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THE MAIN INDICATORS OF BIOSECURITY AND PRESENCE OF HOUSE MOUSE (*Mus musculus* L.) IN ANIMAL HUSBANDRY FACILITIES

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Abstract: Analysis of biosecurity indicators at critical control points intend to prevent undesirable infections in technological chains of production. Product quality is the basis for defining a biosecurity plan under the HACCP concept. General and specific biosecurity measures developed to prevent introductions of infective materials have been at the focus of attention in Serbia in recent years. The house mouse (*Mus musculus* L.) is usually accused for transferring pathogens into objects. The possibility of internal infections can be reduced by removing food sources and discovering their hiding places. The adaptability of *Mus musculus* to various conditions has affected the search for alternatives of their control. The objective of our research was to analyse the most important indicators of biosecurity and presence of *Mus musculus,* the 'cause-and-consequence' characteristics and mice control by environmentally safe substances in facilities with different technological processes. Method of questionnaire was used to define written biosecurity plan, isolation of objects, control of movement and for traffic visitors. Hygiene evaluation, i.e. mechanized cleaning, sanitary washing, facility disinfection, ventilation and facility sanitation, was performed visually. The biosecurity and wellbeing of animals were evaluated by the parameters: animal hygienic conditions of rearing, forage stocks, animal biosecurity and removal of animal carcasses. Longworth traps were used for mice trapping and determination of critical control points. The efficacy of sodium selenite was found in our study to range from 71.4% to 88.8% and it provided a good alternative for *Mus musculus* control in different production units because it does not interfere with technological production processes within facilities or cause animal resistance. Biosecurity measures need to be implemented using clear instructions in order to reduce biorisks and increase product safety.

Key words: HACCP**,** biosecurity indicators, efficacy, environmentally safe substances, *Mus musculus*

Introduction

The use of biosecurity plans *(Pritchard et al., 2005)* raises the level of biological safety of food, quality and volume of production and prevents unwanted situations *(Uhlenhoop, 2007; Bojkovski et al., 2010)*. It is not possible to protect all production elements (*Pinto and Urcelay, 2003*), those exposed to the highest risk should be considered first at critical points (*Anon. 2006*). Biosecurity and rodent control basically mean the prevention of pathogens from penetrating facilities from their external sources *(Klimpel et al., 2007; Fuehrer et al., 2012)*. The use of protective chemicals requires additional control and quality attestation to confirm that food had been produced under HACCP principles *(Pešić-Mikulec and Jovanović, 2003)*. The control of *Mus musculus* using environmentally safe ingredients increases the safety and quality of products when four biorisk stages have been covered (i.e. identification, characterization, exposure, monitoring and database).

There is an increasing need to evaluate the indicators of hygienic conditions *(Hristov et al., 2009)* and presence of *Mus musculus* in various facilities. Due to rodents' fast adaptation to various habitats and grave consequence that they may bring, indicators of their presence (active holes, feces and odour) should be closely monitored throughout the year *(Čamprag, 1983; Ružić, 1983)*. Favourable conditions in production facilities may rapidly increase their populations.

Control measures are normally used when rodents become abundant and damage considerable *(Đedović et al., 2012; Vukša et al., 2012)*. Frequent application of anticoagulants causes detrimental effects and triggers resistance to them *(Jokić et al., 2013)*. Environmentally safe products make a good alternative and rodent mortality can be achieved with one-off treatment.

Our research indicated a considerable significance of biosecurity plans, based on clearly defined indicators of hygienic conditions and presence of *Mus musculus,* and alternative environmentally safe substances for preserving product safety and quality.

Materials and Methods

Localities

Experiments were conducted in 6 agricultural facilities that use different production technologies (marked A, B, C, D, E, F). The facility A and B are storehouses for agricultural products, C and E include two production units (pig farms and storehouses), while D and F incorporate cattle farms and storehouses.

Manufacturers

The active ingredient sodium selenite was manufactured by Alfa Aesar A Jonson Matthey, Paris, France. The product Ekocel C was manufactured by Ciklonizacija, Novi Sad, Serbia. The active ingredient cellulose was manufactured by Natrocell Tecnologies Ltd., Great Britain. The product Natromouse was made by Pinus Plus d.o.o., Slovenia.

Methods

Several methods *(Sundrum et al.,1994; Bartussek et al., 2000; Bracke et al., 2001; Blockuis, 2008)* adapted to domestic conditions, were used for defining and evaluating the parameters and indicators of animal wellbeing and biosecurity. The evaluation scale used in this study *(Hristov and Reljić 2009, Hristov et al, 2009, Hristov and Stanković 2009)* included the following ratings: 5 - excellent (4.50-5.00), 4 - very good (3.50-4.49), 3 - good (2.50-3.49), 2 - satisfactory (2.00-2.49) and 1 - insufficient (0-1.99). *SWOT* analysis was applied to derive complete data for isolation from the biosecurity aspect: S-strength, W-weakness, O-opportunity, T-treatment.

The number of active holes, feces and special odour were indicators of the presence of *Mus musculus* in the facilities and these indicators were evaluated on a scale: weak, medium, strong *(Čamprag, 1983; Ružić, 1983)*.

The mice were trapped using Longworth traps during 400 nights per locality in order to locate critical points.

Experimental methods. Trials were set up according to the method PP 1/114(2) *(EPPO, 2004)*. Baits were laid in boxes for mice in portions of 10 g at 1-2 m distance. Each box was labelled with an ordinal number and product name. According to HACCP standard, a duplicate label was put up also on the wall above each box to be clearly visible and carrying a warning sign *(Bokelman, 1996)*.

The amount of bait eaten was measured daily for the duration of 15 days and fresh baits were laid daily. The abundance of rodents was determined based on the total amount of bait eaten and the ratio of the lowest and highest amounts of eaten bait per day, devided by the daily requirement of mice. Product efficacy was calculated according to Abbott's formula *(Abbott, 1925)*.

Results and Discussion

Table 1 shows the ratings for evaluation biosecurity indicators in 6 facilities considering the existance of formulated plans; the ratings were insufficient in 3 facilities, i.e. C, E and F (1.00) and good in the objects A, B and D (3.00, 3.30 and 2.75, respectively).

Table 1. Evaluation of biosecurity indicators in facilities of different types and capacities

	Evaluation of biosecurity plans*					
Indicators	**A**	**B**	**C**	**D**	**E**	**F**
Facility size (m^2)	800	1000	1200	1700	800	1200
Written biosecurity plan	3.00	3.30	1.00	2.75	1.00	1.00
Isolation of entire facility and its individual production units	3.50	3.80	1.15	2.30	1.10	1.75
Traffic control	3.20	4.00	1.80	3.00	1.20	1.50
Spatial conditions of rearing	-	-	1.00	2.90	1.00	1.75
Animal hygienic conditions of rearing	-	-	1.00	2.60	0.50	1.25
Forage stocks	-	-	0.90	2.60	0.45	0.50
Animal biosecurity	-	-	0.70	2.10	0.40	1.20
Removal of animal carcasses	-	-	1.00	2.50	1.50	2.00
Mechanized cleaning	3.20	3.80	1.20	2.80	1.30	2.50
Sanitary washing	3.40	3.60	1.30	2.30	1.45	2.50
Facility disinfection	3.00	3.60	1.00	1.30	1.00	2.00
Ventilation	3.00	3.00	1.00	2.00	1.20	2.30
Control of rodent populations	2.80	3.20	0.50	1.50	0.70	1.30
Facility sanitation	1.99	4.00	1.50	2.10	1.20	2.30
Average rating per facility	3.01	3.58	1.07	2.41	1.00	1.70
Total rating			2.13			

* Facility interior parameters evaluated

The average indicator ratings were negative in the facilities C and E (1.07 and 1.00, respectively). The surrounding topography on their localities supported rodent intrusion and the number of trapped animals was 95 and 28 (Table 3). Rodent population control also received negative ratings (0.50 and 0.70), which affects various technological processes and the quality of products.

The facility B was the only one given the rating 'very good' for traffic control (4.00) and for hygienic procedures in object (3.80, 3.60, 3.60). Traffic control received better ratings in the facilities A and D (3.20 and 3.00) than C, E and F (1.80, 1.20 and 1.50). Even though visits were limited and entry was not allowed into some critical areas (A and D), a lack of strict and consistent regime was a serious problem. An even greater problem, however, was the dysfunctioning state of disinfection barriers and points, and dress change upon entry.

Animal hygienic conditions of rearing were given the rating 'insufficient' in the facilities C, E and F (1.00, 0.50 and 1.25, respectively), and 'good' in the facility D (2.60). Forage stocks and animal biosecurity received negative ratings on the farms C, E and F (0.90 and 0.70, 0.45 and 0.40, and 0.50 and 1.20,

respectively), while forage stocks were 'good' on the farm D (2.60) and its animal biosecurity 'satisfactory' (2.10).

Hygiene within facility – mechanized cleaning, sanitary washing and disinfection - were very good in the facility B (3.66), good in A (3.20), satisfactory in D and F (2.13 and 2.33, respectively) and insufficient in C and E (1.16 and 1.15, respectively). Cleaning and washing are not necessarily thorough, and a good waste management is central *(Gibbens et al., 2005)* for providing good hygiene, which makes disinfectants more effective in reducing rodent populations (Table 3, facilities A and B, 2 and 5 animals, respectively). Sanitary procedures have been frequently disregarded, especially on the pig farms C and E.

Regular sanitation leads to success, as in the facility B (4.00). In all other facilities, the ratings were insufficient or satisfactory, which increase the possibility of introducing infective materials *(Stanković et al., 2011)*. Sanitation was the most important biosecurity measure for Spanish farmers in a similar study *(Casal et al., 2007)*, while all other indicators received mostly moderate ratings.

The total average rating of indicators was satisfactory (2.13), while facility isolation from potential sources of infection was an important protective measure *(Stanković and Hristov, 2009)*.

Table 2. **Active ingredients and their properties in control of *Mus musculus***

	Active ingredient	
	Sodium selenite	Cellulose
Molecular formula a.i.	$(C_6H_{10}O_5)n$	Na_2O_3Se
(%) a. i. in bait	0.1	45
Mode of activity	exchange of S-H group of functional cells with S-S bonds	metabolic disorder (water retention)
Mortality time	4-8 days	8-10 days
Bait acceptibility	good	weak
Bait alatability	weak	weak
Human hazard	weak	weak
Animal hazard	weak	weak
Control of *Mus musculus*	good	weak

The most important characteristics of these environmentally safe products and their active ingredients are given in Table 2, and it shows their different modes of activity and mortality times. Neither product poses a risk to humans or animals and they can be applied repeatedly over the year. Differences were evidenced in bait acceptibility and sodium selenite was better in that respect in all examined facilities. Palatability should be improved, particularly of the cellulose product, in order to significantly enhance bait uptake.

Table 3. The number of *Mus musculus* animals trapped, indicators of their presence and efficacy of active ingredient in six facilities

Facility	*Mus musculus*		Efikasnost (%)	
	No. of trapped animals	Indicator of presence*	Sodium selenite	Cellulose
A	2	*	83.3	•
B	5	*	81.8	•
C	95	***	88.8	70.6
D	11	**	81.1	66.7
E	28	***	71.4	60.6
F	30	***	75.0	•

* Classification of parameters (*Čamprag, 1983; Ružić, 1983*): weak*, medium** high***
• Active ingredient efficacy below 20 %.

Consistent implementation of the HACCP standard and formulated biosecurity plans were preconditions for having a low abundance of rodents (2 and 5 animals) and product efficacy of 83.3% and 81.8% in the facilities A and B. The facility B required repairs of its loft and closure of holes in walls, which should reduce mice numbers. The cellulose product had an efficacy below 20% in the facilities A, B and F.

Both products showed the highest effectiveness in the facility C (88.8% and 70.6%), and the high numbers of trapped animals and parameters of their presence indicated that control of *Mus musculus* had not been practiced for a long time. Active holes in walls inside and outside facility revealed the IV and V categories of presence (*Ružić, 1983*).

The facility D had good physical barriers. The trapped rodents (11) were caught at critical points near entrances. The indicators of rodent presence were estimated as medium. The efficacy of sodium selenite was 81.1%, while cellulose was weaker 66.7%.

In the facilities E and F, the indicators of presence of *Mus musculus* were high, the number of animals trapped was 28 and 30, respectively, with a growing tendency due to favourable conditions for their hiding and reproduction. Alternative food sources were available. The efficacy of sodium selenite in those facilities was 71.4% and 75.0%, respectively, while cellulose had 60.6% efficacy in the facility E, which requires a general repairs and regular preventive measures. The object F, farm of Holstein Friesian cattle breed with tether housing system required thorough cleaning of pads from mud deposits. Places for silage were not properly isolated and interior temperature was appropriate for reproduction of *Mus musculus*.

Conclusion

The present data demonstrate the biosecurity status of facilities.

The following conclusions may be inferred from the presented data:

- The objective of introducing a biosecurity plan in an agricultural production facility is to raise the level of biological security of food, its quality and production volume. The average rating of good (3.01) and very good (3.58) in the facilities A and B are indicative of well-implemented HACCP measures. The farms C, D, E and F received lower ratings and need to improve their animal biosecurity.
- The responsibility for production processes lies with the facility staff and they should especially rely on clearly formulated instructions and databases that are able to predict certain risks.
- Presence indicators of *Mus musculus* should follow over the year, especially in those objects with outdated constructions.
- All potential threats should be evidenced and adequate protection formulated. Different qualities of production require different levels of protection and corresponding control.
- The abundance of *Mus musculus* and critical control points should be determined by trapping. Environmentally safe products should be given preference as they have no effect on various segments of the environment and rodent resistance. Palatability should be improved, especially of the cellulose-based product.

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Najznačajniji indikatori biosigurnosti i prisustva domaćeg miša (*Mus musculus* l.) u objektima stočarske proizvodnje

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Rezime

Analiza indikatora biosigurnosti na kritičnim kontrolnim tačkama je namenjena sprečavanju neželjenih infekcija u tehnološkom lancu proizvodnje. Kvalitet proizvoda je osnov definisanja plana biosigurnosti po HACCP konceptu. Opšte i posebne mere biosigurnosti kojim se sprečava unošenje infektivnog materijala su teme prezentacija poslednjih godina u našoj zemlji. Za prenosioce patogena u objekte često se smatra domaći miš (*Mus musculus* L.). Uklanjanjem izvora hrane i otkrivanjem skloništa smanjiće se mogućnost infekcija unutar objekta. Sposobnost prilagođavanja različitim uslovima uticala je na pronalaženje alternativa kontrole *Mus musculus*. Cilj naših istraživanja su analize najznačajnijih indikatora biosigurnosti i prisustva *Mus musculus,* uzročno-posledične karakteristike i kontrola ekološkim materijama u objektima različitih tehnoloških procesa proizvodnje. Postavke pisanog plana biosigurnosti, izolacija, kontrola kretanja i promet posetioca utvrđivana je metodom upitnika. Ocene higijenskih uslova: mehaničko čišćenje, sanitarno pranje, dezinfekcija objekta, ventilacija i sanitacija objekta prikazane su vizuelnom metodom. Metodama biosigurnosti i dobrobiti životinja ocenjeni su parametri: higijenski uslovi odgoja životinja, zaliha hrane za životinje, biosigurnost životinja, uklanjanje uginulih leševa.Izlovljavanje jedinki *Mus musculus* i utvrđivanje kritičnih kontrolnih tačaka vršeno je klopkama tipa Longworth. .

Istraživanjima smo utvrdili da je efikasnost preparata na bazi natrijum selenita od 70,6% do 100% i da je dobra alternativa u kontroli *Mus musculus* u objektima različitih proizvodnih jedinica, jer ne ostavlja posledice na tehnološke procese proizvodnje i pojavu rezistentnosti. Potrebno je sprovoditi biosigurnosne mere po jasno definisanim uputstvima kako bi se smanjio biorizik i povećala bezbednost proizvoda.

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