests on its capacity to establish persistent infection. Viral persistence is established during a "window of opportunity" early in gestation andis associated with immunotolerance to the infecting viral strain. Different from persistent infectionsby herpesviruses and lentiviruses, persistent infected(PI) animals remain free of antibodies to BVDV(Chase et al., 2004), which calls for detection of viral antigen or viral RNA as thesole methods for diagnosing persistent infection. Although transiently infected animals maybe capable of transmitting virus to susceptible cattle to a limited extent, only PI animals areresponsible for viral persistence in the host population. Typically, about one percent of thecattle population is PI and some 60 percent are seropositive when the infection has reachedan equilibrium (Houe, 1999; Hessmanet al., 2009). Calves born to seropositivecows receive colostral antibodies against BVDV (Peterhans et al., 2010). These antibodies decrease in titer overtime and the calves become susceptible for infection. The time span of colostral protectiondepends on the antibody titer and the level of infectious pressure to which the animals areexposed. Older animals are more likely to be seropositive, due to a longer time during whichthe animals are at risk of being exposed to PI animals. In contrast, many heifers may still beseronegative during their first pregnancy. When exposed to PI animals during the critical periodof development, fetuses may be infected to become PI, thereby assuring viral persistencein the next generation.

The economic losses due to BVDV infection are considerable (Weldegebrieletal., 2009). These losses, and the epidemiological insight that removalof PI animals efficiently truncates chains of infection, have encouraged control programs atthe national and regional levels. Life vaccinesmay cause fetal infection or even trigger mucosal disease (Becheret al., 2001), whereasinactivated vaccines are safe but confer insufficient protection, especially against fetalinfection (Van Oirschotet al., 1999). Consequently, the cornerstone of allcurrent eradication programs is detection and elimination of PI animals. The approachesused to achieve this goal however differ. PI animalsmay be detected by determining herd immunity to BVDVin countries where seroprevalence is low. High seroprevalence signalsthe presence of PI animal(s) in the herd, which may then be identified by virus detection. Thisapproach is blunted by a high overall seroprevalence in the cattle population.

Ukraine currently has 4.5 million cattle (http://minagro.gov.ua, 2013). These animals are kept in 4350 herds, of which 10 to 20% have 900 and more animals, with the biggest ones approaching 7000 animals. In addition to the very large herds, there are an unknown number of small privately owned herds in Ukraine. About 60% of the herds produce milk and about 30% are in mixed milk and beef production. The rest are beef herds. The extent of animal traffic between the herds is not known in detail but most likely a large fraction of male calves end up in beef herds because only a relatively

small number of bulls are required for natural breeding and artificial insemination. The intensity of import and export is not known at this time. There is no systematic testing for BVD, nor is there a systematic approach to controlling BVD. Inactivated and attenuated BVDV vaccines are in use, but the extent of use and the efficacy of these vaccines are unknown.

This study was focused on detection of BVDV specific antibodies by ELISA and viral RNA by real-time PCR,identification of persistently infected animalsin selected cattle farmsand with followed genetic typing of selected BVDV isolates.

MATERIAL AND METHODS

*Cattle and sample collection.*1080 blood samples of cattle from 3 different farms in North-East territory of Ukraine were used for this study. The samples were collected from November 2011 to June 2012. Animals were selected of different ages from newborns.

A total number of animals are 815 cattle in the first farm, 900 and 5431 cattle in the second and the third farm. A detailed questionnaire was completed for each herd with the owner's support. The variables of interest related to individual animals as well as to the herd and comprised the type of farm, animal movements, general management, feeding, prophylactic health measures, disease incidence, and BVDV disease awareness.

Antibodies capture ELISA. The ELISA test was performed by the commercially available ELISA Kit HerdChek BVDV Ab Test (IDEXX Laboratories, Switzerland), in which microtitre plates were coated with immobilizing BVDV antigen. BVDV antibodies of the sample were bounded to the antigen on the plates. After incubation of the test sample in the well, captured BVDV antibodies are detected by anti-bovine horseradish peroxidase conjugate. Unbound conjugate is washed away and a substrate/chromogen solution is added. In the presence of enzyme, substrate is converted into a product which reacts with the chromogen to generate a blue color. The reaction was terminated by the addition of stop solution to each well and finally the absorbance at 450 nm was monitored in ELISA reader (BIO-TEK Instruments, Inc. Winooski, VT, USA). The result could be read visually where the optical density (OD) value was measured at450 nm. Positive and negative controls were used as indicated in the kit. The presence or absence of BVDV antibody in the sample is determined by the corrected OD value (S/P) for each sample was considered as follow:

$$\frac{S}{P} = \frac{Sample\ A_{450} - NC\bar{x}A_{450}}{PC\bar{x}A_{450} - NC\bar{x}A_{450}}$$

$NC\bar{x}$- is negative control;
$PC\bar{x}$- is positive control.

Samples with S/P values than 0.3 were classified as negative and samples with S/P values higher than 0.3 were classified as positive for BVDV antigen.

After serological testing, molecular analyses were conducted.

*Real-time RT-PCR.*RNA extraction from the serum was performed using QIAamp Viral RNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instruction

(vacuum protocol), with the following modifications. Serum samples were combined for five into one pool of 140 µl. The RNA was eluted in 60 µl RNA storage solution. The real-time RT-PCR was applied, using *cador*BVDV RT-PCR Kit (QIAGEN, Germany) and carried out in a total volume of 50 µl, containing 20 µl of the eluate from the RNA isolation and 30 µl of the Master Mix and applied the following program in the ABI Prism 7700 Sequence Detection System: 1 × 30 min 50 °C (RT-step), 1 × 10 min 95 °C, and 45 × 30 sec. 95 °C; 1 min 55 °C (cycling).

*Phylogenetic study.*Samples, determined as positive in PCR were studied with sequencing.Phylogenetic analysis in 5'-UTR (245 bp fragment) was used for the genetic typing of BVDV isolates into subgenotypes. Phylogenetic trees were constructed by Neighbor Joiningand Maximum parsimony algorithms. Pair distance was determined by Murakami algorithm. All phylogeny trees buildings and analyses were done with modules of MEGA 5 software.

*Statistical analysis.*Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp.One Microsoft Way, Redmond, WA, USA) for analysis.Using NCSS 07.1.21 statistical software (NCSS, LLC, Kaysville, Utah, USA).

RESULTS AND DISCUSSION

As the first step of our study BVDV specific antibodies were detected by ELISAin 713 of 1059 samples analyzed (67.3%). This number is in agreement with findings in many cattle herds around world. However the number of positive samples differed in the herds. While 57 samples out of 283 (20.1%) were identified in the first herd, 400 out of 475 (84.2%) and 256 out of 301 (85%) animals were positive in the second and the third herd.

The real-time PCR assay detected BVDV RNA in 5 of 1068 samples analyzed (0.5%). 4 positive samples out of 490 (0.8%) and 1 out of 301 (0.33%) were found in the second and the third herd. The genetic materials of BVDV were not found in the first herd.

Animals that were virus-positive in the real-time RT-PCR but antibody-negative in ELISA were considered to be persistently infected. Based on these criteria, the results obtained with the antibody detection method and the real-time RT-PCR were concordant in 1047 of the 1080 animals. All 5 virus-positive samples were serological negative. Consequently, 5 of these 1047 (0.48%) animals were persistently infected. The 5 virus-positive animals were 2, 4, 5 and 8 month old.

The genetic typing of viral isolates revealed that only BVDV type 1 viruses were presented. The phylogenetic analysis confirmed two BVDV-1 subtypes, namely b and f (Fig. 1) and revealed that all 4 viruses from second farm were typed as BVDV-1b and all of them were absolutely identical in 5'-UTR, but virus from third farm were typed as BVDV-1f.

The genetic diversity, demonstrated in the study, releases the belonging of characterized viruses to BVDV-1b strains with the distance not more 2-4 %. This is typical in the current genetic studies of worldwide characterized viruses. Allocated viruses of this subtype are truly same inside this clade of Ukrainian viruses.

Another detected subtype was 1f. This group of BVDV-1 was also detected in several countries of the Central and Western Europe, so they are not unique. Characterized isolate had 4.5 % differences among subtype-belonged related viruses of BVDV-1f

genotype.

Current scientific literature explains the significant role of the BVDV-1 in the epidemiology of bovine viral diarrhea all over the World. It demonstrates distribution in all European countries, only several countries have been eradicated this disease by the implementation of the eradication strategies based on PI-animals elimination and/or vaccination of susceptible animals.

Viral genetic divergence studies allows to study the molecular diversity of virus for the creation of effective prevention means, and gives the opportunity to determine viral origin and source for recognition of the epidemiology of bovine viral diarrhea and its eradication strategy development.

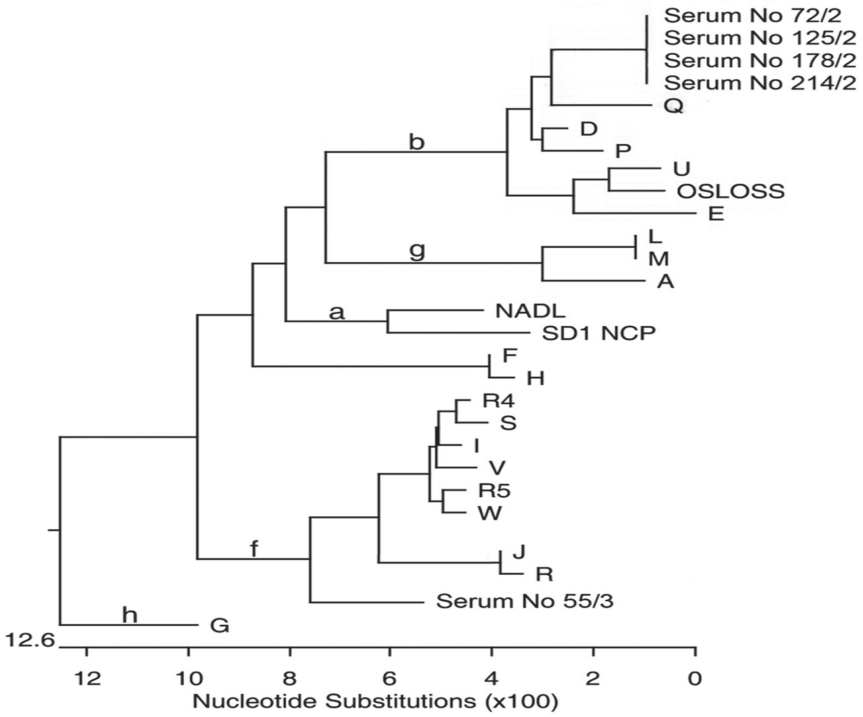

Fig. 1.Genetic typing of BVDV isolates in the 5'-UTR region

CONCLUSION

High seroprevalence levels for BVDV (20.1 - 85%) were demonstrated in the cattle herds.The real-time PCR assay detected BVDV RNA 0.33 - 0.8% of cattle have been tested. Detected viruses belonged to BVDV-1 type, subgenotypes 1b and 1f by 5'-UTR region sequences.

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EPIDEMIOLOŠKA ANALIZA BVDV INFEKCIJE NA FARMAMA GOVEDA U HARKOVSKOJ OBLASTI, UKRAJINA

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Izvod

Bovina virusna dijareja je oboljenje goveda, svetske distribucije, izazvano virusom bovine virusne dijareje (BVDV), koji pripada rodu *Pestivirus* i familiji *Flaviviridae*. Virus se održava u populaciji goveda jedinstvenom kombinacijom tranzitorne i perzistentne infekcije. Perzistentno inficirane (PI) životinje mogu razviti oboljenje sluznica, koje se karakteriše lezijama na gastrointestinalnom traktu sa letalnom ishodom. Ovo istraživanje se odnosi na identifikaciju PI životinja na farmama goveda u Harkovskoj oblasti, Ukraina. Prikupljenih 1080 uzoraka krvi, sa tri različite farme,

ispitano je na prisustvo specifičnih antitela na BVDV pomoću ELISA testa, kao i na prisustvo genoma BVDV primenom real-time RT-PCR testa. U ovoj studiji je registrovano 5 PI životinja na dvema farmama. Za genotipizaciju otkrivenih BVDV izolata u subgenotipove korišćena je filogenetska analiza 5'-UTR (245 bp fragment). Genetska tipizacija ukazala je da su sva 4 virusa sa druge farme klasifikovana kao BVDV-1b i imali su identičan region 5'-UTR. Virus sa treće farme klasifikovan je kao BVDV-1f.

Ključne reči: bovina virusna dijareja, real-time RT-PCR, ELISA, genotipizacija, filogenetska anliza, goveda.

Received / *Primljen*: 06.03.2013.
Accepted / *Prihvaćen*: 21.03.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 53-60, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 581.141

FLORISTIC COMPOSITION AND WEED SEED BANK IN INTENSIVE AND EXTENSIVE CULTIVATION OF VINE GRAPE

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SUMMARY: Floristic composition of weed species occurs as the companion of cultivated plants, including vine grape as a perennial culture. Knowledge of weed seed banks in agro-eco systems of certain region enables better choice of cultivation practices, as well as rational herbicide use. In the period 2011-2012 studies of floristic composition and weed seed banks in vine grape grown both intensively and extensively were performed. The aim of the study was to determine the relation of floristic composition and weed seed bank in vine grape plantations with different cultivation practices. In addition to the farming operations of soil tillage, intensive cultivation method would involve also herbicide use and extensive farming would involve all cropping operations without herbicide use. Soil samples for determination of weed seed bank were taken at the beginning and at the end of the growing season from each plot in ten replications. Samples were taken from different depths of the arable soil layer, i.e. from 0-10 cm, 10-20 and 20-30 cm (Conn, 1987; Sharratt, 1998). Despite great diversity of weed species, whose seeds were determined from the samples, only few weed species dominated with a greater number of seed, and these were Amaranthus retroflexus L., Portulaca oleracea L., Chenopodium album L., Stellaria media (L.) Vill. and Lamium purpureum L.

Key words: *weed seed bank, vine grape, method of growing, extensive, intensive.*

INTRODUCTION

Weed seed bank in the soil represents the past and potential future of the size of weed seed community above the soil surface (Swanton and Booth, 2004). According to Baker (1989), definition of ''seed bank'' is that ''it is seed quantity in the soil capable to

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Original scientific paper / *Originalni naučni rad*
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germinate, to give new life to annual and perennial plants''. The type of soil and cultivation have great influence on the quantity of weed seed in the soil. Understanding of weed seed bank is necessary for improvement of studies of weed population dynamics or for establishment of weed control programs (Ambrosio et al., 2004). According to its floristic composition and structure, vineyard weed community belongs amid communities of weed field row crops and orchards (Konstantinović, 2011). The grapevine (*Vitis vinifera* L.), whose numerous varieties are used for production of grapes belongs to the family *Vitaceae*. Weeds that occur in vineyards have extremely adverse effect both on the vineyards that are in establishment, as well as on the older vineyards. Cultivation practices include autumn and spring inter-row soil cultivation, as well as cultivation during a vegetation period. Studies of weed seed bank size and composition can provide information on past and present weed populations, which contributes to development of forecast system of future potential problems in agricultural production. The study showed that weed communities, in addition to the methods applied for weed control on agricultural production areas are also under great influence of external factors (Buhler *et al*., 2000; Cardina *et al*., 2002). Weed seed density, as well as the quantity, to a great extent depend upon soil type, previous crops, soil cultivation, and certainly upon herbicide use (Konstantinović *et al*, 2008). The aim of this study was to determine differences in weed seed bank of extensive and intensive cultivation of vine grapes, as well as differences in floristic composition of these two ways of growing. Despite different ways of cultivation in the vine rows and in between vine rows in both systems of vine grape growing, the method of sampling was the same, as well as the method of analysis.

MATERIAL AND METHODS

During 2012 at site Erdut, the analysis of weed floristic composition was performed in several vineyard localities, of both extensive and intensive vine grape cultivation. With the aim of establishing weed seed bank, in addition to the analysis of floristic composition, spring soil sampling was performed within the lines and between vine grape lines. Soil sampling on one plot was performed in ten replications, and always from depths of 0-10, 10-20 and 20-30 cm. Each sample contained approximately 3 kg of the soil that was sieved through a series of copper sieves of 0.25 mm in diameter. This was followed by separation of weed seeds from samples, and their determination (Skender *et al*., 1998). Determination was done by microscopes and weed identification guides. Data processing was performed according to the method of Conn (1987) and Sharratt (1998).

RESULTS AND DISCUSSION

At the studied site in intensive and extensive vineyards 33 weed species were found: *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Convolvulus arvensis* L., *Cynodon dactylon* (L.) Pers., *Datura stramonium* L., *Echinochloa crus-galli* (L.) Beauv., *Euphorbia ciparissias* L., *Euphorbia helioscopia* L., *Galium verum* L., *Geranium dissectum* L., *Hibiscus trionum* L., *Iva xanthifolia* Nutt., *Lamium purpureum* L., *Papaver rhoeas* L., *Poa annua* L., *Polygonum aviculare* L., *Polygonum persicaria* L., *Portulaca oleracea* L., *Setaria glauca* (L.) Beauv., *Setaria*

viridis (L.) P. B., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers., *Stachys annua* L., *Stellaria media* (L.) Vill., *Veronica arvensis*. *Taraxacum officinale* Web., *Viola tricolor* L.,*Sinapis arvensis* L., *Rumex crispus* (L.)-Medic., *Capsella bursa-pastoris* L., and *Anthemis arvensis* L. In terms of life forms, 22 weed species were represented by therophytes (T), 4 by geophytes (G), 3 by hemicryptohpytes (H) and 4 by therophytes/ hemicryptophytes (Th) (Table 1).

Table 1. Floristic composition of weed species in extensive and intensive grapevine plantations

WEED SPECIES	Extensively	Intensively	Life form
Amaranthus retroflexus L.	+	+	**T**
Ambrosia artemisiifolia L.	+	+	**T**
Chenopodium album L.	+	+	**T**
Convolvulus arvensis L.	+	-	**G**
Cynodon dactylon (L.) Pers.	+	+	**G**
Datura stramonium L.	+	+	**T**
Echinochloa crus-galli (L.) Beauv.	+	+	**T**
Euphorbia ciparissias L.	+	+	**H**
Euphorbia helioscopia L.	+	+	**T**
Galium verum L.	-	+	**G**
Geranium dissectum L.	+	+	**T**
Hibiscus trionum L.	+	-	**T**
Iva xanthifolia Nutt.	+	+	**T**
Lamium purpureum L.	+	+	**Th**
Papaver rhoeas L.	+	+	**T**
Poa annua L.	+	+	**T**
Polygonum aviculare L.	+	-	**T**
Polygonum persicaria L.	+	+	**T**
Portulaca oleracea L.	+	+	**T**
Setaria glauca (L.) Beauv.	+	+	**T**
Setaria viridis (L.) P. B.	+	+	**T**
Solanum nigrum L.	+	+	**T**
Sorghum halepense (L.) Pers.	+	+	**G**
Stachys annua L.	+	+	**T**
Stellaria media (L.) Vill.	+	+	**Th**
Veronica arvensis	+	+	**T**
Veronica hederifolia L.	+	+	**T**
Taraxacum officinale Web.	+	+	**H**
Viola tricolor L.	-	+	**Th**
Sinapis arvensis L.	+	+	**T**
Rumex crispus (L.) – Medic.	+	+	**H**
Capsella bursa-pastoris L.	+	+	**Th**
Anthemis arvensis L.	+	+	**T**

(+) *Determined*, (-) *Undetermined*. (+) Pronađeno, (-) Nije pronađeno.

Soil cultivation can have great influence on seed distribution in the soil profile (Anderson *et al.* 1998; Sosnoskie *et al.* 2006). On the chosen sites of Erdut in 6 vineyards, seeds of the following weeds in weed seed bank were not found: *Taraxacum officinale* Web., *Viola tricolor* L.,*Sinapis arvensis* L., *Rumex crispus* (L.)-Medic., *Capsella bursa-pastoris* L., and *Anthemis arvensis* L. The following fourteen weed species were determined: *Portulaca oleracea* L., *Amaranthus retroflexus* L., *Stellaria media*

(L.) Vill., *Chenopodium album* L., *Echinocloa crus-galli* (L.) R. et Sch., *Hibiscus trionum* L., *Polygonum aviculare* L., *Datura stramonium* L., *Setaria glauca* (L.) Beauv., *Setaria viridis* (L.) Beauv., *Stachys annua* L., *Ambrosia artemisiifolia* L., *Cynodon dactylon* (L.) Pers., and *Lamium purpureum* L. In the top soil layer of 0-10 cm there was a significantly higher number of seeds per m², including three weed species: *Portulaca oleracea* L. (5627), *Amaranthus retroflexus* L. (5263) and *Chenopodium album* L. (4125). In the last five years in intensively cultivated vineyards weed control has been performed in the vine rows, by herbicides glufosinate-ammonium and glyphosate. Annually, treatments were performed once with the allowed concentrations of glyphosate for perennial crops, as well as three times by herbicide glufosinate-ammonium. In the top soil layer the number of seeds per m² was 16669, while in the other two layers the number of weed seeds was almost equal, but singificantly lower in comparison to the top layer. In the layer of 10-20 cm the number of weed seed was 11.538 per m^2, and in the layer of 20-30 cm it was 11.454 weed seeds per m^2.

Between vine rows 10 weed species were found: *Portulaca oleracea* L., *Amaranthus retroflexus* L., *Stellaria media* (L.) Vill., *Chenopodium album* L., *Iva xanthifolia* Nutt., *Poa annua* L., *Veronica hederifolia* L., *Setaria viridis* (L.) Beauv., *Cynodon dactylon* (L.) Pers., and *Lamium purpureum* L. In the top soil layer, it was five weed species that had a higher number of seeds in relation to other species and layers of the sampled soil, i.e. *Portulaca oleracea* L. (3931), *Amaranthus retroflexus* L. (3712), *Stellaria media* (L.) Vill.(2871), *Chenopodium album* L.(2987), and *Lamium purpureum* L.(1755). In the top soil layer the seed quantity was significantly higher, numbering 15974 seeds per m^2, in comparison to the other two layers of 10-20cm and 20-30 cm of depth. In the soil layer of 10-20 cm 9139 seeds per m^2 were found, while in the layer of 20-30 cm 11831 seeds per m^2 were found.

In systems with reduced tillage, weeds have a tendency to produce a significantly higher number of seeds in top layers than in systems with intensive cultivation (du Croix Sissons *et al.* 2000; Dyer 1995; Soriano *et al*. 1968). In systems with no-tillage, seeds remain on the soil surface, exposed to inconvenient conditions that reduce or increase germination capability, depending on the species (Chauhan *et al*. 2006). In the row of extensively grown vineyard 16 weed species were found: *Portulaca oleracea* L., *Amaranthus retroflexus* L., *Stellaria media* (L.) Vill., *Chenopodium album* L., *Euphorbia helioscopia* L., *Galium verum* L., *Polygonum persicaria* L., *Datura stramonium* L., *Setaria glauca* (L.) Beauv., *Solanum nigrum* L., *Stachys annua* L., *Geranium dissectum* L., *Veronica arvensis* L., *Euphorbia ciparissias* L., *Convolvulus arvensis* L., and *Sinapis arvensis* L. In the top soil layer of 0-10 cm a significantly higher number of seeds had three weed species, *Portulaca oleracea* L., numbering 7736 seeds per m^2, *Amaranthus retroflexus* L., numbering 3669 seeds per m^2, and *Chenopodium album* L. with 5264 seeds per m^2, in relation to 10 other species that were also found in this soil layer. In comparison to other two layers, the top soil layer contained significantly higher quantity of weed seed numbering in total 19380 weed seeds per m^2. In the vine row of extensive vineyard the number of weed seeds decreased with increased soil depth.

In between line area of extensive vineyard, 14 weed species were found: *Portulaca oleracea* L., *Amaranthus retroflexus* L., *Stellaria media* (L.) Vill., *Chenopodium album* L., *Polygonum persicaria* L., *Polygonum aviculare* L., *Papaver rhoeas* L., *Geranium dissectum* Jusl., *Solanum nigrum* L., *Stachys annua* L., *Euphorbia ciparissias* L., *Convolvulus arvensis* L., *Datura stramonium* L., and *Ambrosia artemisiifolia* L.

According to the number of seeds in the top layer of 0-10 cm two weed species, *Portulaca oleracea* L. and *Amaranthus retroflexus* L. proved dominant, numbering 6620 and 7577 weed seeds per m^2, respectively. The top soil layer contained significantly higher quantity of weed seeds, i.e. 15712 per m^2 in comparison to the other two layers. In the soil layer of 10-20 cm 12601 seeds per m^2 were determined, while in the layer of 20-30 cm 13.638 seeds per m^2 were determined.

In the vine row of extensive vineyard there was a greater number of weed species among which the following three were dominant: *Portulaca oleracea* L., *Amaranthus retroflexus* L., and *Chenopodium album* L. A higher number of weed species occurring between vine rows in intensive vineyard suggests that soil cultivation itself equalized and reduced numbers of seeds in the seed bank, while in the vine row there were three weed species with a significantly higher number of seeds in the seed bank in relation to the seed bank in between vine rows of intensive vineyard. In between rows area, simultaneously with herbicide application with glyphosate and glufosinate-ammonium, vine covering and uncovering were performed on a regular basis.

In the vine row of extensive vineyard, according to the number of determined seeds, the following three weed species proved to be dominant: *Portulaca oleracea* L., *Amaranthus retroflexus* L. and *Chenopodium album* L. Two weed species, *Portulaca oleracea* L., and *Amaranthus retroflexus* L., which were dominant in comparison to other weed species, occurred in between vine rows, but in abundance they were almost equal to the number of weed seeds in the vine row. Soil tillage in extensive vineyard was reduced to periodical cultivation or mulching, in the vine rows, as well as between them. Cardina *et al.* (1991) stated that weed seed density in cultivated soils was always composed of several dominant weed species in a high number and several weed species in a moderate or lower number. Results of Uremis *et al.* (2003) suggest that weed seed bank consists of several dominant weed species, but *Amaranthus* sp. was always among them. Results of this study confirmed dominance of weed species *Amaranthus retroflexus* L., but Portulaca *oleracea* L. proved to be dominant as well.

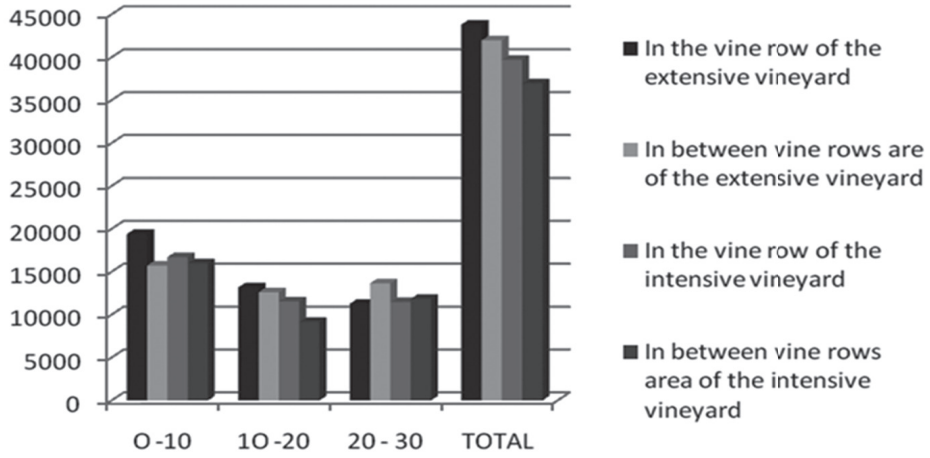
In the rows of the extensive and intensive vineyards, a higher number of weed seeds was found in comparison to the area between vine rows in both vineyard growing systems. In the extensive vineyard, a higher number of weed seed was distributed per m^2 (Graph 1).

In the vine row of the intensive vineyard, up to depth of 0-30 cm per m^2, there were 39683 seeds of weed species.

In between vine rows area of the intensive vineyard, up to depth of 0-30 cm per m^2 there were 36944 seeds of weed species.

In the vine row of the extensive vineyard, up to depth of 0-30 cm per m^2 there were 43785 seeds of weed species.

In between vine rows are of the extensive vineyard, up to depth of 0-30 cm per m^2 there were 41951 seeds of weed species.



Graph 1. Total number of weed seeds per m² in the vine row and in between vine row area of the extensive and intensive vineyard.

CONCLUSION

The results of this study suggest that weed seed bank potential was high, with 27 sampled and indentified weed species. The number of weed species in the intensive vineyard in the vine row and in between line row area was lower in comparison to the extensive vineyard. It was concluded that intensive mechanical cultivation in between vine row area reduced the number of weed species. The number of weed seeds was also significantly lower in intensive system of vine grape cultivation than in the vineyard of extensive vine growing.

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FLORISTIČKI SASTAV I BANKA SEMENA KOROVA U INTENZIVNOM I EKSTENZIVNOM GAJENJU VINOVE LOZE

BRANKO KONSTANTINOVIĆ, MILAN BLAGOJEVIĆ,
BOJAN KONSTANTINOVIĆ, NATAŠA SAMARDŽIĆ

Izvod

U agroekosistemima poznavanje banke korovskih semena na određenom području omogućava bolji izbor agrotehničkih mera, kao i racionalniju primenu herbicida. Tokom 2011-2012 godine vršena su ispitivanja floističkog sastava i banke semena korova u zasadu vinove loze, koja je uzgajana po principu intenzivnog i ekstenzivnog uzgoja. Intenzivan uzgoj bi podrazumevao pored svih agrotehničkih operacija obrade zemljišta i upotrebu herbicida. Ekstenzivan uzgoj bi podrazumevao sve agrotehničke operacije bez upotrebe herbicida. Uzorci zemlje za određivanje banke semena korova uzimani su na početku i pred kraj vegetacije, i to sa svake parcele u deset ponavljanja,

iz oraničnog sloja zemlje, posebno sa dubine od 0-10 cm, 10-20 cm i 20-30 cm. Uzeti uzorci zemljišta su ispirani vodom kroz bakarna sita određenih promera i sušeni na sobnoj temperaturi, a zatim je usledilo izdvajanje semena korova i njihova determinacija. I pored velike raznovrsnosti korovskih vrsta, čija su semena determinisana iz ispitivanih uzoraka, samo nekoliko vrsta dominiralo je sa većom brojnošću semena: *Amaranthus retroflexus* L., *Portulaca oleracea* L., *Chenopodium album* L., *Stellaria media* (L.) Vill. i *Lamium purpureum* L.

Ključne reči: banka semena korova, vinova loza, ekstenzivno, intenzivno.

Received / *Primljen*: 12.03.2013.
Accepted / *Prihvaćen*: 04.04.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 61-68, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 636.09:626.24

POTENTIAL BENEFITS OF SERUM PROFILING FOR RESPIRATORY DISEASE CONTROL

IRENA GOLINAR OVEN[1]

SUMMARY: On one Slovenian large pig farm 36 litters were selected from a herd to make serum profiles to selected (important) respiratory pathogens for preparation of specific control measures. 36 serum samples from breeding sows and 342 serum samples from pigs (38 serum samples; 9 samplings) were tested for antibodies to Porcine Circovirus Type 2 (PCV2), Swine Influenza Virus (SIV), Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae and Haemophilus parasuis. The same pigs were bled at 2, 4, 6, 8, 11, 14, 17, 22 and 28 weeks of age. Commercial ELISA kits of different producers were used. In breeding sows seroprevalence to SIV, A. pleuropneumoniae was 100 %, to PVC2 94 %, to M. hyopneumoniae 83,3 % and to H. parasuis 36 %. Colostral antibodies in pigs against SIV and PCV2 persisted for about 4 weeks. The lowest seroprevalence was detected in 6 weeks old pigs against both viruses. According to serum profiles vaccination of sows against SIV and PCV2 can be proposed. Colostral antibodies against A. pleuropneumoniae persisted for almost 8 weeks (94,8 % prevalence). The lowest prevalence was detected in 14 weeks old pigs (53,8 %). According to serum profiles vaccination against A. pleuropneumoniae around 11-14 weeks of age can be proposed. Till 8 weeks of age pigs were seronegative against H. parasuis. The seroprevalence increased at 11 weeks of age. Vaccination against H. parasuis around 8-9 weeks of age can be proposed. The seroprevalence against M. hyopneumoniae at 6 weeks of age decreased to 0 % and at 11 weeks of age started to increase. Second vaccination between 11.-14. weeks of age can be proposed.

Key words: *pig, respiratory diseases, serum profile, control measure.*

INTRODUCTION

Respiratory infections occur with a high prevalence in all swine-producing areas (Sörensen et al., 2006). The economic impact of respiratory diseases is considerable,

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Original scientific paper / *Originalni naučni rad*

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mainly due to reduced growth and feed efficiency (Stärk, 2000).

Clinically significant disease is seldom the result of an infection with one pathogen. Several pathogens are very often involved in respiratory diseases (Sörensen et al., 2006). Combinations of bacteria and viruses work synergistic in producing more severe respiratory diseases than those induced by each individual agent (Choi et al., 2003).

In developing intervention strategies the interaction between different pathogens on a farm should be considered (Thacker, 2001). Although the structure of modern pig production is rapidly changing, preferably toward all-in/all-out and multisite systems, farrow-to-finish operations still exist. Continuous flow of animals through the system leads to steady transmission of respiratory pathogens from sows to piglets, and from older to younger pigs (Sörensen et al., 2006).

The main respiratory pathogens of concern are Porcine Circovirus Type 2 (PCV2), Swine Influenza Virus (SIV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis*.

Serological data in studying respiratory-disease dynamic in herds may be useful. Clinical occurrence of disease is of little value because most pigs in chronically infected herds are subclinically infected (Andreasen et al., 2000). Serum profiles are those serial studies performed in order to know the immunological status of a farm. Serum profiles are based on the detection of circulating antibodies. The same serum can be tested against specific antibodies for different respiratory pathogens (Golinar Oven and Valenčak, 2010). In practice, cross-sectional serological testing of different age groups from different housing units is the easiest way to gather such information (Andreasen et al., 2000). We chose to use a longitudinal study design which is more precise.

The objective of this study was to make the serum profiles to selected (important) respiratory pathogens for preparation of specific control measures on one Slovenian large pig farm.

MATERIAL AND METHOD OF THE STUDY

A farm was one site unit from farrow-to-finish production with 7000 breeding sows at the time of collection of samples. Pigs were vaccinated at the age of 10-14 days against *M. hyopneumoniae*. Blood samples were collected between December 2007 and July 2008.

36 litters were selected from a herd. Piglets were randomly selected (using a computer-generated list of random numbers) from each of these litters. A cohort consisted of pigs born within the same week. The sows were chosen according to the week they farrowed.

Piglets were individually identified at 2 weeks of age with numbered ear tags. The same pigs were bled at 2, 4, 6, 8, 11, 14, 17, 22 and 28 weeks of age. The last blood sampling (at 28 weeks of age) was done in a slaughterhouse. 38 pigs finished the trial. One blood sample was taken from each sow at weaning.

Blood samples were drawn from the anterior *vena cava* by venipuncture. Serum was harvested by centrifugation for 10 min at 3000 rpm and stored at - 20°C until testing for the presence of antibodies.

36 serum samples from breeding sows and 342 serum samples from pigs (38 serum samples; 9 samplings) were tested for antibodies to PCV2, SIV, *M. hyopneumo-*

niae, *A. pleuropneumoniae* and *H. parasuis*.

Commercial ELISA kits of different producers were used:

- INGEZIM CIRCO IgG (Ingenasa) – indirect ELISA,
- INGEZIM INFLUENZA PORCINA (Ingenasa) – indirect ELISA,
- INGEZIM MHYO COMPAC (Ingenasa) - blocking ELISA,
- CHEKIT-APP-ApxIV ELISA Test Kit (IDEXX),
- *Haemophilus parasuis* Antibody Test Kit (ELISA) Swinecheck® HPS (Biovet).

Ingezim Circo IgG: Samples with optical density (OD) higher than positive cut off value were considered positive to PCV antibodies. Samples with OD lower than negative cut off value were considered negative.

Ingezim Infuenza Porcina: Samples with S/P values greater or equal 0,2 were positive for antibodies to influenza A viruses. Samples with S/P values less than 0,2 were considered negative for antibodies to influenza A viruses.

Ingezim Mhyo Compac: The results of the test were expressed as OD value. A blocking percentage of sample was calculated. Samples were considered positive when the OD value was equal or lower than 40% of negative control. Samples were considered negative when the OD value was equal or higher than 45% of negative control.

Chekit -APP-ApxIV ELISA: The diagnostic relevance of the result was obtained by comparing the OD of the samples, with OD of the positive control. Samples were considered positive when the value (%) was equal or higher than 40%. Samples were considered negative when the value (%) was lower than 30%. If the samples were suspect ($\geq$30% to <40%) they were tested in a second run.

Haemophilus parasuis ELISA: For each sample and control we subtracted the OD obtained in the well containing antigen from the well without antigen. A ratio was calculated. Sample ratio less than 0,6 was considered negative and sample ratio greater or equal to 0,9 were considered positive. Sample ratio less than 0,9 but greater or equal to 0,6 was considered suspicious and sample was tested in a second run.

RESULTS

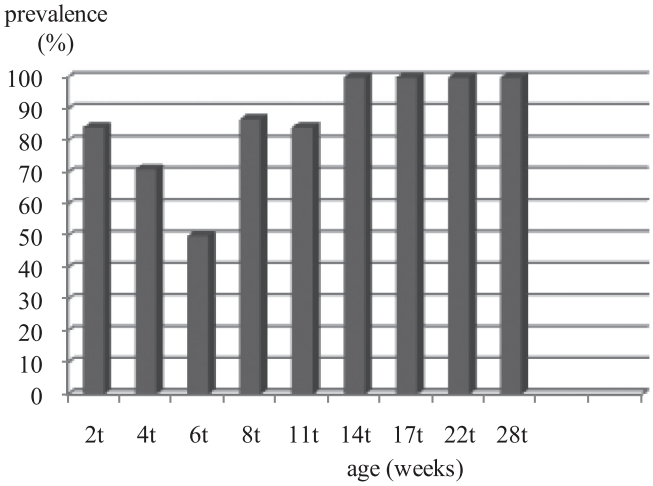
Breeding sows

Seroprevalence to SIV, *A. pleuropneumoniae* was 100 %, to PVC2 94 %, to *M. hyopneumoniae* 83,3 % and to *H. parasuis* 36 %.

Piglets

PCV2

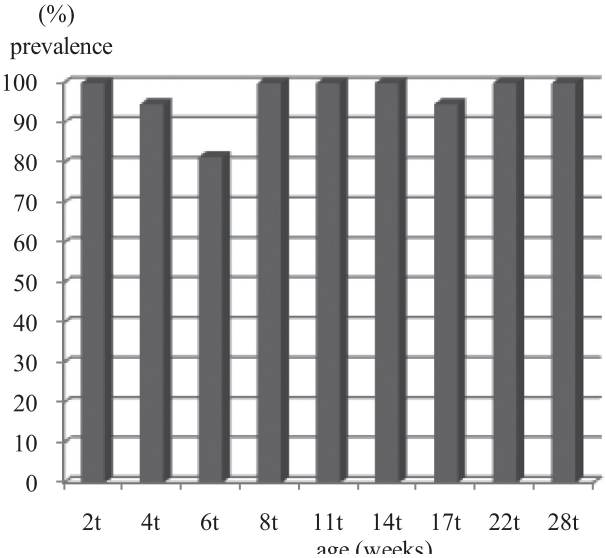
At 2 weeks of age the seroprevalence against PCV2 from 84,2 % decreased to 50 % (at 6 weeks of age). At 8 weeks of age increased to 86,8 % and at 14 weeks of age to 100 %.



Graph. 1. Serum profile of PCV2.

SIV

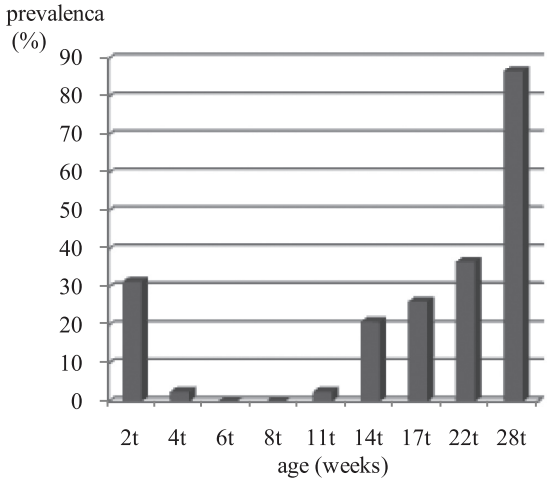
The seroprevalence against SIV was 100 % at 2 weeks of age, 94,7 % at 4 weeks of age; the lowest seroprevalence was at 6 weeks of age (81,5 %). The seroprevalence then increased to 100 %, only at 17 weeks of age was a little lower (94,7 %).



Graph 2. Serum profile of SIV.

M. hyopneumoniae

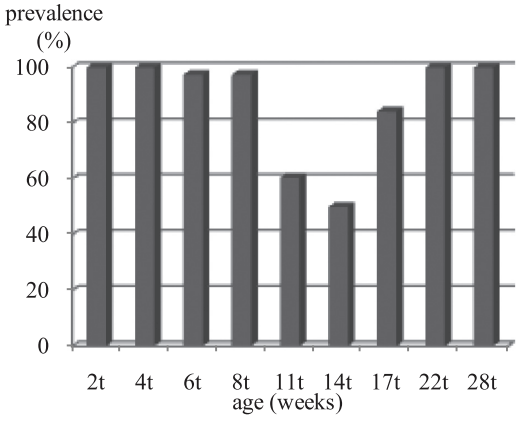
The seroprevalence against *M. hyopneumoniae* was 31,5 % at 2 weeks of age and decreased to 0 % at 6 and 8 weeks of age. At 11 weeks increased to 2,6 % and 28 weeks of age increased to 86,8 %.



Graph 3. Serum profile of *M. hyopneumoniae.*

A. pleuropneumoniae

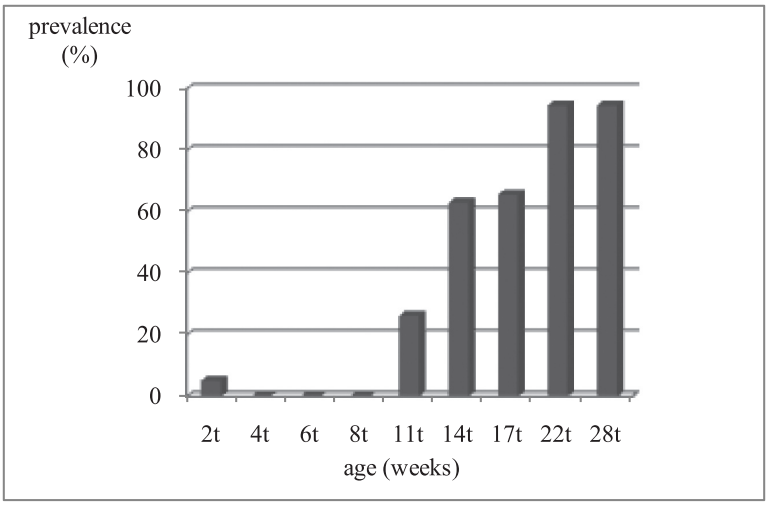
All pigs had specific antibodies against *A. pleuropneumoniae* at 2 and 4 weeks of age. The seroprevalence was lower at 6 and 8 weeks of age (97,4 %), and decreased to 50 % at 14 weeks of age. At 17 weeks of age increased to 84,2 % and at 22 and 28 weeks of age to 100 %.



Graph. 4. Serum profile of A. pleuropneumoniae.

H. parasuis

Only 5,2 % of piglets had specific antibodies against *H. parasuis* at 2 weeks of age. Pigs were seronegative against *H. parasuis* at 4, 6 and 8 weeks of age. The seroprevalence increased to 26,3 % at 11 weeks of age and to 97,4 % at 22 weeks of age.



Graph. 5. Serum profile of *H. parasuis*.

DISCUSSION

Serum profiling is a method which can contribute to creation of an effective vaccination program on farm against selected agents.

Colostral antibodies against SIV and PCV2 persisted for about 4 weeks. The lowest seroprevalence was detected in 6 weeks old pigs against both viruses. The animals were probably infected around 6 weeks of age with both viruses. According to serum profiles vaccination of sows against SIV and PCV2 can be proposed to prolong colostral immunity and better protect animals against postweaning multisystemic wasting syndrome (PMWS) and swine influenza.

Colostral antibodies against *A. pleuropneumoniae* persisted for almost 8 weeks (94,8 % prevalence). The lowest prevalence was detected in 14 weeks old pigs (53,8 %), and the highest at 22 and 28 weeks old pigs (100 %). According to serum profile the best time for vaccination against *A. pleuropneumoniae* is around 11-14 weeks of age.

Samples were examined for antibodies against *A. pleuropneumoniae* using ApxIV-ELISA. ApxIV is expressed by all serotypes of *A. pleuropneumoniae* only after infection of pigs, but not under in vitro conditions (Dreyfus et al., 2004).The ApxIV-ELISA does not differentiate serotypes of *A. pleuropneumoniae*. The previous survey in years 1997-2001 showed that in Slovenia are present pathogen serotypes. The advantage of ELISA for *A. pleuropneumoniae* over other serological tests is that it does not cross-react with other bacterial species and the test differentiate between pigs infected with *A. pleuropneumoniae* and vaccinated pigs against *A. pleuropneumoniae* (Dreyfus et al., 2004).

Colostral antibodies against *H. parasuis* were found only in 5,1 % at 2 weeks

old piglets. Circulating antibodies as result of natural infection were detected from 11 weeks (25,6 % prevalence) to 28 weeks old pigs (94,7 % prevalence). According to serum profile the best time for vaccination in this farm against *H. parasuis* is around 8-9 weeks of age.

Specific antibodies against *M. hyopneumoniae* found at 2 weeks of age are maternal antibodies, but prevalence (31,5%) in piglets is unexpectedly low according to prevalence in sows. Following vaccination with one dose product frequently no serum antibodies are detected. Serum antibody levels decline and pigs frequently become seronegative 4-6 weeks following vaccination (Thacker and Thanawongnuwech, 2002). This is also evident from our serum profiling. Up to 25% of the pigs may express antibodies to *M. hyopneumoniae* at the age of 10-12 weeks. At the time of slaughter approximately 90% of the animals become seropositive to the microb (Wallgren et al., 1998). In our case the seroprevalence started to increase at 11 week of age and at time of slaughter 86,8% animals had specific antibodies. Increasing of antibodies to *M. hyopneumoniae* should be due to decreasing immunity against *M. hyopneumoniae* and result of infection with *M. hyopneumoniae.* According to serum profiling second vaccination around 11-14 weeks of age can be proposed.

CONCLUSION

In our study was established, that cross-sectional testing is sufficient for preparation of control measures for the farm, but only with supposition that no new disease is entering the farm. Cross-sectional method must be repeated quarterly or at least twice a year, to follow the efficiency of performed measures.

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POTENCIJALNA KORIST ANALIZE SERUMA U KONTROLI RESPIRATORNIH BOLESTI

IRENA GOLINAR OVEN

Izvod

Na jednom slovenačkom velikoj farmi svinja, odabrano je 36 legala za analizu serumua na respiratorne patogene, radi pripreme mera kontrole. Uzeto 36 uzoraka seruma priplodnih krmača i od 342 uzorka seruma prasadi (38 uzoraka seruma, po 9 uzorakovanja). Uzorci su testirani na antitela svinje na Circovirus Tip 2 (PCV2), virus svinjskog gripa (SIV), Micoplasma hiopneumoniae, Actinobacillus pleuropneumoniae i Haemophilus parasuis. Krv je uzimana od istih prasadi, 2, 4, 6, 8, 11, 14, 17, 22. i 28. nedelje starosti. Komercijalni ELISA kompleti različitih proizvođača su korišćeni. Kod priplodnih krmača seroprevalencija na SIV i A. pleuropneumoniae je bila 100%, na 94% PVC2, M. hiopneumoniae na 83,3% i na H. parasuis 36%. Kolostralnih antitela u svinja protiv SIV-a i PCV2 traje već oko 4 nedelje. Najniži seroprevalencija je otkrivena kod 6 nedelja stare prasad protiv oba virusa. Prema serumskom profilu krmača, vakcinacija protiv SIV-a i PCV2 može se predložiti. Kolostralnih antitela protiv A pleuropneumoniae traje već skoro 8 nedelja (94,8% prevalenca). Najniža prevalencija je otkrivena kod prasadi starih 14 nedelja (53,8%). Prema serumu profilu, vakcinisanja protiv A. pleuropneumoniae oko 11-14 nedelja starosti može se predložiti. Do 8 nedelja starosti, prasad imaju seronegativni profil protiv H. parasuis. Seroprevalencija se povećana sa 11 nedelja starosti. Vakcinacija protiv H. parasuis oko 8-9 nedelja starosti može se predložiti. Seroprevalencija protiv M. hiopneumoniae sa 6 nedelja starosti se smanjila na 0%, a sa 11 nedelja starosti je počela da raste. Druga vakcinacija između 11.-14. nedelja starosti može se predložiti.

Ključne reči: svinja, respiratorne bolesti, serum profil, kontrolne mere.

Received / *Primljen*: 22.04.2013.
Accepted / *Prihvaćen*: 15.05.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 69-75, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 658.2:582.628.091

EFFECT OF SOIL ON NURSERY-GROWN WALNUT PLANTS

SVETLANA M. PAUNOVIĆ, RADE MILETIĆ, JELENA LUKOVIĆ[1]

SUMMARY: This experiment evaluates the effect of two soil types viz. an alluvial loamy deposit and a leached vertisol on survival, percentage of first-class plants, growth and diameter of nursery-grown grafted walnut. Plants grown on the alluvial loamy deposit showed higher rate of survival at the end of the first growing season and increase in percentage of first-class plants at the end of the second season compared to plants grown on leached vertisol. The average growth and diameter of plants on the alluvial loamy deposit increased at the end of the first and second growing season compared to plants grown on leached vertisol.

Key words: *plant, walnut, alluvial loamy deposit, leached vertisol.*

INTRODUCTION

Nursery production of walnut plans is a complex process dependent upon a range of factors. Apart from favourable environmental conditions, the production of high quality planting stock is also determined by adequate soil selection. Soils selected for walnut production should be permeable, deep, loose, rich in both humus and nutrients. Compact, stony, dry, strongly podzolic, too moist, salinated, strongly alkaline or rather acid soils are not suitable for walnut cultivation (Stanković and Jovanović, 1983). Walnut grows best on soils with medium to fine textures such as loam or sandy loam with good internal drainage, whereas soils that are poorly drained, droughty, and sandy should be avoided (Ponder, 2004). Permeable loose soils with favourable air and temperature regimes and a pH of 6.5-7.5 are ideal for the species (Šapa, 2002; Solar and Štampar, 2004). There is practically not a single study that deals with the effects of soil on the quality of walnut plants.

The objective of this study was to evaluate the effect of two soil types viz. an alluvial loamy deposit and a leached vertisol on survival and growth of nursery-grown walnut plants.

Original scientific paper / *Originalni naučni rad*

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*This study was financially supported by the Ministry of Education and Science, Republic of Serbia, Project Ref. No. TR-31064: Development and preservation of the genetic potential of temperate zone fruits.

MATERIAL AND METHOD

The experiment was conducted during 2010 and 2011 at two locations of the Fruit Research Institute, Čačak. Five walnut cultivars viz. Šeinovo (control), Šampion, Elit, G-286 and G-139 were used. Planting was performed in the second third of May, involving a sample of 30 grafted walnut plants per cultivar. The experiment was laid out in a randomised block design (5 cultivars x 2 soil types x 3 replications). In terms of their physicochemical properties, the soils used for the research are classified as an alluvial loamy deposit and a leached vertisol.

Plant survival and percentage of first-class plants were determined at the end of the first and second growing season, respectively. Upon shoot emergence, vegetative growth and plant diameter were measured.

The results obtained were statistically analysed using Fisher's model of analysis of variance - ANOVA. The significance of differences between the means of the control and those of the other test cultivars at $P \leq 0.01$ and $P \leq 0.05$ was defined using Dunnett's test (Dunnett, 1955). LSD test was performed at $P \leq 0.05$ to test the significance of differences between soils, as well as interaction means. The results are given in tabular form.

RESULTS AND DISCUSSION

The soil at location I is an alluvial soil in terms of morphology and origin, and an alluvial loamy deposit in terms of physicochemical properties. The soil is relatively coarse in texture, and slightly acid. In the 0-20 cm layer, the soil has a good content of humus and readily available phosphorus and potassium, and a medium nitrogen content (Table 1). Their contents decrease with increasing depth. In the 0-100 cm layer, the soil has 58.2% total sand and 42.5% total clay, on average. The other soil particles occur within the narrow range across profile depth: coarse sand 1.0-3.0%, fine sand 54.0-58.6%, silt 20.9-26.2% and clay 17.0-18.8% (Table 2). The soil at location II is a leached vertisol, according to its morpohological, textural, and agrochemical propertis. The soil is acid in reaction. In the 0-20 cm layer, the soil has a good supply of humus and readily available phosphorus and potassium, and a medium supply of nitrogen (Table 3). It is a medium-textured soil. In the 0-100 cm layer, there is 33.6 % total sand and 66.3% total clay, on average. The content of the other particles across profile depth is as follows: coarse sand 0.0-4.75%, fine sand 28.6-33.3%, silt 16.1-25.4% and clay 28.6-38.1% (Table 4). The leached vertisol contains much larger amounts of clay compared to the alluvial loamy deposit.

Table 1. Agrochemical properties of walnut nursery soil - location I

Tabela 1. Agrohemijske osobine zemljišta rastila oraha – lokalitet I

Depth/*Dubina* (cm)	pH in KCl/*pH u KCl*	K_2O mg/100g air-dry soil/ *K_2O mg/100g v.s.z.*	P_2O_5 mg/100g air-dry soil/ *P_2O_5 mg/100g v.s.z.*	Humus/ *Humus* (%)	N/*N* (%)
0-20	6.35	28.25	17.80	2.76	0.13
20-40	6.28	12.60	7.40	1.50	0.06
40-60	6.25	9.25	3.95	1.30	0.05
60-80	6.20	8.85	3.10	1.30	0.05
80-100	6.23	8.25	3.00	0.97	0.04

Table 2. Physical properties of walnut nursery soil – location I

Tabela 2. Fizičke osobine zemljišta rastila oraha – lokalitet I

Depth/ Dubina (cm)	Coarse sand/ Krupan pesak (%)	Fine sand/ Sitan pesak (%)	Silt/Prah (%)	Clay/ Glina (%)	Total sand/ Ukupan pesak (%)	Total clay/ Ukupna glina (%)
0-20	3.0	58.1	20.9	18.0	61.1	38.9
20-40	2.0	54.0	25.6	18.4	56.0	44.0
40-60	1.0	58.3	22.3	18.4	59.3	40.7
60-80	1.0	58.6	23.4	17.0	59.6	40.4
80-100	1.0	54.0	26.2	18.8	55.0	45.0

Table 3. Agrochemical properties of walnut nursery soil – location II

Tabela 3. Agrohemijske osobine zemljišta rastila oraha – lokalitet II

Depth/ *Dubina* (cm)	pH in KCl/ *pH u KCl*	K_2O mg/100g air-dry soil/*K_2O mg/100g v.s.z.*	P_2O_5 mg/100g air-dry soil/ *P_2O_5 mg/100g v.s.z.*	Humus/ *Humus*(%)	N/*N* (%)
0-20	4.44	25.74	17.31	2.78	0.13
20-40	4.26	25.16	3.60	1.56	0.07
40-60	4.28	25.19	1.31	1.11	0.05
60-80	6.65	25.40	2.95	1.06	0.05
80-100	7.05	22.40	6.30	0.85	0.04

Table 4. Physical properties of walnut nursery soil – location II

Tabela 4. Fizičke osobine zemljišta rastila oraha – lokalitet II

Depth/ *Dubina* (cm)	Coarse sand/ *Krupan pesak* (%)	Fine sand/ *Sitan pesak* (%)	Silt/*Prah* (%)	Clay/ Glina (%)	Total sand/ *Ukupan pesak* (%)	Total clay/ *Ukupna glina* (%)
0-20	2.75	33.3	21.7	40.2	38.1	61.9
20-40	4.75	28.6	19.8	46.8	33.3	66.6
40-60	3.25	32.4	16.1	47.9	35.6	64.4
60-80	1.00	31.6	25.4	42.0	32.6	67.4
80-100	00.0	28.6	31.0	40.4	28.6	71.4

Dunnett's test ($P \leq 0.01$ and $P \leq 0.05$) showed that cv. Šeinovo had a highly significantly higher rate of survival at the end of the first growing season, and a higher percentage of first-class plants at the end of the second growing season, as compared to the other cultivars tested. As regards plant height at the end of the first and second growing seasons, no difference was observed between the control and G-286 and Šampion, whereas the control exhibited highly significantly higher growth compared to cvs. Elit and G-139. Šeinovo gave plants that were highly significantly superior in terms of diameter at the end of both growing seasons, compared to the other cultivars. LSD-test ($P \leq 0.05$) revealed the survival rate, percentage of first-class plants, growth and diameter to be highly significantly higher in plants grown on alluvial loamy deposit than in plants on leached vertisol (Tables 5 and 6).

Table 5. Survival, growth and diameter of walnut plants in the first growing season

Tabela 5. Prijem, porast i prečnik sadnica oraha u toku prve godine gajenja

Treatment/*Tretman*	Cultivar /Soil/ *Sorta /Zemljište*	Plant survival at the end of the 1st growing season/ *Broj primljenih sadnica na kraju I vegetacij*e (%)	Plant growth at the end of the 1st growing season/ *Porast sadnica na kraju I vegetacije* (cm)	Plant diameter at the end of the 1st growing season/ *Prečnik sadnica na kraju I vegetacije* (mm)
Cultivar/*Sorta* (A)	Šampion	78.3±0.90 **	18.5±0.52 ns	7.1±0.22 **
	Elit	74.5±0.66 **	17.2±0.52 **	7.0±0.29 **
	G-139	77.0 ±0.90 **	17.5±0.44 **	7.1±0.24 **
	G-286	78.0±0.62 **	18.3±0.59 ns	7.2±0.16 **
	Šeinovo	86.1±0.56	19.2±0.48	7.8±0.10
Soil/*Zemljište* (B)	Leached vertisol/ *Smonica u lesiviranju*	74.2±0.45 b	17.0±0.41 a	7.1±0.22 a
	Alluvial loamy deposit/ *Aluvijalno ilovasti nanos*	83.4±0.41 a	19.2±0.36 b	7.6±0.15 b
ANOVA				
Cultivar/*Sorta* (A)		**	**	**
Soil/*Zemljište* (B)		**	**	**
A x B		**	**	**

- A and B stand for treatments for cultivars and soil, respectively
- *A i B predstavljaju tretmane za sorte i zemljište*
- The asterisks in vertical columns indicate significant differences between the means at P≤0.05 and P≤0.01 according to Dunnett's test and ANOVA (F-test) results; ns- non-significant
- *Zvezde u vertikalnim kolonama obeležavaju značajne razlike između sredina za P≤0.05 i P≤0.01 na osnovu Dunnett testa i rezultata ANOVA (F-test); ns- nije značajno*
- The values designated with same small letters within columns for years and interaction means do not differ significantly at P≤0.05 according to LSD test
- *Vrednost u kolonama za godine i interakcijske sredine označene istim malim slovima značajno se ne razlikuju za P≤0.05 na osnovu LSD-testa*

Table 6. Growth, diameter and percentage of first-class walnut plants in the second growing season

Tabela 6. Porast, prečnik i broj sadnica oraha I klase u toku druge godine gajenja

Treatment /*Tretman*	Cultivar /Soil/ *Sorta /Zemljište*	Percentage of first-class plants at the end of the 2nd growing season/ *Broj sadnica I klase na kraju II vegetacije* (%)	Plant height at the end of the 2nd growing season/ *Visina sadnica na kraju II vegetacije* (cm)	Plant diameter at the end of the 2nd growing season/ *Prečnik sadnica na kraju II vegetacije* (mm)
Cultivar/*Sorta* (A)	Šampion	62.7±0.91 **	194.5±5.72 ns	18.7±0.21 **
	Elit	56.3±0.67 **	185.5±5.82 **	19.4±0.26 **
	G-139	59.8 ±0.92 **	186.7±4.58 **	19.9±0.25 **
	G-286	64.5±0.57 **	193.8±7.52 ns	18.5±0.25 **
	Šeinovo	73.7±0.63	197.6±7.86	21.7±0.16
Soil/*Zemljište* (B)	Leached vertisol/ *Smonica u lesiviranju*	58.1±0.46 b	179.9±4.09 a	17.7±0.14 a
	Alluvial loamy deposit/ *Aluvijalno ilovasti nanos*	68.7±0.42 a	203.4±1.29 b	21.6±0.13 b
ANOVA				
Cultivar/*Sorta* (A)		**	**	**
Soil /*Zemljište* (B)		**	**	**
A x B		**	**	**

- A and B stand for treatments for cultivars and soil, respectively
- *A i B predstavljaju tretmane za sorte i zemljište*
- The asterisks in vertical columns indicate significant differences between the means at P≤0.05 and P≤0.01 according to Dunnett's test and ANOVA (F-test) results; ns- non-significant
- *Zvezde u vertikalnim kolonama obeležavaju značajne razlike između sredina za P≤0.05 i P≤0.01 na osnovu Dunnett testa i rezultata ANOVA (F-test); ns- nije značajno*
- The values designated with same small letters within columns for years and interaction means do not differ significantly at P≤0.05 according to LSD test.
- *Vrednost u kolonama za godine i interakcijske sredine označene istim malim slovima značajno se ne razlikuju za P≤0.05 na osnovu LSD-testa*

Bulatović (1985) found that the most suitable soils for walnut cultivation contain the following: 4.30-7.33% coarse sand, 59.26-65.70% fine sand, 14.53-15.26% clay, 66.60-72.75% total sand, 31.13-25.96% total clay, 5.05-11.56 mg phosphorus in 100 g air dry soil, 5.66-9.66 mg potassium in 100 g air dry soil, 0.69-1.48% humus, 0.05-0.10% nitrogen and pH 6.5-8.0 The same author reported higher growth of walnut plants when grown at pH7 than at pH 6 and pH 8. Korać et al. (1998) gave priority to soils having 3% humus, 250-300 ppm K_2O, 80-100 ppm P_2O_5 and pH 7-7.5, as confirmed by Solar and Stampar (2004). Šapa (2002) recommended soils containing 2.5-3% humus, 250-300

ppm K_2O and 100-120 ppm P_2O_5, whereas Šoškić (2007) suggested soils with 2.3-3% humus, 1.5-3% potassium, and 0.12-0.13% phosphorus as most suitable for walnut production.

There is practically not a single study that deals with the effects of soil properties on the quality of walnut plants. In an experiment on an alluvial loamy deposit, Paunović et al. (2010a) reported survival rates of 77.3% and 74.3%, and average vegetative growth of 14.3 cm and 171.6 cm at the end of the first and second growing season, respectively. These results are not in agreement with the findings from the present experiment on aluvial loamy deposit.

In the present study, mid-season cultivars (Šeinovo, Šampion and G-286) exhibited a higher survival rate, higher growth and a higher percentage of first-class plants, on average, compared to mid-late (G-139) and late-season (Elit) cultivars. These results are consistent with the reports of Paunović et al. (2010b) who suggested that survival and growth of walnut plants are significantly influenced by genetic traits of a cultivar, with cultivars having superior survival and growth during the first year in the nursery show improved survival and growth, and produce high quality plants in the second year.

Overall, the results obtained on the two soils are in agreement with those of other authors. Stanisavljević and Mitrović (1997) and Paunović et al. (2011) reported the following values, depending on cultivar, under Čačak conditions: survival rate 67.2–86.5% and growth 14.7-17.8 cm at the end of the first growing season; percentage of first-class plants 50.9-63.4% and growth 170-172.3 cm at the end of the second season. At the end of the second growing season, the survival rate of plants was 43.0%-73.0% in a study by Solar et al. (2001) and 69.9-95.5% as obtained by Erdogan (2006). In terms of plant growth, 40.0-50.0% first-class plants reaching a height of about 150 cm can realistically be obtained at the end of the second growing season (Korać et al., 1998). Plant growth in the second season was 168-172 cm and 179.7-244.5 cm, as reported by Achim and Botu (2001) in Romania and Ozkan and Gumus (2001) in Turkey, respectively.

CONCLUSIONS

Alluvial loamy deposits and leached vertisols can be used for the production of high quality walnut plants, under adequate fertilisation and regular cultivation conditions.

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UTICAJ ZEMLJIŠTA NA SADNICE ORAHA U RASTILU

SVETLANA M. PAUNOVIĆ, RADE MILETIĆ, JELENA LUKOVIĆ

Izvod

U ogledu je praćen uticaj dva tipa zemljišta, aluvijalno-ilovasti nanos i smonica u lesiviranju na prijem, broj sadnica I klase, porast i prečnik okalemljenih sadnica oraha u rastilu. Na zemljištu tipa aluvijalno ilovasti nanos ostvaren je veći prijem sadnica i broj sadnica I klase, veći porast i prečnik sadnica na kraju prve i druge vegetacione sezone u odnosu na sadnice gajene na smonici u lesiviranju.

Ključne reči: sadnica, orah, aluvijalno ilovasti nanos,smonica u lesiviranju.

Received / *Primljen*: 12.04.2013.
Accepted / *Prihvaćen*: 26.04.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 76-82, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 578.82:582.926.2

TOMATO AND CUCUMBER VIRUS DISEASES AND CONTROL OF VECTORS

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SUMMARY: The tomatoes and cucumbers in greenhouses in Albania are of particular importance. The viruses of tomato that were found are as follows: Tomato Mosaic Virus (ToMV) and strain of internal necrosis fruit, virus strike of tomato, Cucumber Mosaic Virus in tomato (CMV), CMV-necrotic strain, CMV- strain, dwarfism of the tomato, the wilt virus with defilement of tomato fruits TSWV (Tomato Spotted Wilt Virus), Potato Virus Y in tomato (PVY). In cucumbers the detected Cucumber Mosaic Virus (CMV), Tomato virus (TSWV) are part of the quarantine list (EPPO, 2004), the presence of these viruses shows the risk of damage which is expected to be in cultivated plants. The major viruses infecting tomatoes were TSWV and ToMV followed by PVY and CMV. The incidence of TSWV virus has been increased lately so the management measures of control will be of great significance in the future. The major virus infecting cucumber was CMV. The treatment with insecticides has reduced aphid populations.

Key words: *virus, monitoring, vector, ELISA, control.*

INTRODUCTION

The cucumber and tomato cultivation in greenhouses in Albania is increasing, due to the tradition of their being widely used by our customers. The area under greenhouses planted with these crops was 731 and 728 ha in 2008 and 2009, respectively. Main crops is tomato (57.8%) and cucumber (23.7%) (Statistical Yearbook, 2009). There is increased vegetable production, especially under plastic covers. However, most farms are still small and fragmented, and use old production systems (Finetti-Sialer, 2005).

Vegetable production is affected by many diseases, some of which are of great

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Original scientific paper / *Originalni naučni rad*

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economic importance. Tomato production has suffered from many pests and disease problems, including those caused by viruses which substantially reduce yield and quality (Golnaraghi et al., 2004).

A recent survey on fungal diseases showed the presence of several diseases of Solanaceae and Cucurbitaceae (Merkuri et al., 2002), but so far there have been no reports on the presence of virus diseases of vegetables in Albania. In this context, an investigation was carried out in the greenhouses in the central part of our country and the results are reported in this paper. The yield and quality of tomatoes and cucumbers cultivated in greenhouses is decreasing due to infections from viral diseases. The symptoms produced by different viruses on tomato cultivars include leaf and fruit deformations, stunting, necrosis of stems and leaves, vein discoloration, general yellowing of leaves, systemic chlorotic and necrotic leaf spots, general mosaic and mottle on leaves, purple leaf veins, vessel browning, development of light green concentric spots with black centers on immature fruits, yellow spot discolorations on ripe fruits and subsequent wilting and complete collapse of plants (Choi et al., 2004; Jeon et al., 2006; Lee et al., 2007; Lee et al., 2008; Oh et al., 2008).

The most important viruses affecting these cultures, the incidence of viral diseases and also vectors such as *Frankinella occidentalis* (EPPO 2004) are defined.

MATERIAL AND METHODS

Surveys were conducted in the years 2008 and 2009 in greenhouses, especially in the intense area, periodically during plant vegetation for the appearance of symptoms caused by diseases and pests. The study was focused on determining the viral diseases that affect tomato and cucumber cultures. The survey was done in greenhouses in the following sites: Peza - Tirana, Shën – Vlash in Durrës, Lushnja, Fushë - Krujë and Mbrostar - Gërcalli of Fier District, during 2008 - 2009.

The leaf and fruit samples were collected from symptomatic plants of tomatoes and cucumbers grown under above mentioned greenhouses according to BBCH-scale (solaneous fruit) Feller (1995), in principal growth stage 5- 8.

The virus-like symptoms most frequently observed were mottle, mosaic, stunting and necrosis. The plants with symptoms were sampled and tested for the presence of viruses in the Laboratory of Viruses in the Department of Plant Protection. The determination of viruses was done by serologic method of ELISA test (Clark and Adams, 1977), by taking taken tomato and cucumber samples, 206 and 50 samples respectively.

ELISA employs polyclonal antisera to trap and monoclonal antisera for detection. Microtitre plates (Nunc Maxisorp Immunoplate) were used. The known infected plants, together with healthy plants of the same species were used as positive control while the test plants were practically used as negative control. Dilute the coating antibody 1000 times in coating buffer. Bring 200 µl of the antibody solution in the wells of the ELISA plate. Cover the plate with a lid and place the plate in a humid box (wet tissue on the bottom of the box). Close the box and incubate the box over-night in the refrigerator at 4 °C or 3 hours at 37 °C. Wash the plate in the plate washer with the washing buffer: 3 washings per plate. Prepare 2 dilutions of plant extract samples in SEB; undiluted sample and 1/10. Dilute the positive control 10 times in SEB. Bring 200 µl of the solutions in the wells of the ELISA plate. Fill 3 wells with the positive control and 3 wells with the negative control. Cover the plate with a lid and place the plate in a humid box.

Close the box and incubate the box over-night in the refrigerator at 4 °C. Wash the plate in the plate washer with the washing buffer: 4 soakings and washings per plate. Dilute the AP conjugate 1000 times in SEB. Bring 200 µl of the conjugate solution in the wells of the ELISA plate. Cover the plate with a lid and place the plate in a humid box. Close the box and incubate the box over-night in the refrigerator at 4 °C or 3 hours at 37 °C. Wash the plate in the plate washer with the washing buffer: 4 soakings and washings per plate. Prepare the substrate (fresh). Bring 200 µl of the substrate in each well of the ELISA plate. Cover the plate with a lid and incubate the plate at room temperature until the positive control and positive samples are coloring yellow. This may take 15 minutes to several hours depending on the concentration of the virus in the samples and the reactivity of the antibodies. Read the plate in the plate reader (405 nm) after 60 minutes of incubation.

Monitoring for the presence of viruses was done in cultivars of tomato planted as the first and cucumber planted as the second crop in greenhouses. The tomatoes cultivars were: Siluete, Platus, Dellos, Jon, Viktor, Berila, Gabriela, 12.07, Bonita and the cucumber cultivars were: Magnum, SG.

Starting from the data on the presence of viruses the vectors for these viruses such as aphids and the presence of trips *Frankinella occidentalis and Thrips tabaci* (EPPO, 2004) were taken into consideration.

Blue/yellow CSALOMON® sticky traps were installed in the above greenhouses. In addition, samples of flowers of tomatoes and cucumbers in greenhouses were collected weekly. The identification of the thrips was based on the EPPO Diagnostic protocol for *Frankliniella occidentalis* (Chatzivassiliou et.al, 2000) and the confirmation by Dr Steve. The characteristics of the CSALOMON® trap (based on tests performed in Hungary): the western flower thrips are predominantly attracted to the blue part of the trap.

Other thrips (e.g. *Thrips tabaci*) are more readily attracted to the yellow part. In this trap type insects are attracted by the visual cue of the bright color of the trap. The trap remains effective as long as the sticky surface is not totally covered by captured insects. This usually happens only after 6-8 weeks of exposure, unless there is a mass outbreak in the glasshouse.

The main pesticides that were used: Nurelle (*chlorpyrifos* + *cypermethrin*), Mospilan (*acetamipride*), Mesurol (*methiocarb*), etc.

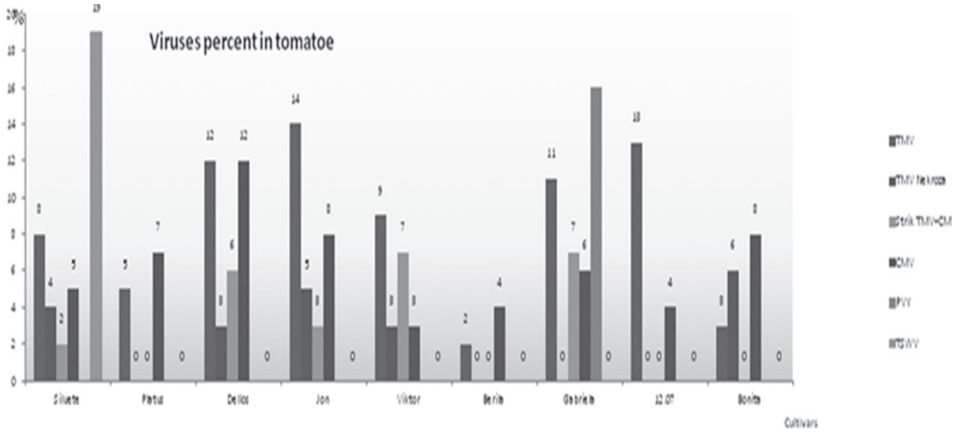
RESULTS

The monitoring was done in the in principal growth stage 5 - 8 of the tomato crop and cucumber plant. The 206 and 50 samples with symptoms for the presence of viruses were analyzed.

The analysis showed the presence of the following viruses in tomatoes: ToMV, CMV, TSWV, and PVY. Some strains of these viruses (CMV necrotic) were detected in our country for the first time. CMV virus was present in the cucumber plant as well. The symptoms were observed and the macroscopic analysis showed that viruses were the cause of these signs. The infected tomato plants were characterized by a light- and dark-green mottling and malformation of leaves. Other symptoms were plant stunting, fruit ripening, and reduced fruit. As mentioned above, the results show that in our tomato culture the severity of viral diseases depend on the cultivars. TSWV, which has several strains (at least 9 species of *Thrips, Frankliniella* and *Scirtothrips*) is the virus

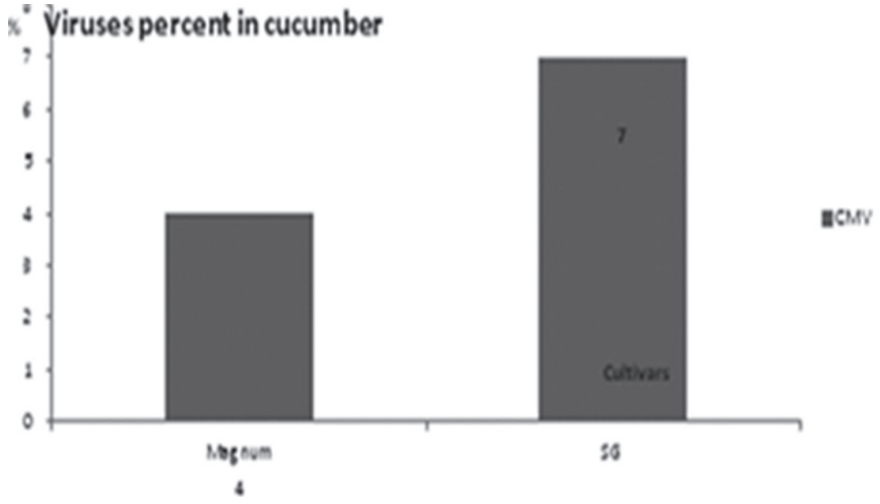
transmitted by thrips: the viruses' incidence; number of plants with symptoms in cv. Siluete (TSWV infection by the virus) is up to 19%. This is due to the growth of aphid populations early in April, where the presence of the vector Trips (*Frankliniella occidentalis*) was found (Çota and Merkuri, 2004).

Also, the cucumber mosaic virus (CMV) in tomatoes displays a higher incidence in cv. Dellos going up to 12%, especially when we have differences in temperatures. When the temperatures increase over 24 ^{o}C, these symptoms for the virus disappear. The cucumber mosaic virus (CMV) is present in cucumber culture so in the SG and Magnum cultivars the incidence of this virus reaches up to 7% (Graph 1-2).



Graph1. Percentage of virus infected tomato plants

The major viruses infecting tomato were TSWV and ToMV followed by PVY and CMV.



Graph 2. Percentage of virus infected cucumbers plants

DISCUSSION

The major virus infecting cucumber was CMV. On some farms it is noted that the control of these diseases caused by viruses is done with pesticides. This comes from the symptoms of viral diseases and in some cases there are similarities with other pathogens. This endangers the safety of consumers and the environment.

Defining viruses in these cultures is a contribution to better knowledge how to organize the control of vectors, as transmitters of the viral diseases, in the future. There are different methods for controlling aphids (*Myzus persicae Sulzer*) and trips (*Frankliniella occidentalis*), all of them relying on the use of chemicals, ranging from 12-14/ vegetative season.

The following plant protection products have bee: commercial: organic - azadirachtin (neem), horticultural oil, insecticidal soap, Mycotrol (fungus), pyrethrins etc. conventional - acetamiprid (Assail), bifenthrin (Brigade), beta-cyfluthrin (Baythroid), esfenvalerate (Asana), dinotefuran (Scorpion), flonicamid (Beleaf), imidacloprid (Provado), malathion, spirotetramat (Movento), thiamethoxam (Actara), zeta-cypermethrin (Mustang), and many more. Home Use Organic products + acetamiprid, bifenthrin, esfenvalerate, imidacloprid, malathion.

It is noted that chemical treatments are not conducted on the basis of the curve of the flight of these insects - vectors, based on the diagnosis and occurrence. It will be assumed that critical levels of aphids are reached 5–10 aphids, mostly wingless, detected per 100 leaves. As a result of intensive chemical treatments that may cause the disappearance of the natural enemies, an increase in aphid population has been observed (Musa et al., 2004). According to some authors (Ruffle and Miller 2002; Epstein et al., 2000), the intensive use of broad-spectrum insecticides contributes to reduce the presence of aphids' natural enemies and increase the environmental pollution and the risk for the customer.

CONCLUSION

In this study, the incidence and distribution of four viruses in the main greenhouses of tomatoes and cucumbers production in Albania is documented. In the tomato plant, the results of serological tests have the presence of new virus strains of ToMV (Tomato Mosaic Virus), such as necrotic strain and aucuba - ToMV. There are also unnoticed previously new strains of CMV virus in tomato for example. CMV - necrotic, CMV - dwarfism of tomato.

These indicate the risk of performance of these viruses in the cultivated tomato plants in greenhouse as well as in the field. The spreading threat is vicious virus of tomato fruits wither. Tomato spotted wilt virus (TSWV), which in some varieties ranges up to 19% and is found in the presence of the vector of dissemination of these viral diseases. Also from serological tests in the tomato plant, the presence of potato virus Y is noted, a virus which has been found in our country for the first time. In cucumber culture, in the SG and Magnum cultivar the mosaic virus CMV is present, the intensity of which reaches up to 7%.

The results presented here suggest that tomato and cucumber viruses' infections should be of special concern to farmers, and they present potential threats to tomato production. For this reason, additional work on characterization of tomato and cucum-

ber viruses, their modes of transmission and best means of their management should be carried out further. Considering that vegetable production is a priority for Albanian agriculture, measures should urgently be taken to avoid the introduction and spread of new and emerging viruses.

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VIRUSNA OBOLJENJA PARADAJZA I KRASTAVCA I SUZBIJANJE VEKTORA VIRUSA

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Izvod

Virusna oboljenja krastavca i paradajza predstavljalju značajan ograničavajući činilac proizvodnje u zaštićenom prostoru u Albaniji. Proučavanjem prouzrokovača oboljenja na paradajzu utvrđeni su sojevi virusa mozaika paradajza (Tomato mosaic virus - ToMV), virusa mozaika krastavca (Cucumber mosaic virus -CMV), virusa bronzavosti paradajza (Tomato spotted wilt virus- TSWV) i virusa crtičastog mozaika krompira (Potato virus Y). Na krastavcu opisani su virus mozaika krastavca (Cucumber mosaic virus-CMV, i virus bronzavosti paradajza (Tomato spotted wilt virus -TSWV). Po intenzitetu oboljenja na paradajzu najznačajniji su bili virusa bronzavosti paradajza i virus mozaika paradajza, nakon toga su po značaju sledili virus crtičastog mozaika krompira i virus mozaika krastavca. Na krastavcu najveće smanjenje prinosa prouzrokuje virus mozaika krastavca. Insekticidni tretman je značajno smanjio populaciju vektora- lisnih vaši.

Ključne reči*:* virusi, monitoring, vektor, ELISA, kontrola.

Received / *Primljen*: 29.04.2013.
Accepted / *Prihvaćen*: 14.05.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 83-90, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 579:597.555.3

CHARACTERISTICS OF SAUSAGES PRODUCED OF CARP MEAT*

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SUMMARY: The aim of this study was to examine sensory, microbiological and chemical properties of sausages produced of carp meat obtained from the fish farm Kakovo, property of the monastery Hilandar. Fish meat and fish products are valuable source of nutrients of great importance for diverse and healthy nutrition. The results of sensory analysis showed that odor and taste were typical for that kind of sausages, free of impurities. At cross sections, sausages stuffing consisted of light brown-orange fragmented mass, characteristic consistency for that type of sausage. Microbiological analyzes didn't show the presence of bacteria L.monocytogenes, E. coli, Salmonella spp., or B. cereus, while the number of aerobic bacteria was 3000 g/ml The total protein content was 16.48%, fat 24.5%, water content was 48.06%, ash content was 4.07%. The amount of calcium was 21.0 mg/100 g and sodium chloride content was 3.10%.

Key words: *common carp, sausages, sensory testing, chemistry, microbiology.*

INTRODUCTION

Fish meat and fish products are valuable source of nutrients of great importance for diverse and healthy nutrition. The optimal ratio of proteins, fats, carbohydrates, vitamins and minerals contributes to the high nutrient value of fish meat (Ćirkovic et al.,

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Original scientific paper / *Originalni naučni rad*

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*This paper is a result of the research within the project TP 31011 "The influence of the quality of the components of food for cyprinid fish species on the quality of meat, losses and the profitability of production", financed by the Ministry of Science and Technological Development, Republic of Serbia.

2011). Recommendations for fish regularly usages in diet are based on the fact that fish meat is the most important nutritional source of n-3 highly unsaturated fatty acids (n-3 HUFA). Consumption of fish meat helps to prevent cancer development, slowing brain aging and development of Alzheimer's disease (Connor, 2000). Also, positive effect of fish meat consumption was confirmed in a case of prenatal development, maintenance functions of the nervous system, eyes and skin (Allen and Harris, 2001). Regular consumption of fish meat causes the reduction of heart disease risk and vascular disease (Kris-Etherton et al., 2002).

Chemical composition of fish varies among species, as well as between individuals of the same species, depending on diet, age, sex, environment conditions and season (Guler et al., 2008; Ćirkovic et al., 2012, Ljubojevic et al., 2013). Proteins from fish meat have desirable amino acid composition with many free amino acids (Buchtová et al., 2010), contain all the essential amino acids for the human body what can make them the only source of animal protein in the human diet (Vladau et al., 2009). Characteristic of fish meat is very good protein digestibility (Untersmayr and Jensen-Jarolim, 2008). Fish meat contains very low amounts of carbohydrates as glycogen, and a high percentage of water (60-86%) (Ćirković et al., 2012b), which has a negative effect because high percentage of water made that fish meat spoils faster. The most abundant minerals in fish meat are phosphorus (170 to 270 mg%), calcium (15 to 100 mg%) and magnesium (20 to 35 mg%) (Stamenković and Dević, 2006). The amount of fat is also very variable, so fish can be divided based on fat content into lean (<5% fat), medium-fat (5-10% fat) and fat (> 10% fat).

Carp is the most dominant fish species on the fish farms in Serbia (Ćirkovic et al., 2007), and the cyprinids are the most frequent in the total world production of freshwater fishes (71.9%, 24.2 million tons in 2010) (FAO, 2012).

Consumption of fish meat is increasing, primarily due to the fact that fish is recommended as an essential ingredient of healthy human diet. According to the latest FAO data (2012), Serbia is one of the countries where the average consumption of fish ranges between 5-10 kg per capita per year, which is significantly below European and world average. As reasons for low fish consumption Baltić et al. (2009) blame low standard of the population, but also weak and undiversified offer of fish and fish meat products in the local market. Manufacturing industry in fish processing sector is still underdeveloped. Fish processing and development of new fish products can provide better sale of fish, not only in traditional fish markets, but also in all other consumer goods stores.

Technological processes of processing, preservation and storage are not the same for fish meat and the mammalian meat. During fish processing, it is very important to know chemical composition and properties of raw fish meat, in order to apply the most appropriate technology procedures adjusted to certain fish species. Minced fish meat and surimi are using as a raw material for the production of finely minced cooked sausages, especially in Asia (Konno, 2005).

Appearance, color, texture, odor and taste of fish sausage present the most important sensory properties of sausage and their quality is based on these properties. From the aspect of food safety, it is very important to determine the microbiological status of the obtained product. Chemical composition of sausage is an important indicator of nutritional quality, as well as checking whether the product is in accordance with the Regulations (Regulations of quality and other requirements for fish, crayfish, shellfish, sea urchins, sea cucumbers, frogs, turtles, snails and their products; 2003).

According to available literature in Serbia, examinations including these types of products were not conducted. The aim of this paper is to show process of production for carp meat sausages, and to determine the sensory, microbiological and chemical characteristics of the resulting product.

MATERIAL AND METHODS

Carp cultured in the Kakovo monastery fishpond in Greece, was overfished and immediately sacrificed. Mean values of the masses of carps were approximately 2850 g. The fish were decapitated and eviscerated, washed with cold water and skin and bones were manually removed. Further processing was done in the monastery facilities. Pieces of fish meat were minced in the meat grinder by using a grid with Ø 5 mm holes. Sausages were produced according to the production procedures for boiled sausages, by the recipe: 60% of fish meat, 15% of smoked fish meat, 15% hydrated soy flakes (1:2) and 5% of ice. In this was added 2% of NaCl, 1% mixture of natural spices and 2% soy isolate. The raw material was stuffed into collagen casings Ø 32 mm and processed in the chamber for heat treatment: heated, dried and smoked at 55 °C and roasted at 75 °C until achieving the temperature of 70 °C in the center of product. The sausages were refrigerated, vacuum-packed, and the samples were stored at a temperature of 4 °C until the end of the analysis.

Analyses of sausages were carried out at the Institute of Meat Hygiene and Technology in Belgrade.

The main chemical composition was evaluated by determining moisture content (ENG ISO 1442), total protein (SRP ISO 937), free fat (ENG ISO 1443) total ash (SRP ISO 936), and the calcium and NaCl content (SRP ISO 1841-1). To determine calcium content, sample preparation was done by destroying of 1 g homogenized carp meat sausage sample by microwave's digestion in a mixture of concentrated nitric acid and hydrogen peroxide in a microwave oven START D (Milestone, Italy). Calcium from solution was determined by flame atomic absorption spectrometry at 422.7 nm on the device SPEKTRAA 220 (Varian, Australia).

Microbiological tests were conducted according to the legislation (Regulation of micro-biological safety of food on the trade, "Offic. Journals of SRJ" no. 26/1993, 53/1995 and 46/2002) by determining: the total number of microorganisms (EN ISO 4833), the presence of *Salmonella* species (EN ISO 6579), *Escherichia coli* (ISO 16 649), *L.monocytogenes* (EN ISO 11290-1) and *Bacillus cereus* in g /ml (EN ISO 7932).

Sensory characteristics of sausages were evaluated by using quantitative-descriptive test (SRPS ISO 6658), at the scale intensity from 1 to 5, and sensory properties of sausages (outward appearance, cut appearance, color, texture, taste, flavor and overall acceptability) were rated. A group of five assessors formed a panel for sensory properties evaluations. Assessors have been previously tested by using a test to sense of taste determination (SRPS ISO 6658), as well as a test for the training of assessors based on detection and recognition of odors (SRPS ISO 3972).

RESULTS AND DISCUSSION

Sensory characteristics of examined sausages were specific to the type of product. On the surface of sausages, the odor was without foreign odors. At intersections, sausage stuffing was consised of light brown-orange fragmented mass and characteristic consistency to the type of sausage. Odor and taste were characteristic for the type of sausage, without foreign flavors and odors.

The paper of Ćirković et al. (2012c) shows the results of chemical composition of carp ciltured in the Kakovo fishpond which was used for sausages production. These results showed how inappropriate rearing technology can effect on the quality of fish meat. The carp was cultured in the earthen ponds of Kakovo (property of the monastery Hilandar, Greece, recipient wellspring) in semi-intensive production system with addition of corn. The results of the analysis showed that sampled carp meat had 37.12% of fat what was higher than in other carp meat results of analysis available from literature. Such a high percentage of fat caused a low percentage of water, (48.8%) in meat, especially for the fish. The amount of protein which was 11.37 %, was lower than in the previous analyses of chemical composition of carp meat.

Meat with a higher fat content required addition of protein products (hydrated extruded soybean flakes). Adittion of smoked carp meat, in which during the heat treatment was reduced the fat and water content, contributed to improvement in taste and correction of the chemical composition of the sausages.

Results of chemical analysis sausages produced from carp meat are presented in Table 1.

Table 1. Results of chemical analysis of sausages produced from carp meat

Characteristic	According to Regulations*	Content
Water content %		48,06±0.93
Total protein content %	min 11%	16,48±0.45
Fat content %	max 25%	24,50±0.76
Ash content %		4,07±0.09
Sodium chloride content %		3,10±0.07
Calcium content mg/100g		21,00±0.11

*Regulations on quality and other requirements for fish, crayfish, shellfish, sea urchins, sea cucumbers, frogs, turtles, snails and their products (2003).

The results of chemical analysis showed that the composition of the sausages were in accordance with regulations on quality and other requirements for fish, crayfish, shellfish, sea urchins, sea cucumbers, frogs, turtles, snails and their products (2003). Lower moisture content is common for products stuffed into permeable collagen casings. The fat content (24.50±0.76) is the result of the use of carps, which were fed with a lot of corn. Increasing of salt content in sausages (3.10%) is a result of smoked carp meat addition, which usually has a high salt content. Salt did not affect a taste correction, because the sausages had lower moisture content and feeling of salinity is most expressed in the products with higher moisture percentage.

Fish sausage produced by Al-Bulushi et al., (2011) contained 12.22% of fat, while

the sausages from the market that thay were tested contained 5.5% of fat. Chuapoehuk et al. (2001) have published results of sausages made of catfish which contained 74.5% of water, 3.16% of fat protein and 13.73% of protein.

Table 2. Results of microbiological analysis of sausages produced from carp meat

Microorganisms	Number of microorganisms
L.monocytogenes in 25 g/mL	not determined
E.coli in g/mL	not determined
Number of erobic colony in g/mL	3 000
Salmonella species in 25 g/mL	not determined
Bacillus cereus in g/ml	not determined

Results of microbiological analysis of sausages produced from carp meat (Table 2) showed that the minimal number of aerobic bacteria (3 000) was found in the sausage. This suggests that the carp meat was hygienic safe and that good manufacturing and hygiene practices in the facilities of monastery Kakovo were correct. Also, a thermal treatment was carried out in a satisfactory way, what resulted in destroying of most microorganisms. It is significant that the presence of any pathogen bacteria was no found.

Used spices did not have bacterial contamination, and contributed to safety of sausage. When considering the microbiological analysis of meat sausages obtained from carp, it is important to consider the amount and type of spices, which are used in the production process. Many spices show antioxidant properties that enhance the stability of fats (Gulcin, 2005), and Yin and Cheng (2003) have found that the antioxidant potential of spices is relatively low and depends on the dose. Spices can also show antibacterial effects against pathogenic bacteria such as *Bacillus cereus*, *Staphylococcus aureus* (Kumudavally et al., 2011), *Salmonella enteritidis* (Benkeblia, 2004), *Shigella* spp. (Bagamboula et al., 2004), *Aeromonas hydrophila* (Fabio et al., 2003). Antibacterial capacity in the reduction of bacteria showed cinnamon, garlic, onion and caraway (Arici et al., 2005; Das et al., 2011).

CONCLUSION

According to presented investigations of sausages produced from carp meat, it can be concluded:

Sensory characteristics ofr examined sausages are specific to the type of product: texture, odor and taste are characteristic for the type of sausage, without foreign impurities.

Microbiological analysis of sausages from carp meat showed the absence of pathogenic bacteria and confirmed that the product was safe and suitable for human consumption.

Chemical analysis showed that the product complies with the Regulations in terms of chemical composition and because sausage contains less fat than sausages produced from the meat of farm animals, is suitable for consumption of human risk groups.

On the facts above, it can be said that by technological process of sausages production from carp meat it was obtained good product.

Presented results can serve as a basis for development of quality standards for home-made fish sausages. In addition, these results may help to develop similar products of different species of fish, which would complement the current offer of fish and fish products in the market, as well as to improve the market value of the product.

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KARAKTERISTIKE KOBASICE PROIZVEDENE OD MESA ŠARANA

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Izvod

Cilj ovog rada je bio da se ispitaju senzorne, mikrobiološke i hemijske osobine kobasice koja je proizvedena od mesa šarana, izlovljenog iz jezera na Kakovu, metoh manastira Hilandara. Riblje meso i proizvodi od ribe predstavljaju vredan izvor hranjivih materija od veoma velikog značaja za raznoliku i zdravu ishranu. Rezultati senzorskih ispitivanja su pokazali da su miris i ukus svojstveni za vrstu kobasice, bez stranih primesa, a na presecima, nadev kobasice se sastojao od usitnjene mase, svetlo smeđe-narandžaste boje, svojstvene konzistencije za vrstu kobasice. Mikrobiološkim analizama nije ustanovljeno prisustvo bakterija L.monocytogenes, E.coli, Salmonella spp., niti B.cereus, dok je broj aerobih kolonija u g/ml bio 3000. Sadržaj ukupnih proteina bio je 16,48%; sadržaj masti 24,5%; sadržaj vode 48,06%; sadržaj pepela 4,07%. Količina kalcijuma je bila 21,0 mg/100g, a sadržaj natrijum hlorida je iznosio 3,10%.

Ključne reči: šaran, kobasica, senzorika, hemija, mikrobiologija.

Received / *Primljen*: 14.05.2013.
Accepted / *Prihvaćen*: 02.06.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 91-98, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 636.09:599.731.1

CURRENT STUDY OF PORCINE CIRCOVIRUS TYPE 2 IN CROATIAN PIG POPULATIONS

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SUMMARY: Porcine circovirus type 2 has been circulating in pigs for many years before being linked to disease. In the last ten years porcine circovirus 2 (PCV2) has moved from the arcane to near center stage in veterinary virology. In Croatia the main epidemic outbreak of PCV2 infection happened in 2004 and caused great economic losses in pig industry. The purpose of this paper was to investigate the current situation regarding PCV2 infection in Croatia. During 2007-2013 a total of 2518 samples of pig blood serum were tested by ELISA. Antibodies to PCV2 were detected in 398 (15.80%) of the tested sera and established in 5 of 9 investigated regions. Virus circulation was confirmed by PCR. The obtained results showed that 100 (53.19%) of 188 examined tissue samples were PCV2 PCR positive.Our results provide information on the current disease exposure to PCV2 infection in Croatian pig populations. PCV2 is not a notifiable disease and obligatory national program is carried out in Croatia but effective monitoring of the health control should be applied.

Key words: *PCV2 (Porcine circovirus type 2), pig, Croatia, vaccine.*

INTRODUCTION

Porcine circovirus type 2 (PCV2) is an economically important swine pathogen which represent a threat to the competitiveness of swine farming worldwide through reduced growth and mortality (McKillen et al., 2007). PCV (Porcine circoviruses) are members of the genus *Circovirus,*family *Circoviridae.* These are very small, non-enveloped icosahedral viruses with a single stranded circular DNA genome of about 1.7 kb organized in an ambience direction (Mankertz et al., 1998). There are two types of

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Original scientific paper / *Originalni naučni rad*
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porcine circovirus: Type 1 (PCV1) is widespread and apparently do not cause clinical problems in pigs and was first reported as a persistent contaminant in laboratory PK15 cell lines (Crowther et al., 2003), and Type 2 (PCV2) considered as an important emerging pathogen associated with a number of different syndromes and diseases in pigs; for instance PMWS (post-weaning multisystemic wasting syndrome), PDNS (dermatitis and nephropathy syndrome), respiratory disease complex, granulomatous enteritis, exudative epidermis, necrotizing lymphadenitis, congenital tremor (Chae, 2005), reproductive failure (Hansen et al., 2010), and infection in immune response (Opriessning et al., 2006). In the last ten years porcine circovirus type 2 (PCV2) has moved from the arcane to near center stage in veterinary virology. This has been associated with dramatic increase in the incidence, to sometimes epidemic proportions, of porcine circovirus diseases (PCDV), notably post-weaning multisystemic wasting syndrome (PMWS). Although tremendous progress has been made since the discovery of PCV2 in 1998, there are many important questions to remain (Meng, 2013).

PCV2 infection in pigs related with PMWS and PDNS in Croatia have been reported by Jemeršić et al. (2004) and Lipej et al. (2005). An epizootic disease characterised by depression, weight loss, respiratory and/or enteritic disorders and erythematous skin lesions of which some had progressed to dermal necrosis, among 4- to 16- week-old pigs (nursery and fattening units) was recognised during 2002 in several farrow-to-finish farms located in eastern and western part of Croatia. The morbidity in all studied farms was from 15-30%, while the mortality ranged from 9.3% to 23.8%. In general, affected animals do not respond to antibiotic treatment. The high prevalence of viral circulation has been confirmed by the use of PCR method. It was assumed that disease was introduced to Croatia by importation breeding stock (gilts and boars) from different European countries, such as Spain, Austria, France and Hungary where the disease was previously diagnosed (Segales and Domingo, 2002). After the first outbreak disease spread within the whole country causing enormous economic losses on commercial swine farms. We don't have information of the total loss during an outbreak of PCV2, but the obtained results in affected farms showed that morbidity varied from 10 to 60% (Lipej et al., 2004). These results lead us to conclude that PCV2 infection had devastating economic impact on the Croatian pig husbandry at that time.

Concerning control of PCV2 vaccination has become very popular during the last 10 years. Benefits of vaccination do not only come from preventing PCV2, but mostly from preventing the effects of PCV2 subclinical infection. Commercial PCV2 vaccines for use in growing pigs and breeding animals were introduced in Croatia in 2009 and to date 3 products are available (authorised: http://www.veterinarstvo.hr/default.aspx?id=140).

The objective of this study was to investigate the current disease widespread of PCV2 infection in pig populations in Croatia.

MATHERIAL AND METHODS

From January 2007 to April 2013 a total of 2518 blood samples from pigs of different age were examined. Samples were delivered to the Virology department, Serology laboratory for viral diseases, of the Croatian Veterinary Institute in Zagreb during regular health program of controlling the disease based on serological testing. Samples were submitted from 22 farms in 9 counties of Republic of Croatia (Table 2). Sera were

obtained by centrifugation for 10 min at 2000 rpm and stored at – 20^0 C until analysis. The test used was commercial enzyme-linked immunosorbent assays (ELISA) Ingezim Circovirus IgG/IgM 11.PCV.K2. (Ingenasa, Madrid, Spain), which detects antibodies against IgG and IgM, allowing the determination of the timing of PCV2 infection: IgM values > IgG values: active infection, IgG values > IgM values: recent infection (between 1-2 month), High IgG values and negative IgM values: old infection.

The test was carried out, and the results were interpreted according to the manufacturer's recommendations. Optical density was read on a Tecan Sunrise Basic spectrophotometer (Austria) at 450 nm.

In addition, 188 samples of lymph nodes, lung and spleen tissue originated from domestic pigs were tested by PCR (Gene Amp® PCR System 9700, Applied Biosystems, USA). Total DNA was extracted from tissue samples using QIAmp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufactures instructions. The oligonucleotide primers (PCV2-2A, PCV2-2B) used for detection of PCV2 DNAs was previously described by Sandvik et al. (2001). The tissue samples were collected during the period 2008 to 2013 in 6 Croatian counties and delivered to the Laboratory for CSF, molecular virology and genetics of Croatian Veterinary Institute.

RESULTS

From the total number of 2518 blood sera tested, PCV2 specific IgG and IgM antibodies were detected in 398 (15.80%). All positive animals had high levels of IgM (IgM>IgG OD value) values suggesting active infection. Antibody prevalence showed difference among counties and investigated period. The highest antibody prevalence of 25% was detected in 2011, followed by 15.64% positive animals in 2012. In contrast, the absence of antibodies to PCV2 was recorded in 2009. According to the county distribution, as expected the antibody prevalence was higher in counties in eastern part of continental Croatia, a region characterized by high density of commercial pig production. The highest antibody prevalence was detected in Osijek and Baranja County where of 1173 sera tested 239 (20.37%) were positive to PCV2. In additionally, in Vukovar and Srijem County we found 108 positive samples (15.14%) out of 713 tested. In other counties was recorded a low prevalence or negative findings to PCV2 antibody. The analysis showed that 5 of 9 (55.55%) regions were positive. These results are summarized in Table 1.

Of 188 examined tissue samples by PCR, 100 (53.19%) were PCV2 PCR positive. The majority of PCV2 positive results were recorded in eastern part of the country. The obtained results showed high level of positive samples in Osijek and Baranja County, especially in 2010 (54.55%) and 2011 (66.66%). The results of PCR PCV2 investigation are shown in Table 3.

Table 1. Results of pig sera tested for specific IgG and IgM antibody to PCV2 in different Croatian counties during the period 2007- 2013

County	Year						
	2007	2008	2009	2010	2011	2012	2013
	tested/ pos	tested/ pos	tested/ pos	tested/ pos	tested/ pos	tested/ pos	tested/ pos
Zagrebačka		92/28	20/0	115/1	2/0	26/2	
Varaždinska					4/0	4/0	
Koprivničko-križevačka	54/0	70/0	34/0	30/0	39/0		
Bjelovarsko-bilogorska	3/2			5/0			
Virovitičko-podravska			6/0				
Brodsko-posavska	108/18			10/0			
Osječko-baranjska	117/29	47/0	64/0	210/39	254/92	389/62	92/17
Vukovarsko-srijemska				120/0	328/65	265/43	
Međimurska	10/0						
Total	292/49	209/28	124/0	490/40	627/157	684/107	92/17

Table 2. Number of pig farms examined to PCV2 antibody during the period 2007- 2013 in different Croatian counties

County	*Year* Godina						
	2007	2008	2009	2010	2011	2012.	2013
The city of Zagreb/		2	1	1	1	2	
Varazdinska					1	1	
Koprivnica and Krizevci/	1	1	1	2	1		
Bjelovar and Bilogora/	1			1			
Virovitica and Podravina/			1				
Slavonski Brod and Posavina	1			1			
Osijek and Baranja	4	3	2	7	5	8	4
Vukovar and Srijem				3	5	2	
Medjimurje	1						
Total	8	6	5	15	13	13	4

Table 3. Results of PCV2 analysis obtained by PCR in different Croatian counties during 2008-2012

County Županija	*Year*				
	2008	2009	2010	2011	2012
	tested/pos	tested/pos	tested/pos	tested/pos	tested/pos
The city of Zagreb					1/1
Osijek and Baranja		5/2	44/24	33/22	69/27
Vukovar and Srijem	15/5	3/3			
Medjimurje		2/0			
Krapina and Zagorje		2/2			
Sisak and Moslavina		14/14			
Total	15/5	26/21	44/24	33/22	70/28

DISSCUSION

Outbreaks of disease in the world's growing livestock sector present significant potential costs – at the farm level through losses in production and productivity, and at the national level by disruption to markets and international trade. Porcine circovirus type 2 (PCV2) is responsible for various symptoms that impair pig growth and are described as PCV2 diseases (PCVD). It has been estimated that PCVD costs (direct and indirect losses) around 600 million Euros per year to the European Union (Segales et al., 2007). Concerning control of PCV2 infection the losses can be limit by strict application of general prophylactic measures. Before the vaccines became available, much focus was on good production practice and on the control of other diseases. Introduction of the PCV2 vaccines indeed change the global situation obtaining significant reductions in mortality in the postweanig area (Baekbo et al., 2011). Since the beginning of observation PCV2 suspected animals in Croatia high percentage of animals with clinical symptoms was recorded (Lipej et al., 2004). PCV2 took a wide spread and reduced pig production. In 2009 Croatia implemented vaccination policy as an important measure for limiting the losses. Presently three commercial PCV2 vaccine are available: one sow vaccine (Circovac, Merial), and 2 piglets vaccines (Ingelvac Circoflex, Boehringer and Porcilis PCV, Intervet). Vaccination and good farm management practice significantly reduced the total losses after an outbreak.

In our study antibody prevalence to PCV2 varied among areas and testing period. During the period 2007-2013 antibodies to PCV2 have been detected in 398 (15.80%) of tested blood samples. The absence of antibodies to PCV2 was recorded only in 2009. The antibody prevalence was higher in Osijek and Baranja County a region important for the Croatian pig industry, because the majority of pig farm are located in this region. We found 239 positive samples (20.37%) to PCV2 of 1173 tested. In other counties was recorded a low prevalence or negative findings for PCV2 antibody. The PCV2 IgG/IgM ELISA as used in this study provides a method to characterize the humoral immune response of PCV2 infected pigs. Animals found positive have experienced ac-

tive infection, as indicated by the presence of specific IgM. IgM are first dectectable 7-10 days following infection and remain detectable until 50-60 post infection. On the contrary, IgG are first detectable 12-15 days post infection and remain detectable for years. During regular health program of controlling the disease in period from 2008 to 2012 188 tissue samples were delivered at Croatian Veterinary Institute and tested by PCR in order to determine whether PCV2 virus is circulating in pig herds. Analysis showed that 100 samples (53.19%) were PCV2 PCR positive. The majority of PCV2 positive results were recorded again in eastern part of the country. The obtained results showed high level of positive samples in Osijek and Baranja County, especially in period 2010 (54.55%) and 2011 (66.66%). In contrast to serological findings, in 2009 with PCR 21samples out of 26 (80.76%) were PCV2 PCR positive. In Croatia PCV2 is not a notifiable disease and obligatory national program is carried out, but about each positive result CVI is obligated to inform Ministry of Agriculture, Veterinary Directorate must be informed.

According to our results based on serology and PCR we can concluded that PCV2 is present in Croatian pig farms. Although the results show that is not so widespread and does not cause great economic losses still remains an important disease having an impact on the swine industry in Croatia. Since 1990 in Croatia the number of farms and pigs has been considerably reduced, and the tendency of declining in pig industry is still continuing. In a region with high pig density and commercial farms a high health status of all pigs must be based on good farm management practice, on the control of the concurrent diseases and introduction of PCV2 vaccine. Although tremendous progress has been made toward understanding PCV2 pathogenesis and immune interactions, many important questions remain. PCV2 is an important pathogen that is causally associated with important emergent disease syndromes in swine.

Due the information obtained from this study it can be concluded that the implementation of the monitoring of the health condition of pigs in large agglomerations and the systematic recording of health indicators is fundamental for control the incidence of infection and prevent it spreading to other animals.

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PROŠIRENOST CIRKOVIRUSA TIP 2 (PCV2) U SVINJOGOJSKIM UZGOJIMA U HRVATSKOJ

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Izvod

Svinjski cirkovirus tip 2 prisutan je u uzgoju svinja diljem svijeta dugi niz godina prije nego što je povezan sa izbijanjem ove bolesti. U zadnjih deset godina PCV2 se polako iz skupine najznačajnih bolesti u veterinarskoj virologiji svrstava u manje značajne

bolesti. Bolest je prvi puta u Hrvatskoj dokazana 2004. godine kada je nanijela velike gospodarske štete. U razdoblju od 2007. godine do 2013. godine pretraženo je 2518 uzoraka krvi svinja na prisustvo protutijela za PCV2 i temeljem seroloških pretaga u njih 398 (15.80%) dokazana su protutijela. Prisustvo virusa potvrđeno je i metodom PCR. Od 188 pretraženih uzoraka tkiva njih 100 (53.19%) bilo je pozitivno na PCV2 virus. Ostvareni rezultati upućuju na činjenicu da infekcija svinjskim cirkovirusom tip 2 i dalje prisutna u svinjogojskim uzgojima u Hrvatskoj. Iako ova bolest nije u „Naredbi o mjerama zaštite životinja od zaraznih i nametničkih bolesti i njihovom financiranju“ i ne postoji nacionalni plan za suzbijanje ove bolesti, dobiveni rezultati ukazuju da postoji potreba programa praćenja ove bolesti u obliku sustavnog pretraživanja stada svinja što bi osiguralo bolji epidemiološki uvid u bolest u populaciji svinja.

Ključne riječi: PCV2 (svinjski cirkovirus tip 2), svinja, Hrvatska, vakcina.

Received / *Primljen*: 20.04.2013.
Accepted / *Prihvaćen*: 17.05.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 99-108, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 636.09

CIRCOVIROSIS IS NOT A DISEASE: REVIEW OF PMWS

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SUMMARY: Postweaning multisystemic wasting syndrome (PMWS) is one of the disease among the porcine circovirus diseases (PCVD). PMWS has a significant economic impact on global pig industry. Porcine circovirus type 2 has been circulating in pigs for many years before being linked to disease. PMWS has been reported from all major pig-producing countries. Infection with porcine circovirus type 2 (PCV2) is necessary for PMWS to develop, but most research has shown that PCV2 needs one or more co-factors for PMWS to develop into severe and even fatal disease. The confirmation of PMWS should be based on certain criteria such are clinical signs (growth retardation and wasting, frequently with dyspnea and enlargement of inguinal lymph nodes and occasionally with jaundice), moderate to severe characteristic histopathological lesions in lymphoid tissues, moderate to high amounts of PCV2 within the lesions in lymphoid and other tissues and mortality in excess of expected or/and historical level for the farm. Introduction of PCV2 vaccines significantly changed the impact of PCV2 on the pig production globally.

Key words: *PCV2, PMWS, risk factors, control, pig.*

INTRODUCTION

Porcine circovirus diseases (PCVD) or porcine circovirus associated diseases (PCVAD) were proposed to group diseases or conditions linked to porcine circovirus type 2 (PCV2) (Baekbo et al., 2012). PCV2 infection is associated with postweaning multisystemic wasting syndrome (PMWS) (Clark, 1996), porcine dermatitis and nephropathy syndrome (PDNS) (Rosell et al., 2000), porcine respiratory disease complex (PRDC) (Kim et al., 2003), and reproductive diseases (Sanchez et al., 2001). PMWS is the most important PCVD and has a significant economic impact on global pig industry (Baekbo et al., 2012).

PMWS in pigs was first identified in western Canada in 1991 (Walker et al., 2000), in Europe was first described in France in 1996. PMWS has been reported from all pig-producing countries, most recently in Australia (Baekbo et al., 2012).

Rewiev Scientific paper / *Pregledni naučni rad*
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PORCINE CIRCOVIRUS TYPE 2

Porcine circovirus (PCV) belongs to the genus *Circovirus* in the family *Circoviridae* (Chae, 2005). PCV, a small, nonenveloped, single-stranded DNA virus was first recognised as a contaminant of the continuous porcine kidney cell line PK-15 in 1974 (Opriessnig et al., 2007). Under experimental conditions, the PK-15–derived PCV isolate did not produce diseases in pigs (Allan et al., 1995). Sequence analyses of the PMWS-associated PCV revealed significant genetic differences compared with the PK15–derived PCV. The pathogenic PMWS-associated PCV was designated as porcine circovirus type 2 (PCV2) and non-pathogenic PCV as porcine circovirus type 1 (PCV1) (Opriessnig et al., 2007).

Analysis of PCV2 viruses from around the world showed close phylogenetic relationships and nucleotide sequence identity greater than 93% (Larochelle et al., 2002). Until now, five genotypes have been described. Most PCV2 genomic sequences fit into two major groups: PCV2 genotypes a and b and seem to have worldwide distribution (Segalés et al., 2008).

PCV2 is regarded as a ubiquitous virus and is present in most pig herds. Domestic and feral swine appear to be the natural host (Segalés and Domingo, 2002).

PCV2 has been found in nasal, tonsillar, bronchial, ocular secretion, faces, saliva, urine, colostrum, milk and semen but oronasal exposure is considered the primary route of transmission (Krakowska et al., 2000). Transmission of PCV2 among pigs can occur by mixing naïve with infected animals by direct contact. Most pigs became infected at 4-11 weeks of age, depending on the farm (Segalés et al., 2012).

DEFINITION OF PMWS

PCV2 needs one or more co-factors for PMWS to develop into severe and even fatal disease (Lohse et al., 2008). Without co-factors PCV2 does not cause any disease, consequently circovirosis is not a disease. Data from different European countries showed almost 100% herd seroprevalence to PCV2 in both PMWS affected and non-affected farms (Rose et al., 2002). The confirmation of PMWS should be based on certain criteria: 1. relevant clinical symptoms (growth retardation and wasting, frequently with dyspnea and enlargement of inguinal lymph nodes and occasionally with jaundice), 2. presence of PCV2-associated microscopic lesions and 3. detection of PCV2 within the lesions in lymphoid tissue of affected pigs (Sorden, 2000). According to Segales (2012) these diagnostic criteria should be: weight loss and paleness of skin (respiratory and/or digestive clinical signs may be present well), moderate to severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues and moderate to high amount of PCV2 in damage tissue. Opriessnig et al. (2007) proposed that in order to put PMWS diagnosis, PCV2 antigen must be revealed in more than one lymphoid tissue (lymph node, tonsil, spleen) and at least one other organ system (lung, lever, kidney, intestines). It is generally accepted that the PMWS diagnosis at herd level should be based on two conditions: 1. a significant increase in mortality associated with clinical signs compatible with PMWS, and 2. an individual diagnosis in at least one of the three to five necropsied pigs (Grau-Roma et al., 2011; Grau-Roma et al., 2012).

DIAGNOSIS

PMWS most commonly affects pigs at 2-4 months of age (Grau-Roma et al., 2009). Morbidity in affected farms is commonly 4-30% (occasionally 50-60%) and mortality ranges from 4% to 20% (Segales and Domingo, 2002). PMWS is characterized clinically by wasting, pallor of the skin, respiratory distress, diarrhea and occasionally icterus. Enlarged subcutaneous lymph nodes are common finding in the early clinical phases of PMWS (Segales et al, 2004).

At necropsy, the typical macroscopic findings are wasting, non-collapsed lungs, pulmonary consolidation and enlargement of at least one lymph node. Microscopic findings in lymphatic tissue include lymphatic depletion, histiocytic infiltration, inclusion bodies and giant cells (Segales et al., 2004). Porcine circovirus type 2 antigens should be present in moderate-to-massive quantity in lymphoid tissues with typical lesions. Based on necropsy of three unthrifty pigs from all herds in a case-control study, pigs with PMWS were found in 78% of case herds and in 26% control herd with no obvious clinical signs of PMWS (Nielsen et al., 2008). Serological assays for the detection of antibodies to PCV2 have been developed, but diagnosis of PMWS using serological techniques is problematic because PCV2 is ubiquitous and seroconversion patterns are relatively similar in PMWS-affected and non-affected farms. PCV2 dynamic are interest because of their potential role in monitoring PCV2 vaccination (Opriessnig et al., 2008). Thus, to be able to classify a herd as PMWS-affected, the clinical appearance (wasting and excess mortality) must be combined with the laboratory findings. Several studies have assessed the diagnostic value of using serology (antibodies) and PCV2 DNA detection (qPCR) for the diagnosis of PMWS (Grau-Roma et al., 2009; Turner et al., 2009; Woodbine et al., 2010). Even though all studies found significantly higher viral load in PMWS pigs compared with non-PMWS pigs, they con currently conclude that neither viral load nor antibodies can be used for diagnosing pigs or herds as PMWS- affected because the diagnostic sensitivity and specificity are too low (Grau-Roma et al., 2009).

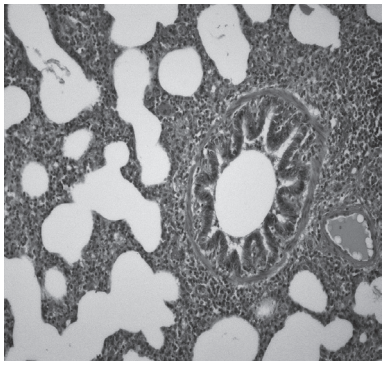

Fig 1. Interstitial pneumonia (Photo: Švara T). Fig 2. Histopathological examination;Interstitial pneumonia (Photo: Švara T).

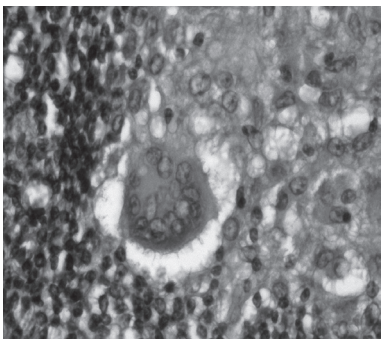
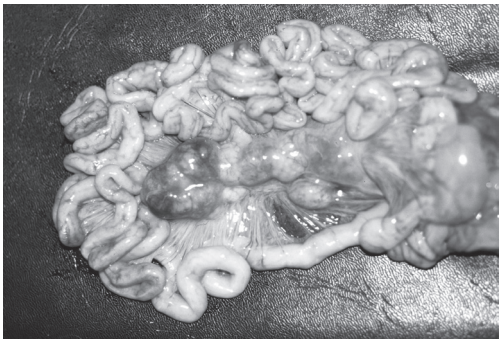

Fig 3. Multinucleate giant cell and macrophages (Photo: Černe M) Fig. 4: Swollen mesenterial lymph nodes. in a medulla of the lymph node (Photo: Švara T).

RISK FACTORS FOR PMWS

Epidemiological studies comparing affected herds with non-affected herds have been carried out in the UK, France, the Netherlands, Spain and Denmark with the objective of identifying factors that either increased or decreased the risk for a herd to be affected by PMWS (Rose et al., 2003; Enoe et al., 2006; Vigre et al., 2006; Woodbine et al., 2007). The most significant factors identified in these studies are summarized in Table 1. Several studies have focused on risk factors for PMWS at the individual pig level. Pigs with low PCV2 antibody titers at 7 weeks of age (and no subsequent seroconversion) and piglets born by seronegative sows were at higher risk of being affected by PMWS (HR = 7.0 and 2.8, resp.) (Rose et al., 2005). Likewise, active infection of the pregnant sows with parvovirus increased the risk (HR = 2.3). It has also been shown that more piglets died from viremic sows than from non-viremic sows (OR = 2.1) and from sows with low antibody titres (OR = 3.0) (Calsamiglia et al., 2007). A longitudinal study in seven PMWS farms showed an increased risk of PMWS if piglets were infected early (before 7 week of age), whereas reduced risk was found if piglets were weaned after 21 days and if they were born of seropositive sows (Rose et al., 2009). The significance of maternal immunity as protective for disease development, as indicated in these studies, was supported by a longitudinal cohort study in 13 Spanish/Danish PMWS farms (Grau-Roma et al., 2011; Grau-Roma et al., 2012).

Table 1. Risk factors for PMWS (Grau-Roma et al., 2011; Baekbo et al., 2012).

	Factors increasing the risk of PMWS	**Factors decreasing the risk of PMWS**
Animals	• Gender (male) • Litter of origin • Low birth weight • Low weaning weigh • Low weight at the beginning of fattening period	• Gender (female)
Facilities	• Large number of sows • Large pens at nursery and growing ages • Proximity to other pig farms	• Separation pit for adjacent fattening rooms • Shower facilities • High level of external biosecurity • Quarantine for purchased pigs and gilts • Change of boots/clothes in entrance room of the farm
Management practice	• High level of cross-fostering • Short empty periods at weaning and fattening • Large range in age and weight entering the nursery • Continuous flow nursery • Purchase of replacement gilts (>500 per year) • Sows with neck injures due to poor injection technique • Early weaning (<21 days of age) • Herd size >400 sows	• Sorting pigs by sex at nursery stage • Greater minimum weight at weaning • Group housing sows during pregnancy • Long empty period (weaners and sows) • Dry sows in collective pens • Visitors with no pig contact for several days before visiting farm • Use of semen from an insemination centre
Vaccination/ treatment/ nutrition	• Vaccination of gilts against porcine reproductive and respiratory syndrome (PRRS) • Vaccination of sows against *E. coli* • Use of separate vaccines against erysipelas and porcine parvovirus (PPV) on gilts • PPV antibodies among finishers • Active PPV infection in pregnant dams • On-farm semen collection	• Vaccination of sows against atrophic rhinitis • Regular treatment for ectoparasites • Use of oxytocin during farrowing • Use of spray-dried plasma in initial nursery ration

PREVENTION AND CONTROL

PMWS is considered a multifactorial disease (Segalés et al., 2012). Before the introduction of commercially PCV2 vaccines, the focus was on good production practice and on control of other diseases (Baekbo et al., 2012). The implementation of „Madec's 20-point plan", a list of management practices lowered the impact of the disease and decreased the mortality in severely affected farms (Madec et al., 2008). These factors still have relevance in the control of PMWS.

Intraperitoneal injection of piglets, five days before weaning with serum from 100 kg pigs, from the same farm (serum therapy) was reportedly successful in reducing losses in growing period (Valenčak and Martinjak, 2005).

Introduction of PCV2 vaccines significantly changed the impact of PCV2 on the pig production globally (Baekbo et al., 2012). In pigs, vaccination improved average daily gain (ADG) and feed conversion, decreased mortality rates, reduced medication costs, and reduced viral loads and PMWS lesions (Pejsak et al., 2009). Even in farms with a subclinical level of PCVD and acceptable low mortality rates, vaccination of piglets increased ADG among grower-finishers (Baekbo et al., 2012). In sows, vaccination increased fertility and reduced returns to service (Kekarainen et al., 2010).

The vaccine success is based on activated humoral and cellular immune responses against PCV2. PCV2 vaccine efficacy in pigs may rely on humoral immunity. Low antibody responses, as well as lack of antibody development after vaccination, do not necessarily correlate with lack of protection. Cell-mediated immunity is also assumed to be important (Segalés et al., 2012).

Four PCV2 vaccines are commercially available in most countries: one sow vaccine (Circovac^{R}, Merial) and three piglet vaccines (Ingelvac^{R} CircoFlex^{R}, Boeringer Ingelheim; Porcillis^{R} PCV/Circumvent^{R} PCV, Intervet/Merial & Circovac^{R}, Merial) (Baekbo et al., 2012). All current vaccines are based on PCV2a strains (Kekarainen et al., 2010).

High levels of maternal-derived antibodies were found to interfere with active seroconversion following vaccination (Fort et al., 2009), even though the vaccine significantly reduced viremia and shedding of virus. It is recommended to avoid vaccination of too young piglets.

CONCLUSIONS

PCV2 is ubiquitous and most PCV2 infections are sub-clinical. PCV2 needs one or more co-factors for PMWS to develop into severe and even fatal disease. Unfortunately no single viral co-factors have yet been identified. Post-weaning mortality is one of the most significant losses in PMWS affected herds, but reduction in growth and poor feed utilization as well as increased consumption of antibiotics add to the cost of the disease. Introduction of PCV2 vaccines significantly changed the impact of PCV2 on the pig production globally.

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CIRKOVIROZA NIJE BOLEST: PMWS

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Izvod

Post-weaning multisystemic wasting syndrome (PMWS) je jedan od oblika cirkovirusnih bolesti (PCVD). PMWS ima značajan ekonomski uticaj na globalnu proizvodnju svinja. Cirkovirus svinja tip 2 je cirkulirao u populaciji svinja mnogo pre nego što je bio povezan sa bolešću. PMWS je izveštavan iz svih zemalja koje su značajne kao proizvođači svinja. Infekcija sa cirkovirusom tipa 2 (SCV2) je potrebna za razvoj PMWS, ali većina od istraživanja pokazala su da SCV2 treba jedan ili više ko-faktora da se PMWS razvije u tešku, pa čak i smrtonosnu bolest. Potvrda PMWS treba da temelji na određenim kriterijumima kao što su klinički znakovi (zaostajanje u rastu i gubitak težine, često sa dispnejom i povećanjem ingvinalnih limfnih čvorova, a povremeno i sa žuticom), umerenim do teškim karakterističnim patohistološkim lezijama

u limfnim tkivima, umerenim do visokim količinama SCV2 unutar lezija u limfnim i drugim tkivima i smrtnost koja je veća od očekivane i/ili istorijske razine za pojedinu farmu. Uvođenje vakcinacije sa SCV2 cepivima značajno je promenilo uticaj SCV2 na svinjogojstvo na globalnoj razini.

Ključne reči: SCV2, PMWS, faktori rizikovanja, kontrola, svinja.

Received / *Primljen*: 18.04.2013.
Accepted / *Prihvaćen*: 30.04.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 109-117, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 636.09

YERSINIA ENTEROCOLITICA- SOURCES AND PATHWAY OF CONTAMINATION PIG CARCASS

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SUMMARY: Y. enterocolitica is a pathogenic microorganism with the possibility of meat and carcasses contamination of pigs which affects human health. This microorganism is at the highest percentage found in the tonsils of pigs and due to poor hygiene practice and inadequate manipulation with knives, can penetrate the carcass and become a source of infection for humans. In EFSA opinion on pork hazards is evaluated comprehensive system of maintaining food safety and quality, and rating hazards relevant to pig carcasses, particular during cooling, including Y. enterocolitica.

***Key words**: Yersinia enterocolitica, pigs, meat, sources, tonsils.*

INTRODUCTION

Yersinia enterocolitica is in the group of foodborne pathogens that causes severe infections in humans. The infection is most frequently associated with pork but studies show that other types of meat can be sources of infection. Pigs are potential reservoirs of *Y. enterocolicica* which is pathogenic for humans. *Y. enterocolitica* is found in tonsils and lymph nodes of pigs but can be also isolated from feces (Thibodeau et al., 1999; Nesbakken et al., 2003). However, the presence of *Y. enterocoticica* in tonsils is six times higher than in feces (Nesbakken et al., 2003). According to data from EFSA (2006), yersiniosis in people, caused by foodborne infections, is at third place in Europe, behind salmonellosis and campylobacteriosis. The disease in humans is characterized by diarrhea, ileitis, arthritis and septicemia. According to the EFSA, among *Yersinia spp.* a special significance in foodborne infections due to consumption of animal products will be related to *Salmonella spp., Toxoplasma spp. and Trichinella spp.*

Review paper / *Pregledni rad*

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Taxonomy and phenotypic characteristics

Genus Yersinia belongs to the family of Enterobacteraceae and has 12 known species of this genus. From these 12 species only three are considered as pathogens: *Yersinia pestis*, especially important as the cause of the plague in people, *Yersinia pseudotuberculosis,* associated with zoonotic infection and *Yersinia enterololitica* (Mills, 2004). In addition to the three pathogenic species there are nine non-pathogenic. In the species there are a wide variety in biochemical features. Such variations inluenced distribution of *Y. enterocolitica* in six biotypes. Wautters (1991) has shown the difference between pathogenic biotypes (1B, 2, 3.4, 5) and non-pathogenic biotypes (biotype 1A) (Table 1.). Biotyping of strains is the most important step of testing *Y. enterocolitica*. *Y. enterocolitica* can be separated into serotypes by using the O-antigen. Over 76 different serotypes are known up to date. In Europe, the most common is biotype 4.

Table 1. Biotype of Y. enterocolitica

Biotype	Serotype (s)	Human virulence	Frequency in Europe
4	O:3	Pathogenic	++++
2	O:9,O:5,27	Pathogenic	++
3	O:3, O:5,27	Pathogenic	+
1B	O:8, O:21, O:13	Highly Pathogenic	0
5	O:3, O:2,3 O:1,2, 3	Pathogenic	0
1A	Numerous	Non-pathogenic	++++

Biotypes 1B, 2, 3, 4 and 5 are pathogenic for humans and animals. Biotype 1A includes a large number of serotypes found in food, environment and can be also found as supporting flora in the digestive tract of animals and humans. These serotypes don't have virulence characteristics. Biotype 1B involves highly pathogenic serotypes O: 8, O: 21 IO: 136. Serotypes O: 4, O: 18 and O: 20 were significantly rare in Europe. Biotype 2 includes two serotype O: 9 and 0:5,27 that are pathogenic for humans. Biotype 3 includes serotypes O: 1, 2, and 3, which were isolated from rodents. Biotype 4 contains only one serotype, O: 3, which is the most prevalent in the world and isolated from pigs.

Characteristics of Y. enterocolitica

Yersinia enterocolitica is the Gram-negative microorganism, rod-forms with a diameter 0.5 to 0.8 μm and length of 1 to 3.5 μm. *Y. enterocolitica* belongs to sporogenic and non-capsular microorganisms. At a temperature of 35-37 ºC is immobile, but at a temperature from 22 to 25 ºC possesses flagellas and is mobile. *Y. enterocolitica* is psychrotrophic microorganism. It has possibility to grow from -2 to 42 ºC (Bercovier and Mollaret, 1984). The optimum air temperature rise of 28 to 29 ºC (Bercovier et Mollaret, 1984). *Y. enterocolitica* has the ability to replicate in food, especially meat, at a temperature below 0 °C (Lee et al., 1981; Sterrn et al., 1980). In food with neutral pH kept at a temperature of 5 °C, there is a possibility that the number of *Y. enterocolitica* with 10 cfu/ml increased to 107 cfu/ml for a period of five days (Bhaduri et al., 1997). The minimum pH for growth of *Y. enterocolitica* is between 4.2 and 4.4. *Y. enterocolitica* has the ability to grow at pH values below 4.2 or above 9. The presence of organic acids reduces the growth ability of *Y.enterocolitica*. *Yersinia enterocolitica* is particularly significant because of their ability to replicate at temperatures of refrigerators, vacuum packaging or in packaging with modified atmosphere (Bercovier and Mollaret,

1984). *Y.enterocolitica* can survive in frozen foods at longer periods. In pork in vacuum packaging, stored between 2 and 7 °C, after five weeks of storage, *Y.enterocolitica* has the ability to grow (Hayashidani et al., 2008). In pork meat during storage at 4 °C in modified atmosphere with a 100% CO_2 *Y. enterocolitica* is suppressed (Bodnaruk et Draughon, 1998). However, Strotmann et al. suggest that different concentrations of CO_2 have no effect on growth of *Y. enterocolitica*. *Y. enterocolitica* grows in the packaging with modified atmosphere with the different concentrations of O_2 and CO_2. Also, in a modified atmosphere, where the ratio of O_2 and CO_2 is 50:50%, it is in pork meat noticed the growth of this microorganism (Strotmann et al., 2007). Its replication is not affect by associated microflora of meat, stored at 10 ºC (Nissen et al., 2001). The high concentrations of O_2 in modified atmosphere of minced pork, shows an inhibitory effect at 4 º C on the growth of *Y. enterocolitica* (Pin et al., 2000).

Today for isolation of *Y. enterocolitica* is used the standard procedure described in ISO standard (10273).

Incedinca disease in humans

According to data from WHO, the incidence of infections with *Y. enterocolitica* increases. The incidence of yersiniosis in European countries is on the decline, with 9533 cases confirmed in 2005 year and 6776 cases in 2010 year, so it is a significant cause of foodborne diseases. Situation in Serbia is unknown but according to unofficial data, in 2010 was recorded 108 cases (Malašević, 2012). The incidence of occurrence is shown in Table 2. The largest number of cases of yersiniosis was recorded in the United States where the number of patients in 1997 year was 87,000 and the incidence of disease 33.4. In Japan, the number of cases of yersiniosis, according to data from 2001 was 4, which is the smallest incedence of yersiniosis (less than 0.01).

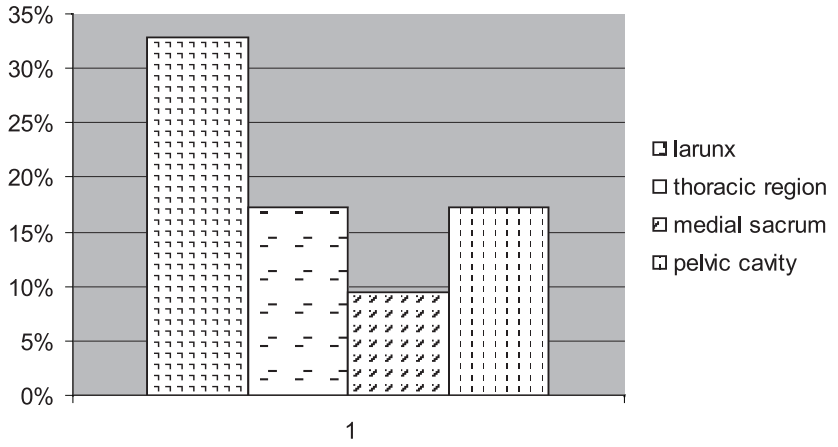
Table 2. Number of registered cases of humans yersiniosis (Nesbakken, 2005)

Country	Cases	Incidence (per 100.000 population)
Australia	73 (2000)	0,6
Austria	94 (1998)	1,2
Belgium	8291 (1994)	8,5
Denmark	7113 (2001)	8,7
Finland	647 (2003)	12,4
Greece	10 (1998)	0,1
Japan	4 (2001)	< 0,01
Norway	862 (2003)	1,9
Špain	425 (1998)	1,1
Šweden	7142 (2003)	8,0
Šwitzerland	51 (1998)	0,7
United Kingdom	27 (2000)	0,05
SAD	87,000 (1997)	33,4
Serbia	108 (2010)	0,9

Sources and pathways of infection

Yersinia enterocolitica is widespread in the environment. It is isolated from the

water (wells, springs), pigs, dogs, cats, ruminants, rodents, molluscs and other invertebrates. However, primarily as a source of pathogenic strains are pigs. There is a connection between the tonsils and the contamination of pig carcasses. Among the tonsils as the most common source of these pathogens (Fondrevez et al., 2010), also other sources were observed (lymph nodes, intestines, liver and heart). As a psychrotrophic microorganism, *Y. enterocolitica* can be replicated in the cold chain of the meat production process as well as in refrigerators at home. Pets, especially dogs, can be either a host or transmission source of infection for children. There is a direct transmission from human to human, which is possible with fecal-oral route and over germ-carriers. However, the first source of infection for humans is raw pork and pork products. An important source of cross-contamination is the content of intestine and tonsils. Observing slaughter line, Van Damme De Zutter (2011) suggest that the most contaminated are medial part of the larunx with this pathogen (32.8%), thoracic region (17.2%), medial sacrum (9.4%) and pelvic cavity (17.2%). These results are shown in graph 1.



Graph. 1. Participation *Y.enterocolitica* in some regions

Participation of positive samples in examined pigs was 57.2% in tonsils and 20% in rectum. Prevalence of *Y.enterocolitica* in sows on the farma is often very low (Gurtler et al., 2005). During breeding, pigs are colonized after the first two months of life (Nesbakken et al., 2005). Initially, this pathogen is present in faeces and tonsils, and later its number significantly increases in tonsils and does not change until slaughter (Nesbakken et al., 2006). Among the tonsils and feces, *Y. enterocolitica* can be isolated from mandibular and mesenteric lymph nodes (Nesbakken et al., 2003). A study in France showed that in slaughterhouse was found 19.8% positive pigs originated from 80% of positive herds (Fondrevez et al., 2010). Risk to the pork safety in terms of this pathogen in slaughterhouses primarily depends on the process hygiene in slaughterhouses, staff practice and their hygiene. In terms of process steps in slaughterhouses, technical aspects of the individual steps may be different between slaughterhouses, but their order is usually similar. It starts with lairage, stunning, slaughter, bleeding, scalding, singeing, washing, evisceration, carcass cutting and cooling. In Europe,the pigs are carriers of

pathogenic strains of *Y. enterocolitica* for people, especially strain biotype 4 (serotype O: 3) and less biotype 2. There are reports that even more than 80% of swine herds can be positive, with a high percentage of positive pigs at slaughter. During the process of slaughter, pig carcasses can be easily contaminated with this pathogen through fecal contamination and from the oral cavity. Particularly strains of biotype 4 (serotype O: 3) are often found on pig carcasses surfaces after slaughter and processing, but before chilling. Slaughter technique and hygiene of premises, equipment and staff who are responsible for this work have a great impact on the incidence of contamination. Fecal contamination can be significantly reduced by technique of rectum binding immediately after evisceration (Andersen, 1988). Oral cavity and tonsils are at high percentage contaminated with *Y. enterocolitica*, so removing the tonsils or cutting carcasses may cause spread of the pathogen that is in this part of carcass. Very often is the muscle M. *digastric* contaminated with *Y. enterocolitica*.

During cutting, further process and distribution of fresh pork and carcass waste is also possible further contamination with *Y. enterocolitica*. However, according to literature data, *Y. enterocolitica* is rarely isolated from chilled pork at retail stores, unless they are cooled tongues as products. *Yersinia enterololitica* has the potential to replicate during storage of meat and meat products. However, the ability to survive, especially at low temperatures and at normal pH is low.

Risk factors

Pigs and pork products are a potential source of *Y. enterocolitica* for humans (Norrung et al., 2009). Genetic characterization showed that the strains of *Y. enterocolitica* that caused the disease of people were identical to the strains that were isolated in the tonsils of healthy pigs (Fredriksson-Ahomaa et al., 2001, 2006). The most common *Y. enterocolitica* is isolated on carcasses and chilled pork and pork products. According to EFSA (2012), a total of 4.1% of pork samples were positive in the European Union in 2010. The main risk factor for the presence of *Y. enterocolitica* in pork is the slaughter of pigs that are carriers of the pathogen. Cross-contamination of carcasses and entrails is possible during slaughter and processing (Fredriksson-Ahomaa et al., 2001). Proper techological operation and hygiene during slaughter have a significant impact on the microbial contamination of pig carcasses and entrails. The major measures affecting the reduction of contamination are: compling with the principles of good manufacturing practices and standard operating procedures (ligation of rectum, handling head and tongue).

Prevention and control in various stages pork meat production

Yersinia enterocolitica is in gastro-intestines, tonsils and skin of pigs that are source of contamination of pig carcasses at slaughter (Nesbakken et al., 2003). The pork is primary source of infection for people. Pigs are asymptomatic carriers of the pathogen. *Yersinia enterocolitica* spreads by objects within farm and along the entire production chain of pork through cross-contamination. This results in the contamination of pig carcasses and human exposure to this pathogen. Control of *Y. enterocolitica* on farms and at slaughterhouses is very important because it is considered that pathogen carrier pigs are the main risk of the presence of *Y. enterocolitica* in pork (Fredriksson-Ahomaa et al., 2001). This pathogen is detected by laboratory testing of pig carcasses. Growing pigs without mixing with other animals from different farms or with different

age groups reduce the occurrence of this pathogen. Also, cleaning and desinfection of objects before inserting the pigs is of particular importance. In order to reduce the spread of infection is necessary to provide a special ventilation system in buildings where pigs are kept, use of hygiene barriers when entering the farm, the use of clean straw as bedding, identification and removal of seropositive animals from the herd, maintaining good hygiene and housing conditions of pigs, protection of other diseases, the use of clean drinking water free from pathogens, prevention of faecal contamination of water and food.

After slaughter, carcass inspection is necessary, which aims to protect human health. However, foodborne pathogens can not visually be detected, including *Y. enterocolitica*. Major foodborne pathogens, including *Y. enterocolitica* can be controlled, in order to reduce the possibility of cross-contamination of carcasses. Cross-contamination is common in the process of palpation and cutting the carcass. That is why process hygiene, sterilization of knives and staff training are very important. Efficient refrigeration and maintenance of the cold chain are primarily the most important measures to reduce the replication of this pathogen. Control of *Y. enterocolitica* in the processing, distribution, retail and consumers is based on similar measures applied in most other foodborne pathogens. Cooling of meat has limited efficiency in control of *Y. enterocolitica* and therefore previous phases in meat production are very important in providing the better microbiological status. Raw meat should be stored separately from other foods. Cross-contamination from raw meat to meat that has been treated with heat regime should be prevented in institutions where it is processed, butcher shops and retail outlets. Knives, equipment and machines used for cutting and processing of raw meat and meat products must be regularly washed and disinfected before they are used for other foods. Also all surfaces that have been in contact with raw meat should be washed and disinfected before next use. Consumption and under-consumption of thermally-treated meat should be kept to a minimum because in these situations the prevalence of foodborne diseases including yersiniosis is much higher.

Examination and monitoring of Y. enterocolitica in slaughter pigs

Distribution and prevalence of *Y. enterocolitica* vary geographically. Pigs are the main carriers of human pathogens *Y. enterocolitica*, especially biotype 4 (serotype 3). Among pigs and other reservoirs have a significant role in the epidemiology of yersiniosis in people. Data suggests that ruminants, particularly cattle may be reservoirs of biotype 2 (serotype ¬ O: 9 and O: 5,27). The presence of *Y. enterocolitica* can be detected in several ways. Cultural methods includes sampling of tonsils and faeces and carcass swabs. When testing large numbers of animals can be used ELISA for screening identification of infected herds and then *Y. enterocolitica* may be confirmed by the cultural method. Serological tests are possible from serum or meat juice during slaughter. Bacteriological examination of feces and tonsils are the long-term, economically non profitable compared to serological testing. On the other hand, serology relies on delayed reaction and positive response does not mean that animal excretes the pathogen (Nesbakken et al., 2006). In pigs for slaughter, prevalence of *Y. enterocolitica* is higher in tonsils than in feces. It is considered that the highest level of pig carcass contamination with this pathogen is at a slaughterhouse. It is also expected high contamination of internal organs, tongue, liver and heart. However, these organs are thermic processed before consuming and *Y. enterocolitica* will be eliminated by this way. In the chain of

production, the number of positive samples with *Y. enterocolitica* decreases. Fresh meat enables replication and survival of pathogenic *Y. enterocolitica*, especially in the case of cross-contamination. For meat products cross-contamination is possible, only in case of contamination in the production or distribution of these products.

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YERSINIA ENTEROCOLITICA- IZVORI I PUTEVI KONTAMINACIJE TRUPOVA SVINJA

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Izvod

Yersinia enterocolitica pripada patogenim mikroorganizmima koji mogu da kontaminiraju trupove i meso svinja što utiče na zdravlje ljudi. Ovaj mikroorganizam se nalazi najčešće u tonzilama svinja za klanje a usled loše higijenske prakse i neadekvatne manipulacije noževima, može dospeti na trup i tako postati izvor infekcije za ljude. EFSA je u svom mišljenju o opasnostima iz svinjskog mesa koje se moraju kontrolisati sveobuhvatnim sistemom osiguranja bezbednosti, ocenila kvalitativno i rangirala relevantne opasnosti na trupovima svinja i momentu nakon hlađenja trupova, uključujući i *Y. enterocolitica*.

Ključne reči: yersinia enterocolitica, svinje, meso, izvori infekcije, tonzile.

Received / *Primljen*: 28.04.2013.
Accepted / *Prihvaćen*: 14.05.2013.

CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 62 (1-2) 118-126, 2013.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 576.89

PARASITES FAUNA OF SWINE AT ORGANIC BREEDING

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SUMMARY: Parasites infection were permanent health problem at swine production at exensive and farm breeding. Organic breeding induced permanent contact of swine and intermeditae host of numerous parasites and those infection were more frequent at these breeding condition.In most cases there were presented biohelminths from genus Metastrongylus, nematode which cause gastritis verminosa (Ascarops strongyllinae, Physocephalus sexalatus and Hyostrongylus rubidus), Oesophagostomum spp. and Macracanthorhynchus hirudinaceus. There were presented at lover rate of infection Acaris suum,. and other helminths.

Key words**:** *biohelminths, swine, extensive breeding.*

INTRODUCTION

Parasitic infections are constant companions of pig production, irrespective of the manner of holding. (Pattison et al.1980., Pavlovic et al.1995, 1996.1997., Schiessl 1990). Disappearance of flow and disease of pigs caused by the presence of the agent, susceptible hosts and the environment condition. Organic farming, which greatly resembles the extensive breeding condition that the pigs have direct contact with many intermediate host of parasites and therefore are in them parasitic infections often (Babic et al..1942., Tričković, 1978., Perez-Brincones and Alvarez- Fernandez 1977., Loskot at al.1988., Pavlovic at al.1996) In this posture the number of parasite species is much higher and the morbidity and mortality caused by them. (Vujic 1976., Pavlovic at al.1997).

Nevertheless, at the organic breeding pigs in the foreground biohelminte and the most important representatives of the genus *Metastrongylus,* verminoznog causes gas-

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Review paper / *Pregledni rad*

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tritis, *Oesophagostomum spp., Acaris suum* and *Macracanthorhynchus hirudinaceus* (Dunn at al.1955, Dun, 1957., Kruse & Ferguson 1980., Pavlovic at al. 2005a, b, 2007, 2010).

METASTRONGYLIDOSIS

In world, a total of 6 *Metastrongylidae* species are established: *Metastrongylus elongatus, M.pudendotectus, M.salmi, M.confusus, M.madagascariensis* and *M. tschiauricus* (Ershov et al., in 1963., Soulsby 1977). In Serbia, we established *M.elongatus* and *M.pudendotectus*. Prevalence of both types varies from region to region (Ivancevic,1963. Pavlovic et al. 2005a, b). Metastrongylus are biohelminths who belong to their own development using many types of *Lumbricidae* (rain worm). The eggs are resistant to the external environment and, depending on the external conditions of the eggs are released larvae that are infective for the real host infectivity but are only acquired when they eat earthworms (Dun et al., In 1955., Kruse, 1978). In Serbia, they are: *Eisenia foetida, E.rosea, Dandreobena rubida, Allopbophora caliginosa, A jassyensis, Lubricus terrestris, L.rubbelus, Eisenia veneta, E.tetraedra, Allopbophora longa, Octolasium complanatuum, O.lacteum, O.rebeli, Dendrobaena octaedra, D.subrubicunda, D.mariupoliensis, Bimastus tenius* and *Heledrillus spp.* (Tričković,1978., Pavlović et al 2005a,b).

Infection occurs when pigs infected pigs eat worms. From the digestive tract of the larvae mature in the mesenteric lymph nodes where they molt. In the bronchi and bronchioles larvae grow and after 24 days reaching adult stage. Preparent period is lasts 24-37 days (Dunn et al. 1955., Tričković, 1978). Most susceptible to infection are young pigs aged 2-8 weeks. Maximum productions of parasite eggs are in 5-9 weeks after infection. In the future the number of parasites is reduced but a number remain especially in the distal parts of the lung (Kvachadze, 1975., Vujić, 1976, Pavlović at al.2005a).

The clinical picture depends on the degree of infection. At low infection are poorly expressed - often present with a weak cough severe infections signs of dispnoa and frequent vesicular breathing. At the beginning of the present weak and hoarse cough that later in an attack when animals have stress (started running, etc...). Mucous membranes are pale, the appetite is reduced and the body temperature is elevated only when there are secondary infections (Pavlović at al. 2005a).

The pathological effects of parasites begin their larval migration movement from the pulmonary capillaries to the lung tissue during migration through the lung tissue, and activities during their adult longevity in the bronchi and aspiration of parasite eggs in the bronchioles and alveoli (Dunn et al. 1955., Drozdz and Zalewska-Schönthaler 1987). It builds on the toxic effects of the metabolic products of the parasite by absorption in the blood can lead to a general intoxication. In weak predilection site infections are the last parts diaphragmatic lobe and in severe infections are caught in the other parts of the lung (Ivancevic, 1962, Tričković, 1978, Nakauchi et al. 1991). It can be seen bronchiolar, bronchitis, diffuse pneumonia, alveolar emphysema and connective tissue and cellular proliferation (Pavlovic et al. 2005). Pathological changes are wedge seems on whose basis are the bronchioles and bronchi in which we find parasites in different developmental stages were available in the mucous exudates or encircled cell infiltrate. The parenchyma luck to gray nodules sized 0.6-2mm in which the central section notes yellow or yellow-green field surrounded by a brown zone of connective tissue (Nakauchi et al. 1991).

Gastritis Verminosa

Verminous gastritis is a common name for the disease caused by the increasing number of nematodes belonging to the orders and *Spiruridea* and *Trichostrongylidea*. (Pathison et al.1980, Pavlovic sar.2008). From among *Spiruridea* are represented *Ascarops* (*Ascarops strongyllinae*, *A.dentatum*) and *Physocephalus* (*Physocephalus sexalatus*)

Ascarops strongyllinae (syn.*Arduenna strongyllinae*) are the red parasite. Larval development takes place through the intermediate host, beetle *Aphodius castaneus, A.rufus, Ontophagus Hecate, Gymnopleurus spp.* and others. In them, the eggs release larvae in the body cavity of the beetle and then larvae molt into the second stage and pupate there. If an infected beetle eats random transient hosts - birds, small mammals, amphibians and reptiles, the larvae of the second degree of their digestive system migrated to the muscles where they pupate again. Infection occurs when the pigs eat the beetle's infectious or infected meat accidental intermediate host. Parasites graduate in host stomach, attaching to the lining of the stomach and that they are found in large amounts of mucus. In a large number can cause diffuse catarrhal gastritis and sometimes form pseudomembrans (Corwin i Stewart.1992).

Physocephalus sexalatus lives in the stomach, and less in small intestine. They infected pig and donkey, camels and cattle. The body of parasites is white more thinly in the front. Larval development takes place through the intermediate host beetles usually: *Scarabeus sacer, S.variolosus, Aphodius castaneus, A.rufus, Gymenopleurus Sturn, Geotrupes Doue, G.stercorarius, Onthophagus misery* and *O.hecate* (Pavlovic and sar.2008). Pigs and other good hosts become infected when they eat beetles or infectious flesh and organs of the infected intermediate host random.

In pigs only when infections are massive we can see inflammatory processes Animals eat less or even stop to eat, restless and often drink water. At sector can be seen in the mucosa of the stomach ulcers and bleeding suffusion. Weak infections go almost unnoticed without visible pathological changes especially in older pigs.

From order Trichostrongylidea here represented genera *Hyostrongylus (Hyostrongylus rubidus*) and *Gnathostoma (Gnathostoma hispidum*) (Pavlović i sar.2008)

Hyostrongylus rubidus are the intense red or red-brown color parasite (Pavlovic and sar.1997). At the time of egg laying protoplasm is furrowed in blastomers. The external environment continues to develop in the first 24 hours caused rabditoid larvae leaving the eggs after 48 hours. The outdoors larvae molt twice and become infective. The larvae require a moist environment to keep the pigs on in dry pens reduce the risk of infection to a minimum.

After oral infection larvae do not migrate from the stomach but remain there and become adult for 17-19 days after infection. Especially invaded are area around the pylorus and the funds. Pathological effects manifest themselves during their larval stage and then hystotrophic adults their mechanical and toxic effects. After infection larvae penetrate the gastric mucosa and glands causing minor bleeding. Later there is a hypertrophy of the gland which is manifested in the form of lumps the size of the lens. Initially flat and look like red spots from which to develop ever more clearly later raised islands of mucosa pearly shine. Later, these places are covered with 1-2 mm thick pseudomembrans that is easily removed. Below it are partially embedded in the mucus, parasites. Since *Hyostrongylus* bloodsucking host severe infections can cause anemia

in young animals

Gnathostoma hispidum lives in the walls of the stomach in pigs and cattle and less and man. It is a large nematode. The male is from 1.5 to 2.6 cm long and 1.1-2mm wide and the female is 2.1 to 4.5 cm long and 2.5 mm wide. Larval development takes place through the intermediate host. Eggs in the external environment due to the two developed blastomers in favorable environmental conditions in which they formed larvae after two coating leaves then penetrates the egg crabs of the genus *Cyclops* where twice molt In developing *Gnathostoma* there are occasional transient hosts - birds, amphibians and reptiles that are infected in the same manner in which larvae migrate to the organs and muscles.

Only to reach the stomach larvae penetrate the lining of the front end generally along the pylorus. At that sites of occurring damage look like a large as a grain of millet, round with sharp red edge. Depending on the number of parasites is the number of ulcers and severe infections of the mucous membranes seems riddled like a sieve. The lining is hemorrhagic and caused chronic inflammation with resultant thickening of the walls of the stomach lining which is rough and wrinkled.

Ascaridosis

Ascaridosis is the most common and most widespread parasitic infection of pig's causes by white and pink nematode *Ascaris suum*. The male is 12-25 cm long and 3 mm wide and the female is 30-35 cm long and 5-6 mm wide. Eggs are oval size 40-50 micrometers. The membrane consists of four layers, brown in color and thick, and the last layer is uneven edges and ascaris provides good protection and easy grip in the external environment. At the time of laying eggs are embryoned and what takes place in the external environment (Olsen, 1986, Rhodes et al.1997).

For infection of susceptible animals are up to one year of age, and over that age of infection might occur very rarely. Egg entered the digestive tract exits developed larvae that are starting hepatopulmonary migratory phase during which the molt four times (in the liver, lungs and intestines) (Douvres et.al.1969, Jakovljevic, 1974, Milivojevic, 1976). After 8-10 days of infection than those smears are swallowed and mature in the gut become adult parasites where there is a male and female mating and laying eggs in the intestinal lumen, where through the feces due to the external environment (Kulišić, 2002)

The clinical picture depends on the number of infective parasite eggs, the age and fitness of the animal. Pronounced clinical picture we have in piglets 4-5 months of age (in this group are possible and significant mortality intensity) in the form of cough, bronchopneumonia (due larvae migration of parasites), weight loss, poor promotion and diarrhea in piglets older than 5 months creates immunity eases and then reversed the clinical picture of the disease is primarily related to the migratory stage larvae. Pigs older than one year have developed immunity to this parasitic, analogous to self-cure mechanism in ruminants (Urban et al.1988).

Typical pathological changes showed liver provides that after the infection gets more continuous or discontinuous silver-gray color with the proviso that in some cases it occurs indurations of its surface is rough and uneven, with hard protuberances and depressions characteristic of interstitial processes of different ages. In young pigs during the larva liver is enlarged, and more or less bleeding beneath the capsule. Due to degenerative-necrotic changes on the surface of the liver can be observed not clearly

defined whitish spots that can conflation so this looks like a liver sprinkled with milky white freckles (Ivetić and sar.2007). In particular, there is a severe generalized infection of cirrhosis of the liver. In the lungs at the surface are visible numerous specks of bleeding especially on the tips of lobes (Milivojevic, 1976). The lungs are collapsed in places and filled with bloody foamy contents in which a large number of larvae. Found in the intestines of parasites and the resulting adults of catarrhal enteritis and possible rupture of the bowel due to blockage of a large number of parasites (Pavlovic and Andelic-Buzadžić, 2011). In the case of intestinal perforation peritonitis occurs. In severe infections the parasites seen in bile duct and excretory ducts of the pancreas and the pigs they can vomit.

Oesophagostomosis

The causes of this disease in Serbia are *Oesophagostomum dentatum* and *Oe.longicaudatum*. In India and North America are being met and *Oe.maplestoni, Oe.brevicaudum* and *Oe.georgianum*. Parasites are whitish in color, females are 7-14 mm long and male's 6-10 mm. parasite development is direct. From eggs to larvae outdoors out with the first dressing for 3-4 days and the other for 5-6 days occur when infective larvae. Pigs become infected when they eat larvae (Pavlovic and Andelic-Buzadžić, 2011)

Upon infection, the larvae come to the colon, there to rally in the lining and create nodes. Nodules consist of peripheral, reactive and central zones where detritus mass and eosinophyls occurred. After 5-10 days larvae leave the nodes, leaving the lumen of the intestine and become sexually mature parasites

Pathological changes in the intestinal mucosa which is red covered with a gray slime. Nodules protruding from the larvae after leaving in the intestine (Babic and sar.1943, Pavlovic sar.1997) also meet diphtheritic deposits sometimes with deep necrosis. The clinical picture depends on the degree of infection. At low rate of infection is poorly expressed - often present mucous diarrhea

MACRACANTHORHYNCHOSIS

Macracanthorhynchus hirudinaceus is the Acanthocephala whose body length response length names. These are roller parasites strong growth and clearly manifest sexual dimorphism. The front end of the proboscis is armed with a round shape hooks that are bent backwards. Females lay eggs long oval 60-100 and 50-56 microns wide, which is due feces in the external environment. They embryo is surrounded by four membranes, which is armed with several small hooks. Other parasite development are necessary transitory hosts, the beetle family *Scarabidae - Melolontha melolontha* and *M.vulgaris, Cetonia aurata Polyphylla fullo, Anomala vitis, Scarabeus* (*Ateuchus*) *sacer, Tropinota* (*Epicometis*) *hirta Poda Anisoplia segetum, Amphimallon solsititialis, Phylophaga Vehemens* et al. (Olsen, 1986; Crompton and Nickol, 1995) When the larvae eat eggs skarabida makrakantarhinhusa from them in the digestive tract, releasing larvae (acantor) and it will remain throughout the stages of metamorphosis beetle (Pavlovic and sar.2010).

Infection occurs when pigs ingest infected pigs beetle puppets, larvae or adults. In the digestive tract of pigs from acantela and adult parasites occur within two months. Parasites occurred in the small intestine, especially the duodenum and rarely in the

colon. They drilled their rostra bowel wall to the sub mucosa and in those places ulcer caused by grain size to millet head pins. Bottom of ulucus is necrotic and the edge is thickened due to chronic inflammation, which leads to the formation of visible lumps with external the bowel wall (Crompton, 1992).

Clinical symptoms of diseases are not specific. In severe infections observed digestive disorders, apathy, in appetence, vomiting, diarrhea, failure to thrive, weight loss and occasional quantity in pain. Diarrhea alternating with constipation what supplements tympanites. Then there is bloody diarrhea with cramps of abdominal muscle tremors. At this stage often leads to mortality of piglets. Acute course of the disease is seen in young and elderly chronic pigs. On dead animals are observed cachexia. At autopsy, noticed by dark yellow or dark brown nodules on the outside mucus cancer. They point to the place of fixation of parasites. Around each of the nodes is observed bright red hyperemic area causing a thickened bowel wall. In the inside of the intestine can be observed or catarrhal hemorrhagic enteritis and a number of parasites attached to the mucous membranes or free in the lumen. In the case of perforation of the intestinal wall is observed peritonitis.

PREVENTION AND THERAPY

Metastrongylidosis, *gastritis verminosa, macracanthorhynchosis, oesophgostomosis* and*, ascaridosis* are disease of pigs reared on pasture, in backyards and even at outlets or organic and extensively, but occur in farm animals kept in conditions where they are kept at the outlet. With free pig keeping the most important but least feasible preventive measure is keeping them separate different categories of animals. Preferably avoid contaminated pastures and considering the longevity of rain worm (live 2-7 years) it is also difficult to achieve and to avoid mixing of wild and domestic pigs as the peak even harder. The most effective preventive dehelmintization are autumn that is performed 3-4 weeks after withdrawal from the pasture and spring with the expulsion of the stall. From anthelmints that interfere with neuromuscular coordination using cholinesterase inhibitors (organophosphate - coumaphos, dichlorovos, halokson, naphtalphos and trichlorophen), cholinergic antagonists (imidazole-levamisole, tetrmisol, pyrimidine-morantel and pyrantel) and antagonists of mediators GABA (ivermectin, cyadectin, doramectin). We must treated all animals in infected heards (Pavlović et al.2002,2004).

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PARAZITSKA FAUNA SVINJA U ORGANSKOM DRŽANJU

IVAN PAVLOVIĆ, BOŽIDAR SAVIĆ, DRAGAN ROGOŽARSKI,
JOVAN BOJKOVSKI, VOJIN IVETIĆ, OLIVER RADNOVIĆ,
MILENKO ŽUTIĆ, SLAVONKA STOKIĆ-NIKOLIĆ,
NEMANJA JEZDIMIROVIĆ, ĐORĐE CVETOJEVIĆ

Izvod

Parazitske infekcije predstavljaju stalne pratioce svinjarske proizvodnje, nezavisno od načina držanja. Organsak proizvodnja i držanje uslovljavaju da svinje imaju direktan dodir sa mnoštvom prelaznih domaćina parazita a samim tim da su kod njih parazitske infekcije češće. U najvećoj meri ovde se javljaju biohelminti od kojih su najvažniji predstavnici roda *Metastrongylus*, uzročnici verminoznog gastrita (*Ascarops strongyllinae, Physocephalus sexalatus* and *Hyostrongylus rubidus*), *Oesophagostomum spp.* i *Macracanthorhynchus hirudinaceus.* Ovde se takođe javljaju i *Acaris suum,* i drugi helminti u manjem obimu.

Ključne reči: biohelimnti, svinje, ekstenzivno držanje.

Received / *Primljen*: 20.04.2013.\
Accepted / *Prihvaćen*: 17.05.2013.

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Tromesečno. – Engleski, izvodi na srpskom jeziku. – Raniji naziv publikacije: Poljoprivreda Vojvodine

ISSN 0350-1205

COBISS.SR-ID 256386