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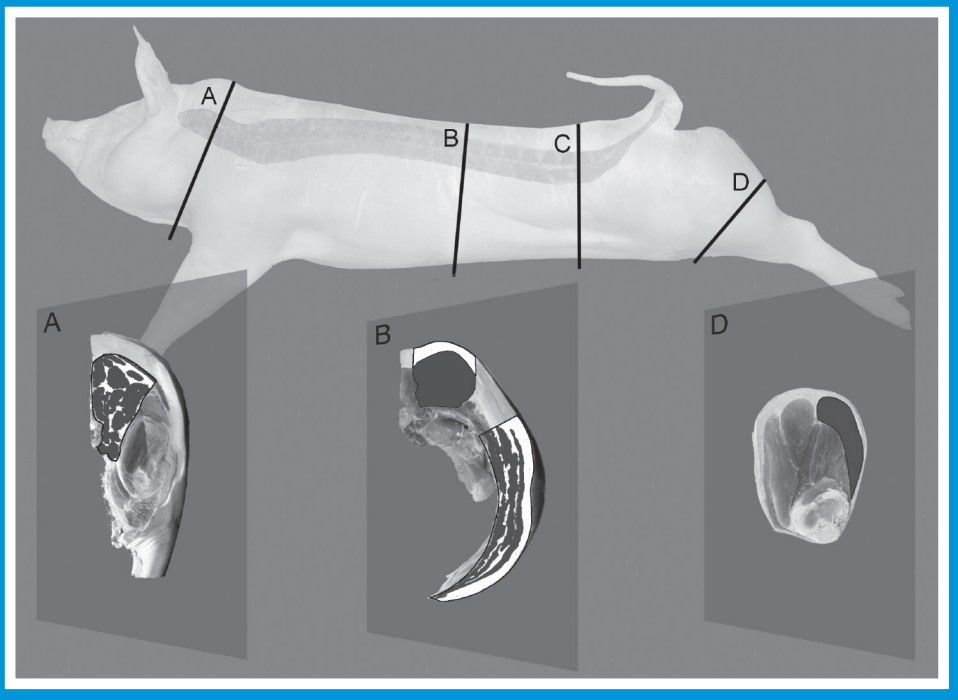

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Review Article

OUTBREAKS OF BLACKLEG OF CATTLE IN NORTHERN NIGERIA (1964-2003)

Nicodemus Maashin Useh[1]*, Sani Adamu, Najume Ibrahim[1], Andrew Jonathan Nok[2], King Akpofure Nelson Esievo[1]

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Summary: Blackleg is an endemic disease in both developed and developing countries and is a well-known cause of financial loss to cattle raisers in many parts of the world. The disease is prevalent in the United States of America, India and other parts of Asia and Europe, Latin America, and Africa. In Nigeria, the economic losses of Zebu cattle alone to the disease have been estimated at US $4. 3 million (~600 million naira) annually. A retrospective study was carried out to ascertain the current status of the disease in Northern Nigeria, and it was observed that the traditional style of livestock production by the transhumance Fulani pastoralists of rural Nigeria, who own livestock resources in the country pose a great challenge to the control of the disease. It was concluded that blackleg is still endemic in Nigeria, and its effective control can only be achieved if the traditional livestock production system of the nomads of rural Nigeria is improved.

Key words: blackleg; cattle; Northern Nigeria; outbreaks; retrospective study

Introduction

Blackleg is a disease affecting cattle, sheep and other ruminants, caused by *Clostridium chauvoei* and was first reported in 1870 (1). In Nigeria, the disease was first reported in 1929 and has remained a major problem of cattle in the country (2). Although vaccination against the disease has been carried out in Nigeria since 1930, many sporadic outbreaks have been recorded annually (3).

Blackleg is endemic in both developed and developing countries and is a well-known cause of financial loss to cattle raisers in many parts of the world (4). The economic losses of ruminants to the disease have not been quantified in most parts of the world, but in Nigeria, the losses of Zebu cattle alone to the disease have been estimated at US $4.3 million annually (~600 million naira) (5). In the United States of America, Latin America, India and other parts of Asia and Europe, the economic losses of ruminants to blackleg have not been estimated, but it has been reported that the disease causes major economic losses in cattle and minor losses in sheep (4,6-7). High annual rainfall has been associated with increased outbreaks of the disease in ruminants (3,8). In this study, we present outbreaks of the disease for about 40 years (1964-2003) and the possible ways of effecting efficient control of the disease in Nigeria.

Materials and methods

A 40 year retrospective data on annual outbreaks of blackleg (1964-2003) was collected from the Federal Ministry of Agriculture and Natural Resources Archives at Kaduna and Abuja, Nigeria and the National Veterinary Research Institute (NVRI), Vom, Nigeria. Similar data of annual outbreaks of the disease in 12 states of Northern Nigeria was also collected from the Veterinary Services Division of these states. The data was recorded according to the number of outbreaks (N. O.), number of cattle in herds infected (N. C. I.) and mortality (M).

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Results

The outbreaks of blackleg of cattle in Northern Nigeria are presented in Tables 1-3. Outbreaks were low in some years and states and high in others. During many of the years investigated, there were few records of outbreaks of all diseases including blackleg.

Table 1: Blackleg outbreaks in the provinces of Northern Nigeria (1964-1970)

Year		Provinces												
		Kano	Sokoto	Katsina	Borno	Sardauna	Bauchi	Zaria	Adamawa	Plateau	Niger	Benue	Kabba	Ilorin
1964	N.O.	4			14	24	19	2	22	6	4	31		1
	N.C.I.	230			2409	-	1452	132	3353	1020	741			12
	M	65			81	50	39	2	164	15	13			1
1965	N.O.	1			17	12	38	1	12	9	3			3
	N.C.I.	70			3298	1087	7126	144	2611	1632	698			573
	M	6			198	38	262	1	105	33	3			33
1966	N.O.	8	3	1	38	1	49	10	16	11	15	1		
	N.C.I.	945	414		3444	30	5502	1785	4300	1923	5127	300		
	M	44	18		181		241	73	159	37	194	10		
1967	N.O.	4	3		12	7	36	2	17	9	9	3		
	N.C.I.	523	186		1824	2430	3995	390	2888	1140	1558	484		
	M	20	5		50	39	109	14	105	23	49	24		
1968	N.O.	12	1	5	11	12	20	12	3	25	8	24		
	N.C.I.	1230	502	315	1445	2031	1214	2734	716	11284	817			
	M	66	35	22	43	72	62	53	26	124	39	6		
1969	N.O.	13		2	1	8	28	17	2	16	8	9		
	N.C.I.			26	8	7655	3254	3878	314	456	2554	498		
	M	35		7	1	60	98	240	14	100	109	50		
1970	N.O.				9	13	28	56	10	14		2		
	N.C.I.				1722	-	2756	11388	1138	1391				
	M				79	42	107	46	77	85		11		

N.O. → Number of outbreaks
N.C.I. → Number of cattle in the herds infected
M → Mortality (Number of dead animals)

Table 2: Blackleg outbreaks in 5 states of Northern Nigeria (1971-1975)

Year		North Eastern state	North Western state	Benue Plateau state	North Central state	Kwara state
1971	N.O.	11	21	26	80	1
	N.C.I.	2460	4180	468	1375	280
	M	9	216	17	187	12
1972	N.O.	18	10	12	24	
	N.C.I.	3490	5419	620	4089	
	M	120	42	15	120	
1973	N.O.	8	1	3	16	1
	N.C.I.	214	200	418	2184	412
	M	22	11	19	34	1
1974	N.O.	22	12	10	20	
	N.C.I.	5180	4112	384	4010	
	M	18	216	11	218	
1975	N.O.	1	12	6	27	
	N.C.I.	211	4020	2116	4124	
	M	84	118	25	296	

Table 3: Outbreaks of blackleg in 11 states of Northern Nigeria (1976-2003)

Year		Gongola	Benue	Plateau	Bauchi	Niger	Kaduna	Kano	Sokoto	Borno	Gombe	Taraba
1976	N.O.		2									
	N.C.I.		149									
	M		4									
1977	N.O.											
	N.C.I.											
	M											
1978	N.O.			1		1			1			
	N.C.I.			170		63			1			
	M			2		-						
1979	N.O.			4	1	2	7			1		
	N.C.I.			168	40	144	206			144		
	M			3	2	8	23			1		
1980	N.O.			2		5						
	N.C.I.			1080		153						
	M			75		11						
1981	N.O.			1		1						
	N.C.I.			1000		74						
	M			20		13						
1982	N.O.											
	N.C.I.											
	M											
1983	N.O.			1		4						
	N.C.I.			66		231						
	M			2		17						
1984	N.O.			1		1	1					
	N.C.I.			44		132	96					
	M			1		6	4					
1985	N.O.			2	1	5	4					
	N.C.I.			58	20	320	506					
	M			2	1	22	34					
1986	N.O.			2	3	2						
	N.C.I.			60	154	76						
	M			2	3	4						
1987	N.O.			2			1					
	N.C.I.			78			1					
	M			3			1					
1988	N.O.			3	4			3				
	N.C.I.			98	211			259				
	M			2	5			17				
1989	N.O.			4	4	3		2				
	N.C.I.			100	667	210		63				
	M			3	5	13		10				
1990	N.O.			1	1		2					
	N.C.I.			36	31		80					
	M			2	2		13					
1991	N.O.	3		1		1		2				
	N.C.I.	1092		46		75		176				
	M	48		3		4		15				

(Tab. 3, continuation)

1992	N.O.			1		3						
	N.C.I.			20		59						
	M			5		2						
1993	N.O.			1		1		1				
	N.C.I.			32		70		27				
	M			3		3		5				
1994	N.O.			1		4	18			56		
	N.C.I.			38		127	450			1580		
	M			2		5	29			85		
1995	N.O.			1	3	3			3			151
	N.C.I.			44	376	83			1999			742
	M			1	12	4			18			55
1996	N.O.			1	2	2	2				1	
	N.C.I.			40	55	50	205				162	
	M			2	5	3	25				42	
1997	N.O.			1	2						4	
	N.C.I.			56	88						102	
	M			2	4						2	
1998	N.O.		19	1	4						7	
	N.C.I.		842	68	456						1179	
	M		12	2	26						33	
1999	N.O.		48	1			3				2	
	N.C.I.		680	51			1840				11	
	M		23	1			64				5	
2000	N.O.		10	1		1					1	
	N.C.I.		728	28		130					55	
	M		20	3		4					3	
2001	N.O.			1	6							18
	N.C.I.			34	108							652
	M			1	5							20
2002	N. O.			1				1				
	N.C.I.			56				180				
	M			2				3				
2003	N. O.											50
	N.C.I.											1870
	M											58

Discussion

The results of this study suggest that there are still pockets of annual outbreaks of blackleg of cattle in Northern Nigeria. The Fulani pastoralists of rural Nigeria are the custodians of livestock resources in Nigeria, with about 70-80% ownership of livestock production in the country (9). Some states investigated in the study had few blackleg outbreaks, because of poor record keeping. It is therefore possible to insinuate that outbreaks of the disease may have occurred in those states during the period investigated, but were not recorded.

In Nigeria, the control of blackleg has been difficult because of ineffective vaccination policy, lack of adequate logistics such as vehicles to carry out vaccination activities, lack of facilities to maintain the cold chain for vaccine storage (hence inadequate potent vaccines) and lack of disease reporting by the nomads (3). Livestock disease control in Nigeria is the responsibility of government and therefore, if reports of outbreaks are not made to the authorities,

it is difficult for government to know the status of blackleg in a given locality. Unfortunately, in the face of outbreak or rumour of one, the nomads move away in mass, from the so called danger areas, and even those of them whose cattle are infected move away, thereby serving as a source of infection to other supposedly healthy herds (3). The nomadic Fulani pastoralists prefer to use herbal remedies to treat diseases of their livestock, including blackleg (10), and only report disease if their herbal preparations do not provide the desired therapeutic results. In most cases, disease outbreaks are reported only if there is uncontrollable cattle mortality. The present study on the outbreaks of blackleg of cattle in Northern Nigeria is important because, in Africa, there is migration of cattle between neighbouring countries, and a disease that is endemic in one country is a potential danger to neighbouring countries. Other authors (3) suggested that to achieve effective control of blackleg in Nigeria and the rest of Africa, governments of the sister African states must intensify awareness campaigns among the nomads, through the Nomadic Education Commissions of the respective countries. It is concluded that blackleg is still endemic in Nigeria, and its effective control can only be achieved if the traditional style of livestock keeping by Nomadic Fulani pastoralists who own most of Nigeria's livestock resources is improved. This is possible if they are settled and encouraged by government to reduce transhumance, with adequate support and provision of modern livestock rearing facilities.

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IZBRUHI ŠUMEČEGA PRISADA (GANGRENA EMPHYSEMATOSA) PRI GOVEDU V SEVERNI NIGERIJI (1964 - 2003)

N. M. Useh, S. Adamu, N. Ibrahim, A. J. Nok, K. A. N. Esievo

Povzetek: Šumeči prisad je endemična bolezen v razvitih ter državah v razvoju in je dobro poznan vzrok finančnih izgub pri rejcih goveda v več delih sveta. Bolezen je prevladujoča v Združenih državah Amerike, Indiji in drugih delih Azije, v Evropi, Latinski Ameriki in Afriki. V Nigeriji so bile ekonomske izgube pri govedu Zebu ocenjene na 4,3 miljona dolarjev (~600 milijonov nigerijskih niarov). Z retrospektivno študijo smo želeli potrditi trenuten status bolezni v severni Nigeriji in ugotovili, da je tradicionalni način govedoreje s sezonskim potovanjem čred pastirjev Fulani v ruralnih delih Nigerije, ki premorejo večino živine v državi, velik izziv za kontrolo bolezni. Šumeči prisad je v Nigeriji še vedno endemična bolezen. Učinkovito kontrolo pa bo mogoče doseči le z izboljšanjem tradicionalne reje govedi nomadov na podeželskih področjih Nigerije.

Ključne beside: šumeči prisad; Severna Nigerija; izbruhi; retrospektivna študija

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Original Scientific Article

DETECTION OF *SALMONELLA* IN POULTRY FAECES BY MOLECULAR MEANS IN COMPARISON TO TRADITIONAL BACTERIOLOGICAL METHODS

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Summary: Comparison of traditional (cultivation-dependent) and molecular (nucleic acid-based) bacteriological methods was performed to detect *Salmonella* in reference capsules containing quantified amounts of *Salmonella enterica* subsp. *enterica* serovars Panama, Typhimurium or Enteritidis and in poultry faeces that was naturally contaminated with *Salmonella* or *Salmonella*-negative but spiked with reference materials. Traditional techniques were performed according to ISO 6579 using different enrichment (MSRV, MKTTn and RVS, respectively) and isolation plating media (XLD, BGA and Rambach agar, respectively). Molecular detection was preceded by the pre-enrichment step. Detection efficiency of two DNA isolation kits, namely High Pure foodproof I Kit (Roche Diagnostics, Germany) and QIAamp DNA Stool Mini Kit (Qiagen, Germany), in combination with classical and real-time PCR assay was compared. Results showed that traditional and molecular detection of *Salmonella* was unambiguous for reference control capsules, but was hindered for faecal samples. RVS medium was less appropriate than MSRV and MKTTn. Combination of MKTTn with Rambach agar plates generated the highest number of positive results with traditional approach. However, recommendation of using the semi-solid MSRV medium was confirmed as it enabled detection of *Salmonella* in high proportion of samples, which was the least variable depending on the selection of isolation plating media. In contrast to culture-based methods, the molecular approach, especially a combination of High Pure foodproof I Kit and real-time PCR assay, enabled successful detection in all *Salmonella*-positive samples and should therefore be considered an important supplement to traditional protocol for *Salmonella* detection in foodstuffs.

Key words: *Salmonella*; foodstuffs; faeces; detection; cultivation; molecular; PCR

Introduction

The routine microbiology laboratories for detection of different bacterial pathogens are complementing traditional diagnostic assays with continually evolving molecular techniques as they are not negatively affected by the presence of growth inhibitory compounds and enable rapid detection (1-3). Surveillance of alimentary zoonoses, diseases that are transmitted from animals to humans through food, and early detection of their causative agents in food producing animals and their environment are very important for the assurance of safe food. Food safety is a growing public health issue, since it was estimated that up to 30 % of the population in industrialized countries is suffering from foodborne illnesses (4). Salmonelloses are the second most frequently reported human zoonoses in the European Union and can cause relatively vast economic damage due to chronic effects of the infections (5). The common reservoir of *Salmonella* is the intestinal tract of animals, however they can be detected in a wide variety of foodstuffs and food ingredients (5). Animal-to-human transmission occurs when bacteria are introduced into the food preparation process or through direct contact with infected animals and faecally contaminated environments.

In-country laboratory-based monitoring of foodborne pathogens is being promoted (4). Traditional microbiological methods offer standardized proce-

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dures for their detection (*e.g.* ISO standards), but are time consuming and not always compatible with short-time-to-result demand. Therefore, food microbiology aims for supplementation of classical methods with molecular techniques based on detection of microbial nucleic acids in foodstuffs, which shorten the analysis time and lower the limit of detection. It was shown previously that the polymerase chain reaction (PCR) has a great potential to speed-up the detection of *Salmonella* in food (6) and can be performed in a manner equivalent to the standard ISO 6579 culture method, which is set as the golden standard for *Salmonella* detection in food and feedstuffs (7,8).

The main objective of our study was to evaluate the detection efficiency for different contamination levels of *Salmonella* spp. in the presence of competitive microorganisms. As a complex matrix, poultry faeces was selected for the starting material. The use of molecular methods polymerase chain reaction (PCR) and real-time PCR was compared to traditional, cultivation-dependent bacteriological methods.

Materials and methods

Reference materials (RMs) and poultry faeces were used. The RMs consisted of gelatin capsules containing a quantified amount of sub-lethally injured *Salmonella* strains of serovars Panama (SPan), Typhimurium (STM) or Enteritidis (SE) as spiked spray dried milk prepared by the Community Reference Laboratory (CRL) for *Salmonella* (9). The levels of contamination were SPan 5 (5 colony forming particles per capsule [cfp/caps]), STM 10 (10 cfp/caps), STM 100 (100 cfp/caps), SE 100 (100 cfp/caps) and SE 500 (500 cfp/caps). Faeces, negative or positive for *Salmonella* spp., and reference capsules were stored at –20° C till use. We examined (*i*) 24 poultry faecal samples (numbered FC-1 to FC-24; 10 g each, negative for *Salmonella* spp.) in combination with a blank capsule (five samples) or a capsule containing STM (five samples STM 10 and four samples STM 100) or SE (five samples SE 100 and five samples SE 500), (*ii*) 20 faecal samples which were naturally contaminated with *Salmonella* and not spiked with capsules (numbered F-1 to F-20; 10 g each), and (*iii*) 10 control samples (numbered C-1 to C-10; no faeces added) consisting of two blank, two SPan 5, three STM 10 and three SE 100 capsules, respectively. In addition, two negative control samples were examined: procedure control (*i.e.* C-11; no faeces or capsule added) and negative faeces control (*i.e.* C-12; 10 g of faeces negative for *Salmonella* spp.). The sample outline is summarized in Table 1.

Traditional bacteriological methods

Cultivation-dependent detection of *Salmonella* was performed according to ISO 6579:2002 (10), including Amendment 1:2007 (11), and the instructions provided by CRL for *Salmonella* (9). In brief, detection involved the following stages: (*i*) overnight sample pre-enrichment in a nonselective broth medium BPW (Buffered Peptone Water), (*ii*) 24- (for the first isolation) and 48-hour (for the second isolation) enrichment in selective broth media MKTTn (Muller Kauffmann TetraThionate-novobiocin broth), RVS (Rappaport Vassiliadis medium with Soya) and MSRV (Modified Semi-solid Rappaport Vassiliadis medium; 11), (*iii*) isolation of colonies presumed to be *Salmonellae* on solid selective and differential plating media BGA (phenol red/Brilliant Green Agar), XLD (Xylose-Lysine-Deoxycholate agar) and R (Rambach agar; 12), and (*iv*) biochemical screening of *Salmonella* isolates on the confirmation media TSI (Triple Sugar/Iron agar), UA (Urea Agar) and LDC (1-Lysine decarboxylation medium). If colonies grown on the isolation media were not well separated, single colony isolation was performed on NA (Nutrient Agar) plates after 24-hour incubation at 37° C and followed by the aforementioned confirmation. For each of the samples from the three selective enrichment media, at least one individual colony, considered to be typical or suspect for *Salmonella*, was examined biochemically. If the selected colonies were not confirmed as *Salmonella*, maximum of five additional typical colonies were tested from the original isolation medium stored at 5° C. Sample was denoted with positive result if growth of *Salmonella* spp. was present at least on one of the isolation media. If not stated otherwise, media and reagents were prepared according to Annex B of ISO 6579:2002.

Molecular methods

Molecular detection of *Salmonella* involved the isolation of microbial DNA that was followed by *Salmonella*-specific PCR and real-time PCR assays. DNA was extracted from 1 mL of the pre-enrichment broths using two different commercial kits, namely the High Pure foodproof I Kit (Roche Diagnostics, Germany) and QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturers' instructions. The latter was not applied for samples devoid of faeces. Microbial DNA was subjected to PCR amplification using *Salmonella* genus-specific primers ST11 and ST15 (13) that were proved as appropriate

Table 1: Outline of the samples used for the study: control samples (C), naturally contaminated faecal samples (F), and faecal samples supplemented with capsules (FC).

Sample name	Faeces	Capsule	Sample name	Faeces	Capsule	Sample name	Faeces	Capsule
C-1	/	blank	F-1	pos	/	FC-1	neg	SE 100
C-2	/	SPan 5	F-2	pos	/	FC-2	neg	blank
C-3	/	blank	F-3	pos	/	FC-3	neg	STM 100
C-4	/	SE 100	F-4	pos	/	FC-4	neg	SE 100
C-5	/	STM 10	F-5	pos	/	FC-5	neg	STM 100
C-6	/	STM 10	F-6	pos	/	FC-6	neg	SE 500
C-7	/	STM 10	F-7	pos	/	FC-7	neg	SE 500
C-8	/	SE 100	F-8	pos	/	FC-8	neg	blank
C-9	/	SE 100	F-9	pos	/	FC-9	neg	SE 500
C-10	/	SPan 5	F-10	pos	/	FC-10	neg	SE 100
C-11	/	/	F-11	pos	/	FC-11	neg	STM 10
C-12	neg	/	F-12	pos	/	FC-12	neg	blank
			F-13	pos	/	FC-13	neg	SE 100
			F-14	pos	/	FC-14	neg	STM 10
			F-15	pos	/	FC-15	neg	STM 100
			F-16	pos	/	FC-16	neg	blank
			F-17	pos	/	FC-17	neg	STM 10
			F-18	pos	/	FC-18	neg	SE 500
			F-19	pos	/	FC-19	neg	STM 10
			F-20	pos	/	FC-20	neg	blank
						FC-21	neg	STM 10
						FC-22	neg	SE 100
						FC-23	neg	SE 500
						FC-24	neg	STM 100

Legend: neg: faeces negative for *Salmonella*, pos: faeces positive for *Salmonella*, SPan: *Salmonella* Panama (SPan 5: 5 cfp/caps), STM: *Salmonella* Typhimurium (STM 10 and STM 100: 10 and 100 cfp/caps, respectively), SE: *Salmonella* Enteritidis (SE 100 and SE 500: 100 and 500 cfp/caps, respectively), blank: no cfp/caps

for the confirmation of *Salmonella*-colonies obtained by the standard ISO 6579 culture method (14). Amplification was performed according to the optimized touchdown protocol as described previously (15). Real-time PCR was performed using the LightCycler foodproof *Salmonella* Detection Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. Briefly, a 20-µl reaction mixture was composed of foodproof *Salmonella* enzyme solution containing FastStart Taq DNA polymerase, internal amplification control (IC), master mix containing primers and hybridization probes specific for *Salmonella* DNA and *Salmonella*-specific IC, and 5 µl of sample DNA, foodproof *Salmonella* positive control template or PCR-grade water as negative control. Amplification was performed by LightCycler 1.2 Real-Time PCR System (Roche Diagnostics, Germany). The inclusivity of foodproof *Salmonella* master mix for the *Salmonella* genus and exclusivity for other genera was extensively tested by the manufacturer.

Results

Reference materials vs. faecal samples

Detection of *Salmonella* in reference materials was unambiguous with no false negative or positive results regardless of the employed method (samples C in Tables 2 and 3). Both the traditional and the molecular methods in all the tested combinations were equally appropriate with detection limit of 5 cfp/sample or lower. On the other hand, detection of *Salmonella* in samples containing poultry faeces was limited as it depended on the method type and the level of *Salmonella* contamination. Detection limit was impaired for traditional methods (above 10 cfp/sample) in comparison to molecular methods (10

cfp/sample or lower), since one sample of naturally contaminated faeces (F-2) and eight faecal samples containing reference capsules (all five samples supplemented with STM 10 [FC-11, FC-14, FC-17, FC-19 and FC-21], two samples with STM 100 [FC-3 and FC-5], and one sample with SE 100 [FC-1]) were denoted falsely negative but tested positive when molecular detection was performed (Table 3). To detail, *Salmonellae* from capsules STM 10 were detected by traditional methods only from the control samples, but not in samples containing faecal material. However, they were detected in all samples FC (100 %) when applying the molecular methods (particularly, real-time PCR in combination with High Pure foodproof I Kit) (Table 4). *Salmonellae* from samples FC supplied with higher cfp number of *Salmonella* Typhimurium (STM 100) or with *Salmonella* Enteritidis in equivalent cfp number (SE 100) were detected in marked proportions of samples (50 % or 80 %, respectively) by traditional bacteriological methods, but in all cases (100 %) when molecular approach was employed (Table 4). *Salmonellae* from SE 500 capsules with the highest *Salmonella*-contamination level were detected in all samples FC by both the traditional (with MSRV enrichment only) and the molecular approach (Table 4). *Salmonellae* from faecal samples F were detected in 95 % and in 100 % of cases applying traditional (particularly, MKTTn in combination with Rambach agar) and molecular (all combinations) methods, respectively (Table 4).

To summarize, detection of *Salmonella* spp. in reference materials succeeded over the entire experimental range of contamination levels and did not depend on the method type, but was impaired in faecal samples when traditional approach was employed, enabling detection in 30 out of 39 *Salmonella*-positive faecal samples (19/20 for samples F and 11/19 for samples FC, respectively) (Table 3). No samples supplied with blank capsule and negative control samples tested falsely positive.

Traditional bacteriological methods vs. molecular methods

Results of cultivation-dependent detection of *Salmonella* in faecal samples after 24 and/or 48-hour incubation showed that MKTTn and MSRV selective enrichment media generated less falsely negative results than RVS (Tables 2 and 3). MKTTn enabled detection in 19 out of 20 *Salmonella*-positive samples F (19/20) and 10 out of 19 *Salmonella*-positive samples FC (10/19), MSRV in 17/20 and 9/19, and RVS in 14/20 and 3/19 samples, respectively. By traditional approach, 13 of 20 *Salmonella*-positive samples F and only 2 of 19 *Salmonella*-positive FC samples (FC-15 and FC-23) tested positive from all three enrichment media. All the rest tested positive from two (5/20 for samples F and 7/19 for samples FC) or only one (1/20 and 2/19, respectively) selective enrichment medium (Table 3). In the majority of faecal samples, detection failed from RVS enrichment regardless of the isolation medium, in particular with faecal samples FC that were contaminated with serovar Enteritidis (Tables 2 and 3). Although serovar Typhimurium was detected after RVS enrichment in 50 % of FC samples supplied with STM 100, detection of serovar Enteritidis of the same cfp number (SE 100) was completely absent (0 %) or markedly impaired (detection in 20 %) when supplied in higher cfp number SE 500 (Table 4).

The highest number of true positive results (19/20 samples F and 9/19 samples FC, Table 2) was attributed to MKTTn when in combination with Rambach agar. However, the lowest number of positive results was also attributed to MKTTn, namely when it was combined with XLD or BGA isolation plating medium (6/20 or 0/20, respectively for faecal samples F; Table 2). Isolation of *Salmonella* colonies originating from different selective enrichment media was not affected by the selection of plating media with the above mentioned exception of MKTTn, which was likewise the only selective medium that generated higher number of positive cases when results obtained from all the three isolation plating media were combined in comparison to results obtained from individual isolation media (4 positive out of 5 samples FC supplied with SE 100 [4/5] for combined results vs. 3/5 for individual combinations of isolation media with MKTTn, respectively; Table 2). MKTTn and MSRV enrichments were comparably effective regarding the detection level, however only MSRV enabled detection of *Salmonella* in all samples FC supplied with SE 500 (Tables 2 and 3). That was the only case for faecal samples where detection by traditional approach was not limited.

Results of molecular detection of *Salmonella* showed that it was successful, since the presence of *Salmonella* was confirmed not only in control samples but also in all faecal samples (detection level of 100 %; Table 4). However, differences were observed for samples FC regarding the procedure for DNA isolation (using the High Pure foodproof I Kit [protocol HP] or QIAamp DNA Stool Mini Kit [protocol S]) and

the type of PCR reaction (classical PCR or real-time PCR) (Tables 3 and 4). Protocol S enabled detection in more FC samples in comparison to protocol HP when using classical PCR detection (15/19 for S vs. 10/19 for HP), but protocol HP in more samples when performing real-time PCR (19/19 for HP vs. 15/19 for S) (Table 3). Protocol HP enabled detection of *Salmonella* in all samples FC, while protocol S generated false negative result for one sample supplemented with STM 10 capsule (FC-19) and for one with SE 100 (FC-1) (Table 3). Real-time PCR enabled detection in all samples FC, while classical PCR failed for three samples (FC-1, FC-19 and FC-22) (Table 3).

The highest number of positive cases using the molecular approach for samples FC, where detection efficiency depended on the method type in contrast to samples F, was obtained by protocol HP in combination with real-time PCR (19/19), followed by protocol S in combination with either classical or real-time PCR (15/15, however different samples were denoted as positive in four cases [FC-10, FC-11, FC-13 and FC-22] depending on PCR reaction type) (Table 3). The highest number of false negative results was obtained by protocol HP in combination with classical PCR that generated 10 positive cases out of 19 *Salmonella*-positive samples FC (10/19) (Table 3). When classical PCR reaction tested negative, in all but two such cases (samples FC-1 and FC-19 that failed to test positive when applying protocol S) real-time PCR tested positive (Table 3). When applying protocol S, two samples (FC-11 and FC-13) tested positive solely by classical PCR (Table 3).

Table 2: Results of traditional detection of *Salmonella*: number of positive samples after 24 and/or 48-hour incubation in selective enrichment media MKTTn, RVS and MSRV as detected on individual isolation media BGA, XLD and R, respectively

Sample name	Faeces	Capsule	No. of samples	MKTTn				RVS				MSRV			
				BGA	XLD	R	No. of pos. samples	BGA	XLD	R	No. of pos. samples	BGA	XLD	R	No. of pos. samples
C	/	blank	2	0	0	0	0	0	0	0	0	0	0	0	0
	/	SPan 5	2	2	2	2	2	2	2	2	2	2	2	2	2
	/	STM 10	3	3	3	3	3	3	3	3	3	3	3	3	3
	/	SE 100	3	3	3	3	3	3	3	3	3	3	3	3	3
C-11	/	/	1	0	0	0	0	0	0	0	0	0	0	0	0
C-12	neg	/	1	0	0	0	0	0	0	0	0	0	0	0	0
F	pos	/	20	0	6	19	19	14	14	14	14	17	17	17	17
FC	neg	blank	5	0	0	0	0	0	0	0	0	0	0	0	0
	neg	STM 10	5	0	0	0	0	0	0	0	0	0	0	0	0
	neg	STM 100	4	1	1	2	2	2	2	2	2	1	1	1	1
	neg	SE 100	5	3	3	3	4	0	0	0	0	3	3	3	3
	neg	SE 500	5	2	4	4	4	1	1	1	1	5	5	5	5

Note: See Table 1 for details on samples and Table 3 for details on positive samples.

Legend: MKTTn: Muller Kauffmann TetraThionate-novobiocin broth, RVS: Rappaport Vassiliadis medium with Soya, MSRV: Modified Semi-solid Rappaport Vassiliadis medium, BGA: phenol red/Brilliant Green Agar, XLD: Xylose-Lysine-Deoxycholate agar, R: Rambach agar, no. of samples: number of samples used for the study, no. of pos. samples: number of samples positive for the growth of *Salmonella*

Table 3: Results of traditional and molecular detection of *Salmonella*

Sample name	Faeces	Capsule	Traditional			Molecular			
			MKTTn	RVS	MSRV	HP		S	
						PCR	rtPCR	PCR	rtPCR
C-1	/	blank	–	–	–	–	–	/	/
C-3	/	blank	–	–	–	–	–	/	/
C-2	/	SPan 5	+	+	+	+	+	/	/
C-10	/	SPan 5	+	+	+	+	+	/	/
C-5	/	STM 10	+	+	+	+	+	/	/
C-6	/	STM 10	+	+	+	+	+	/	/
C-7	/	STM 10	+	+	+	+	+	/	/
C-4	/	SE 100	+	+	+	+	+	/	/
C-8	/	SE 100	+	+	+	+	+	/	/
C-9	/	SE 100	+	+	+	+	+	/	/
C-11	/	/	–	–	–	–	–	/	/
C-12	neg	/	–	–	–	–	–	–	–
F-2	pos	/	–	–	–	+	+	+	+
F-16	pos	/	+	–	–	+	+	+	+
F-1	pos	/	+	–	+	+	+	+	+
F-3	pos	/	+	–	+	+	+	+	+
F-4	pos	/	+	–	+	+	+	+	+
F-20	pos	/	+	–	+	+	+	+	+
F-9	pos	/	+	+	–	+	+	+	+
F-5	pos	/	+	+	+	+	+	+	+
F-6	pos	/	+	+	+	+	+	+	+
F-7	pos	/	+	+	+	+	+	+	+
F-8	pos	/	+	+	+	+	+	+	+
F-10	pos	/	+	+	+	+	+	+	+
F-11	pos	/	+	+	+	+	+	+	+
F-12	pos	/	+	+	+	+	+	+	+
F-13	pos	/	+	+	+	+	+	+	+
F-14	pos	/	+	+	+	+	+	+	+
F-15	pos	/	+	+	+	+	+	+	+
F-17	pos	/	+	+	+	+	+	+	+
F-18	pos	/	+	+	+	+	+	+	+
F-19	pos	/	+	+	+	+	+	+	+
FC-2	neg	blank	–	–	–	–	–	–	–
FC-8	neg	blank	–	–	–	–	–	–	–
FC-12	neg	blank	–	–	–	–	–	–	–
FC-16	neg	blank	–	–	–	–	–	–	–
FC-20	neg	blank	–	–	–	–	–	–	–
FC-11	neg	STM 10	–	–	–	+	+	+	–
FC-14	neg	STM 10	–	–	–	+	+	+	+
FC-17	neg	STM 10	–	–	–	+	+	+	+
FC-19	neg	STM 10	–	–	–	–	+	–	–
FC-21	neg	STM 10	–	–	–	–	+	+	+
FC-3	neg	STM 100	–	–	–	–	+	+	+
FC-5	neg	STM 100	–	–	–	–	+	+	+
FC-15	neg	STM 100	+	+	+	+	+	+	+
FC-24	neg	STM 100	+	+	–	–	+	+	+
FC-1	neg	SE 100	–	–	–	–	+	–	–
FC-4	neg	SE 100	+	–	+	–	+	+	+
FC-10	neg	SE 100	+	–	+	+	+	–	+
FC-13	neg	SE 100	+	–	+	+	+	+	–
FC-22	neg	SE 100	+	–	–	–	+	–	+
FC-6	neg	SE 500	+	–	+	+	+	+	+
FC-7	neg	SE 500	–	–	+	+	+	+	+
FC-9	neg	SE 500	+	–	+	+	+	+	+
FC-18	neg	SE 500	+	–	+	+	+	+	+
FC-23	neg	SE 500	+	+	+	–	+	+	+

Note: Sample was denoted with positive result if growth of *Salmonella* was detected at least on one isolation medium. Where no faeces was added to samples, QIAamp DNA Stool Mini Kit DNA extraction was not performed. See Table 1 and Table 2 for details.

Legend: HP: High Pure foodproof I Kit (Roche Diagnostics, Germany), S: QIAamp DNA Stool Mini Kit (Qiagen, Germany), PCR: polymerase chain reaction, rtPCR: real-time PCR, + positive result (*Salmonella* detected), – negative result (*Salmonella* not detected)

Table 4: Summary of the results of traditional and molecular detection of *Salmonella*

Sample name	Faeces	Capsule	Traditional			Molecular			
						HP		S	
			MKTTn	RVS	MSRV	PCR	rtPCR	PCR	rtPCR
C	/	SPan 5	**1**	**1**	**1**	**1**	**1**	/	/
	/	STM 10	**1**	**1**	**1**	**1**	**1**	/	/
	/	SE 100	**1**	**1**	**1**	**1**	**1**	/	/
F	pos	/	0.95	0.70	0.85	**1**	**1**	**1**	**1**
FC	neg	STM 10	0	0	0	0.6	**1**	0.8	0.6
	neg	STM 100	0.5	0.5	0.25	0.25	**1**	**1**	**1**
	neg	SE 100	0.8	0	0.6	0.4	**1**	0.4	0.6
	neg	SE 500	0.8	0.2	**1**	0.8	**1**	**1**	**1**

Note: Samples containing blank capsule and negative control samples are not shown. Numbers indicate proportions of samples that were correctly detected as positive (no. of positive samples vs. no. of tested samples). See Tables 1-3 for details.

Discussion

Three types of samples were analyzed within the scope of the present study: artificially prepared gelatin capsules with quantified amounts of *Salmonella* spp. strains of different serovars (samples C), samples containing poultry faeces supplemented with capsules (samples FC) and the least defined samples containing naturally *Salmonella*-contaminated poultry faeces (samples F). Prior to the interpretation of results, faeces used for the artificial contamination was confirmed as negative for *Salmonella* spp. as negative faeces control and faecal samples supplied with blank capsules tested negative by both the traditional and the molecular methods. The first type of samples (samples C) served as a reference material with defined sample parameters to enable determination of the experimental detection limit in optimal conditions. Faecal samples (samples FC and F) were more complex as they contained variable endogenous and dietary components in addition to the faecal microbiota that may interfere with the growth of *Salmonella* or selected bacterial species in *in vitro* conditions (16) and inhibit their detection or quantification by molecular means (17-20). Food samples harboring diverse microflora resemble faecal samples in a sense of exerting a negative effect on the detection of food pathogens, which was previously reported for *Salmonella* (21,22). Culture-based methods using selective agents are not always sufficiently effective in eliminating or suppressing contaminating microflora (23-25). Therefore, negative results have to be interpreted with caution when detection of selected microbial taxa in the environmental samples is being aimed for like foodborne pathogen detection or detection of infectious agents shedding into the environment.

Our results confirmed that the efficiency of detection markedly depends on the sample complexity, since the limit of *Salmonella* detection was lower for reference materials (5 cfp/sample or lower regardless of the method type) than for faecal samples (above 10 cfp/sample for traditional methods and 10 cfp/sample or lower for molecular methods). At the same time, it was confirmed that molecular approach improves detection in environmental samples as cultivation-dependent detection of *Salmonella* was unambiguous only when reference control capsules were considered but was hindered in faecal samples in comparison to molecular detection with markedly lower detection limit. These differences were less apparent for the naturally *Salmonella*-contaminated faecal samples. Therefore, the contamination level of these samples was generally higher than in faecal samples supplied with reference capsules, since it was reported that the recovery of *Salmonella* from environmental samples depends not only on the interference by competing flora but also on the contamination level (26).

Regarding traditional bacteriological methods, results of our study indicate that medium RVS might be considered less appropriate for cultivation-dependent detection of *Salmonella* in poultry faeces than media MKTTn and MSRV. Albeit the enrichment medium MKTTn in combination with Rambach agar plates generated the lowest number of false negative results by culture-based methods in general, the only case of unhindered detection

of *Salmonella* from faecal samples of certain type was supported by the MSRV enrichment, similarly to the efficient detection supported by molecular methods. Results favor the strong recommendation of using the semi-solid MSRV enrichment medium for detection of *Salmonella* contamination in animal samples and samples from food production chains or foodstuffs, since it generated high and consistent detection rate in comparison to MKTTn where detection rate was high but depended on the selection of isolation plating media. Our results are in accordance with the findings of Eriksson and Aspan (27) and with the collaborative studies of CRL for *Salmonella* (9), where it was suggested to replace a selective broth medium in the ISO procedure by a semi-solid medium to obtain a higher detection rate of *Salmonella* in faecal samples. However, the use of more than one enrichment and isolation medium proved important as detection of *Salmonella* in faecal samples was more successful when results from different isolation plating media were combined in one case and depended on the enrichment medium type in other cases.

When aiming for nonselective detection of foodborne pathogens of a certain kind, detection limit for different microbial variants applying a selected method should not differ considerably. However, medium RVS, which generated the highest number of false negative cases in our study, appeared to be selective with regard to *Salmonella* serotype. With RVS enrichment, detection of serovar Enteritidis was impaired in comparison to serovar Typhimurium. Our results were not surprising, since it was reported previously that various *Salmonella* serotypes may perform differently in a given culture medium or sample-medium ratio (28). Therefore, investigation of complex environmental, sanitary or clinical samples by traditional bacteriological methods requires standardized procedures introduced after comprehensive research learning from past deficiencies to introduce critical corrigenda.

Despite the improved detection limit of molecular methods for the selected foodborne pathogens, they can not distinguish between viable microorganisms capable of causing a zoonotic infection and their genetic material present in samples merely as a remnant of formerly live organisms. However, the preenrichment procedures in a non-selective medium enable efficient recovery of viable *Salmonella* from different food samples or matrices to be followed by PCR analysis (29,30). The additional step of bacterial enrichment is therefore strongly recommended to increase the numbers of *Salmonellae* originating from the investigated samples in order to improve the sensitivity of molecular diagnostic techniques (7,30-32). Despite the additional diagnostic procedures applied, isolation of bacteria remains the definitive step for the identification of foodborne pathogens. However, their early detection in breeding animals and their secreta/excreta can help decrease the number of faecal shedders of pathogens like *Salmonella* from animal and food production systems, therefore interrupting the infection-transmission cycle. Many methods for the rapid detection and serotype identification of *Salmonella* in foodstuffs have been developed to date, since rapid and reliable detection of pathogens is crucial for ensuring food safety (27,30,33,34). The specialized, chromogenic culture media and the immuno assays are being employed, but nucleic acid-based assays like PCR and its variants are becoming the most important rapid diagnostic techniques in clinical and food microbiology within the last two decades.

One of the chromogenic media developed to detect *Salmonella* is Rambach agar (12). It is based on a combination of biochemical characteristics and is reported to be highly specific. Among the isolation plating media that were tested in our study, Rambach agar plates proved to be more suitable in combination with the selective enrichment medium MKTTn than XLD plates and these more than BGA plates. Our results are in accordance with the previously reported evaluation of enrichment broths and plating media for the detection of *Salmonella* in poultry (35), where it was reported that the most effective isolation medium was the chromogenic medium CHROMagar (*Salmonellae* were identified in 79.3 % of the positive samples), followed by Rambach agar (48 %) and finally by classical media like XLD (34.5 %) and BGA (13.8 %).

In contrast to traditional cultivation, molecular approach enabled successful detection of *Salmonella* both in reference materials and in faecal samples. Although molecular detection is not dependent on the selection of growth media, its success depends on the selection and appropriate combination of nucleic acid-based methods. The Qiagen's QIAamp DNA Stool Mini Kit was applied for faecal samples as it included a commercial mixture of reagents to ensure targeted removal of faecal metabolites that can degrade the isolated DNA and inhibit the downstream enzymatic applications. On the other hand, all samples were processed by the High Pure foodproof I Kit by Roche, which is optimized to provide

DNA of high quality from *Salmonella*-enrichment cultures of various food samples.

In our study, differences were observed for faecal samples spiked with reference capsules regarding the efficiency of PCR detection of *Salmonella* after DNA isolation procedure using the two selected commercially available kits. In combination with classical PCR detection, QIAamp DNA Stool Mini Kit enabled detection in more samples, but in fewer with real-time PCR, for which the High Pure foodproof I Kit was confirmed to be the optimal choice as reported before (36). Although the latter is formulated to generate highly purified DNA from food samples, it also proved appropriate for faecal samples with similarly high potential for PCR inhibition, possibly due to supplementary enrichment step diluting the inhibitors. The High Pure foodproof I Kit enabled detection of *Salmonella* in all *Salmonella*-positive faecal samples, while the QIAamp DNA Stool Mini Kit surprisingly generated false negative results regardless of the following PCR reaction type.

Real-time PCR proved to exert higher sensitivity than classical PCR reaction since it enabled detection in all faecal samples, however when not preceded by DNA isolation using the QIAamp DNA Stool Mini Kit. One single combination of suitable DNA isolation protocol and PCR test, namely the High Pure foodproof I Kit in combination with *Salmonella*-adapted real-time PCR detection kit (both from Roche Diagnostics, Germany), was sufficient for detection in all *Salmonella*-positive samples.

We can conclude that traditional bacteriological methods, being asserted as the procedure of a choice for the detection of *Salmonella* in foodstuffs for most laboratories around the globe, ought to be supplemented with molecular methods to generate results in shorter time and with lower detection limit. Inconsistency of cultivation-dependent, and to a lesser extent of molecular detection, indicates the importance of using more than one method for confirmation or exclusion of possible contamination of biological materials analyzed in the process of food-safety monitoring. Molecular approach, possibly the combination of real-time PCR assay with the High Pure foodproof I Kit or alternative provided by the manufacturer, can enable detection of *Salmonella* without generating false negative results. Therefore, it should be considered as an important supplement to the traditional protocol. Efforts targeted to early detection of *Salmonella* will contribute to successful control and reduction of foodborne salmonelloses in the future.

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PRIMERJAVA MOLEKULARNIH IN KLASIČNIH BAKTERIOLOŠKIH METOD ZA UGOTAVLJANJE SALMONEL V KOKOŠJEM BLATU

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Povzetek: Primerjali smo klasične (gojiščne) in molekularne (genetske) bakteriološke metode za ugotavljanje salmonel v referenčnih kapsulah, ki so vsebovale znano število bakterij serovarov Panama, Typhimurium ali Enteritidis vrste *Salmonella enterica* subsp*. enterica*, ter v blatu kokoši, ki je bilo bodisi naravno okuženo s salmonelami bodisi negativno na salmonele in okuženo z referenčnim materialom. Pri klasičnih tehnikah smo glede na ISO 6579 uporabili različna gojišča za obogatitev (MSRV, MKTTn in RVS) in za izolacijo na ploščah (XLD, BGA in Rambach agar). Molekularno določanje smo začeli po koraku predobogatitve. Primerjali smo učinkovitost dveh kompletov za osamitev DNK, in sicer High Pure foodproof I (Roche Diagnostics, Germany) in QIAamp DNA Stool Mini (Qiagen, Germany) kompletov, v kombinaciji s klasično in s PCR reakcijo v realnem času. Rezultati so pokazali, da je bilo ugotavljanje salmonel s klasičnimi in z molekularnimi metodami v referenčnih kontrolnih kapsulah neovirano, v vzorcih blata pa omejeno. Gojišče RVS je bilo manj primerno kot gojišči MSRV in MKTTn. Gojišče MKTTn je v kombinaciji s ploščami Rambach dalo največ pozitivnih rezultatov pri klasičnem pristopu. Priporočeno poltrdno gojišče MSRV je omogočalo ugotovitev salmonel pri velikem deležu vzorcev. Ta je bila najmanj odvisna od izbire gojišč za izolacijo, zato smo potrdili njegovo primernost. V nasprotju od gojiščnih metod je molekularni pristop, še posebno kombinacija High Pure foodproof I kompleta s PCR reakcijo v realnem času, omogočal uspešno ugotavljanje salmonel v vseh pozitivnih vzorcih, zato bi ga morali upoštevati kot pomembno dopolnitev klasičnega protokola za ugotavljanje salmonel v živilih.

Ključne besede: *Salmonella*; živila; blato; ugotavljanje; gojenje; molekularno; PCR

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Original Scientific Article

EFFECT OF IMMUNOCASTRATION (IMPROVAC®) IN FATTENING PIGS I: GROWTH PERFORMANCE, REPRODUCTIVE ORGANS AND MALODOROUS COMPOUNDS

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Summary: The effect of the immunocastration (vaccination against gonadotropin releasing hormone using Improvac® vaccine) on growth performance, size of reproductive organs and levels of fat androstenone and skatole was studied in Slovenian pig fatteners. The pigs (50% crosses of Duroc), selected from 35 litters (2 castrates and 3-4 boars per litter) farrowed within two weeks period, were assigned to three experimental groups: boars (n=25), immunocastrated males (n=24) and surgically castrated males (n=25). The vaccinations with Improvac® were performed at the age of 10 and 19 weeks. The trial started when pigs were 12 weeks old, and lasted until 24 weeks of age, when pigs were slaughtered. During the trial, pigs were lodged individually and their daily feed intake (*ad libidum*) and weight (at 12, 19 and 24 weeks of age) were recorded. At the slaughter line, the testes and accessory reproductive glands were removed and weighed. Subcutaneous fat samples were taken for the determination of androstenone and skatole concentration. Results on androstenone and skatole showed that the immunisation was 100% successful. The levels of androstenone were below a detection limit of the laboratory method in all pigs. For skatole, the concentrations were at comparably low level in surgically and immunocastrated males, whereas higher levels were observed in boars. Vaccination strongly reduced the weight of testes and accessory reproductive glands. Regarding growth performance, the immunocastrated males showed comparable feed intake, feed efficiency and growth rate as boars in the period from the start of the experiment until the revaccination; thereafter they were closer to the surgical castrates. In the present study, the beneficial impact of the immunocastration on pig productivity and on the reduction of substances responsible for boar taint was confirmed in Slovenian local conditions.

Key words: pig; immunocastration; growth performance; androstenone; skatole

Introduction

Surgical castration is routinely practiced in male pigs destined for fattening. The aim is to prevent, an unpleasant off-flavour which develops in meat of sexually mature entire males and results in rejection of such meat by the consumers (1). The two main compounds held responsible for boar taint are androstenone (2) and skatole (3). Androstenone is a testicular steroid producing a urine-like smell, while skatole has a faecal-like odour and is a by product of bacterial metabolism of the tryptophan in the intestine. Although the origin of these two compounds is different, the elevated level of androstenone is accompanied by the elevated level of skatole, the correlation coefficient being around 0.3 (4). It seems that their metabolic pathways are interdependent, and it has been hypothesised that androstenone, along with other testicular steroids, affects the metabolism of skatole in the liver (5). Surgical castration has also a positive impact since castrated pigs

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exhibit less aggression and sexual behavior (mounting). However, surgical castration has also drawbacks. Fattening of castrates compared to the entire males is economically less efficient due to their lower feed efficiency and carcass leanness. Surgical castration without anaesthesia has also been criticized from the animal welfare viewpoint (6). A ban on surgical castration without anaesthesia is under consideration by the EU and other European countries. Therefore, the alternative methods for boar taint prevention are of prime interest. One of them is immunocastration, a non-surgical castration by vaccination in which male pigs are actively immunized against GnRH (gonadotropin-releasing hormone). It is a more welfare-friendly alternative to surgical castration without anaesthesia (7). Currently this method is extensively used in a number of countries, including Australia, New Zealand and Brazil. In Europe its use has been approved in Switzerland in 2008 and in the EU in 2009. Immunocastration reduces the weight of testes and accessory sex glands (8) and the production of the substances (androstenone and skatole) responsible for boar taint (9). Moreover, immunocastrated pigs have been shown to exhibit better growth performance as surgical castrates (10).

Due to the recent approval of the vaccine Improvac® in the EU, the tests in local conditions are currently going on in many European countries. The objective of the present study was to test the effectiveness of the vaccine on growth performance, weight of the reproductive organs and fat androstenone and skatole levels in pigs fattened in the controlled environment (individually housing with *ad libidum* feeding) for one of the main commercial hybrids in Slovenia.

Material and methods

Animals and fattening trial

The pigs used in the present study were 50% Duroc crosses raised on commercial pig farm. For the experiment, the pigs were selected from 35 litters farrowed within two weeks period. Pigs (2 surgical castrates and 3-4 boars per litter) were individually marked (with plastic mark and with a tattoo). At the age of ten weeks they were transferred to the experimental stable and individually housed. At that time the first vaccination against GnRH (2 ml of Improvac® vaccine per animal, Pfizer Animal Health) was applied by veterinarian and pigs were assigned to three treatment groups: boars (n=25), immunocastrated males (n=24) and surgically castrated males (n=25, due to the illness, one animal from this group was subsequently removed from the experimental dataset). The second vaccination of immunocastrated males (2 ml of vaccine) was performed at 19 weeks of age (5 weeks prior to slaughter). The trial started when pigs reached 12 weeks of age and lasted for 12 weeks. Pigs were individually housed and fed *ad libitum* a commercial diet BEK-1 (Jata Emona d.o.o., Ljubljana, Slovenia) containing 13.0 MJ/kg of metabolisable energy, 17% crude protein, 2.6% crude fat, 4% crude fibre, 6% crude ash and 1% lysine. Feed intake was individually recorded and pigs were weighed at 12, 19 and 24 weeks of age, when they were sent to slaughter. Additionally, daily gain and feed conversion ratio were calculated. Animals were slaughtered in a commercial abattoir according to standard procedure (app. 1 hour transport, 2 hours of lairage, CO_2 stunning). At the slaughter line, testes and accessory male reproductive glands were removed and taken to the laboratory for dissection and weighing.

Skatole and androstenone determination

Samples of subcutaneous fat were taken at the level of last rib and sent to the laboratory (CCL Nutricontrol, Veghel, Netherlands) accredited according to SIST EN 17025 for the determination of androstenone and skatole concentration. Briefly, the fat of the samples (25 g) was liberated by heating the sample in a microwave oven, and the fat extracted with an organic solvent. For skatole determination, the extracts were analysed by HPLC on a reversed phase column with fluorescence detection using an external standard. For androstenone, the fat was extracted using organic solvent with known amount of internal standard of androstenone, and the extracts analysed by GSMS in SIM mode. The lower limit of sensitivity was 0.04 μg androstenone/g fat.

Statistical analysis

One-way analysis of variance (procedure GLM of SAS, SAS Inc., Cary, NC, USA) was used to evaluate the effect of treatment group (*i.e.* surgically castrated, immunocastrated males or boars) on growth performance, weight of the reproductive organs, and skatole and androstenone concentrations. When a significant group effect ($P<0.05$) was detected, means were compared using a *Tukey* test.

Results and discussion

Reproductive organs

Vaccination strongly reduced testes and accessory male reproductive glands (Table 1, Figure 1) in immunocastrated males compared to the entire males (3 to 15 times lighter in case of testes with epididymides and vesicular gland, respectively). In surgical castrates the accessory reproductive glands were even more severely atrophied.

Table 1: Weight of the reproductive organs (mean ± se) in surgically castrated, immunocastrated males and boars

	SURGICALLY CASTRATED MALES	IMMUNO-CASTRATED MALES	BOARS	*P*-value
Number of animals	24	24	25	
Testes with epididymides, g		223.2 ± 18.5[a]	701.3 ± 38.8[b]	<0.000
Epididymides, g		23.6 ± 1.92[a]	88.9 ± 4.05[b]	<0.000
Bulbo-urethral gland, g	6.9 ± 0.32[a]	36.6 ± 3.34[b]	117.8 ± 6.2[c]	<0.000
Vesicular gland, g	1.7 ± 0.06[a]	12.2 ± 0.76[a]	175.4 ± 13.6[b]	<0.000
Prostate, g	1.3 ± 0.05[a]	2.1 ± 0.12[b]	11.0 ± 0.38[c]	<0.000

Means with different letters within one row are significantly different (P<0.05).

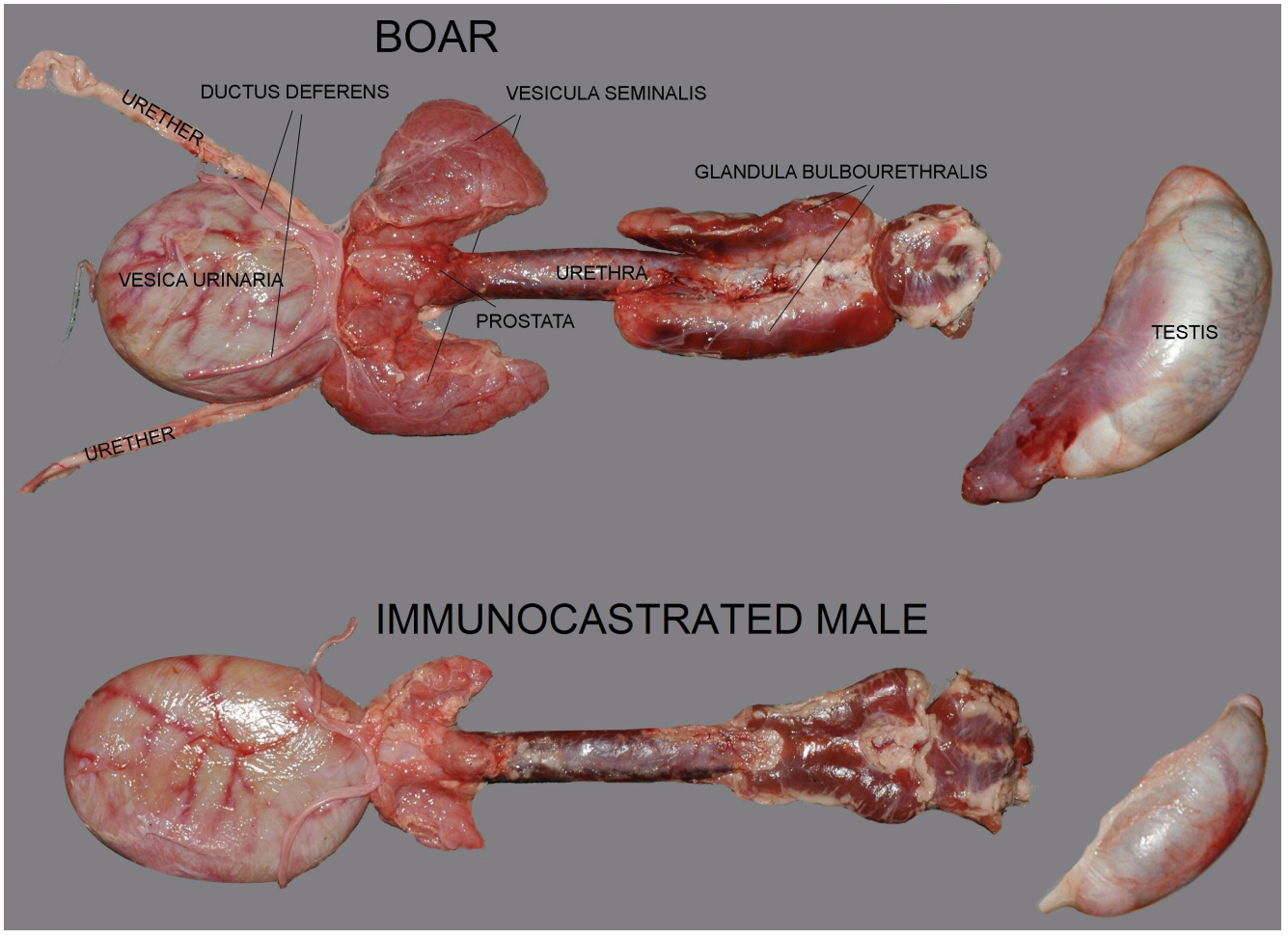

Figure 1: Representative image of testes and accessory reproductive glands belonging to a boar or an immunocastrated male

The effectiveness of anti-GnRH vaccine is known from numerous studies, and for the efficient inhibition of the testes function, two applications of vaccine are needed (9, 10). It was demonstrated (9, 10) that plasma testosterone level (an indicator of testes function) remained unaffected until the second vaccination, whereas at the time of the slaughter (only 15 days later), the level of testosterone was seven- to twenty five-fold lower in immunised pigs, which was also consistent with the rise of anti-GnRH antibody titres at that time. Along with a decline of the androgenic hormone level, the literature acquaints a reduction of reproductive organs. In various studies (8, 9, 10, 11, 12, 13, 14, 15, 16) a drastic reduction was reported for the testes (16% to over 60%), bulbourethral glands (50% to over 90%) and seminal vesicles (36% to over 90%) in immunocastrated males when compared to entire males. The results in the present study show a similar degree of reduction of the reproductive organs as reported in the literature. The difference in the weights reported is, however, greatly related to the onset of the second vaccination and the time elapsed until the slaughter.

Boar taint compounds

Consistent with a reduction of the size of reproductive organs observed in the present experiment, the levels of fat tissue androstenone and skatole were strongly affected (Table 2). The concentration of androstenone in fat was above the laboratory method detection limit (*i.e.* 0.04 μg/g of fat) only in the case of boars. Regarding the skatole, its level was fairly low and not statistically different between surgically castrated and immunocastrated males, whereas its concentration was almost six-fold higher in boars. These results prove the efficiency of vaccination against GnRH. Moreover, we can consider that the vaccination was successful in the prevention of boar taint, since, proposed sensory threshold (4) concentrations of androstenone (0.5 or 1.0 μg/g fat) and skatole (0.2 or 0.25 μg/g fat) were not exceeded in the present experiment. It has been shown, that fat androstenone concentration drops very sharply after the cessation of testes function (17), its half life being in the range of a few days. However, a complete clearance of androstenone from the fat tissue requires a period of at least 3 weeks (18). Recent study (19) interested in the effect of different timing of immunocastration has shown, that a revaccination performed only two weeks prior to slaughter should be sufficient for the prevention of boar taint (*i.e.* decline of androstenone and skatol below threshold levels). In agreement with our results, the effectiveness of the anti-GnRH vaccine has been proven by numerous studies (9, 10, 11, 12, 13, 14, 15). However, the absolute values of the concentrations of malodorous compounds reported are, again, difficult to compare due to the differences in the experimental designs but also due to the differences in analytical procedures which need harmonisation (20).

Table 2: Subcutaneous fat skatole and androstenone concentrations (mean ± se)

	SURGICALLY CASTRATED MALES	IMMUNO-CASTRATED MALES	BOARS	*P*-value
N	24	24	25	
Skatole (ng/g)	38.2 ± 4.44[a]	39.2 ± 3.08[a]	231.4 ± 76.1[b]	0.006
Androstenone (μg/g)	*bd*	*bd*	1.48 ± 0.19	

bd – below detection limit (0.04 μg/g fat); means with different letters within one row are significantly different (P<0.05).

Growth performance

Results on growth performance (Table 3) show no difference between experimental groups in the average body weight at the beginning of the experiment (12 weeks) and at the time of the revaccination (19 weeks). However, the differences became significant at the end of the experiment, when we could note lower body weight of surgically castrated as compared to the immunocastrated males or boars. As a consequence, we noted lower daily gain for surgically castrated than immunocastrated males or boars, however the differences were insignificant. Feed intake and feed efficiency (considering the whole experimental period from the first vaccination until slaughter) differed between surgically cas-

trated males and boars. Surgically castrated males had higher overall feed intake (10%) and lower feed efficiency (15%) compared to boars. The immunocastrated males took intermediate position in both cases, but were not significantly different from either of the control groups. Considering the separate experimental phases, feed intake and feed efficiency of the immunocastrated males resembled those of boars in the first part of the experiment (until revaccination), whereas after the second vaccination a shift was noted and the immunocastrated males were more similar to the surgical castrates.

Table 3: Body weight, daily gain, feed intake and feed efficiency (mean ± se) in surgically castrated, imunocastrated males and boars, according to the phase of the experiment

	SURGICALLY CASTRATED MALES	IMMUNO-CASTRATED MALES	BOARS	*P*-value
Number of animals	24	24	25	
Body weight, kg				
12 weeks	29.8 ± 0.84	31.6 ± 0.89	31.3 ± 0.89	0.309
19 weeks	82.6 ± 0.87	85.4 ± 1.26	85.6 ± 1.25	0.146
24 weeks	117.1 ± 0.96	121.0 ± 1.37	121.9 ± 1.77	0.049
Daily gain, g/day				
Birth-12 weeks	364 ± 8	385 ± 11	381 ± 10	0.279
12-19 weeks	1010 ± 19	1031 ± 20	1038 ± 20	0.584
19-24 weeks	978 ± 23	1012 ± 23	1034 ± 27	0.273
12-24 weeks	998 ± 13	1022 ± 13	1038 ± 18	0.174
Birth-24 weeks	697 ± 6	719 ± 10	725 ± 12	0.112
Feed intake, kg/day				
12-19 weeks	2.77 ± 0.035^{a}	2.6 ± 0.026^{ab}	2.58 ± 0.065^{b}	0.032
19-24 weeks	3.77 ± 0.058^{a}	3.72 ± 0.090^{a}	3.37 ± 0.077^{b}	<0.000
12-24 weeks	3.21 ± 0.035^{a}	3.06 ± 0.036^{ab}	2.93 ± 0.061^{b}	0.008
Feed efficiency, kg feed/kg gain				
12-19 weeks	2.76 ± 0.062^{a}	2.54 ± 0.026^{b}	2.49 ± 0.036^{b}	<0.000
19-24 weeks	3.89 ± 0.089^{a}	3.72 ± 0.090^{a}	3.28 ± 0.061^{b}	<0.000
12-24 weeks	3.23 ± 0.057^{a}	2.99 ± 0.036^{b}	2.82 ± 0.032^{c}	<0.000

Means with different letters within one row are significantly different (P<0.05); 12 weeks - start of experiment; 19 weeks - revaccination; 24 weeks - end of experiment, when animals were slaughtered.

It is well known that boars exhibit better growth performance in comparison with the surgically castrated males (*i.e.* lower feed intake, higher feed efficiency, higher weight gain), which was reconfirmed in the present study. Concerning growth performance of the immunocastrated males, the literature reports are not completely coherent, which we can ascribe to different experimental conditions. The early experiments (8) demonstrated that immunoneutralization of GnRH in boars resulted in the castration-like effects, without any major influence on growth performance (average daily gain, body weight). Some studies, comparing only the immunocastrated males and surgically castrated males (13, 21, 22) also found no difference in growth rate between surgically and immunocastrated males, although some trends for better average daily weight gain (13, 22) and better overall feed efficiency (21) of the immunocastrated males were observed. Several studies (10, 23, 24, 25) reported Improvac® treated pigs to grow faster and present higher feed intake as boars in the period following a revaccination (besides being also superior to the surgical castrates). This could be related to the relatively high (boar-like) levels of growth hormone in immunocastrated males (26) and the fact that the experimental animals were not housed individually. Namely, boars housed in groups waste more energy due to their social behaviour (aggressiveness, mounting), which could, combined with lower feed consumption, be responsible for lower performance as compared to their castrated counterparts (15, 24, 27, 28). The key reason for the observed differences in the literature is related mainly to the timing of the immuniza-

tion; the longer the time elapsed between the second vaccination and slaughter, the greater the difference between the immunocastrated males and boars. It was, for example, shown (11) that pigs who responded earlier to the anti-GnRH immunisation exhibited growth performance which was comparable to the surgically castrated males, whereas late responders were more similar to the boars. It is only after the second vaccination that an immune reaction with antibody titres against GnRH is elicited (29). As a consequence a production of gonadal steroids is reduced, leading the immunocastrated males to start acting like surgical castrates in regard to the social behaviour, feed consumption and consequently growth performance (24). This is supported by the results of the present study, where the feed intake of immunocastrated males increases (along with a decrease of feed efficiency) from that similar to the boars to the one similar to surgical castrates after the revaccination.

Conclusions

Present study on the individually housed Slovenian commercial fatteners confirmed the efficiency of Improvac® vaccine in regard to the elimination of malodorous compounds. The study also confirmed the benefits of the immunocastrated males as compared to the surgical castrates in growth performance, but mainly feed efficiency.

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UČINEK IMUNOKASTRACIJE (IMPROVAC®) PRI PRAŠIČIH PITANCIH: I. RASTNOST, REPRODUKTIVNI ORGANI IN SUBSTANCE, ODGOVORNE ZA VONJ PO MERJASCU

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Povzetek: Preučevali smo vpliv imunokastracije (cepljenja proti gonadotropin sproščajočemu hormonu s cepivom Improvac®) na rastnost, velikost reproduktivnih organov ter na raven androstenona in skatola v maščobnem tkivu. Poskusni prašiči (50% križanci pasme durok) so bili izbrani iz 35 gnezd (po 2 kastrata in 3-4 merjasci na gnezdo), rojeni v obdobju dveh tednov. Živali smo razdelili v tri poskusne skupine: merjasce (n=25), imunokastrate (n=24) in kirurške kastrate (n=25). Cepljenje z Improvacom ® smo opravili pri starosti 10 in 19 tednov. S poskusom smo pričeli pri starosti 12 tednov in je trajal do 24. tedna starosti, ko so bili prašiči zaklani. Med poskusom so bili prašiči individualno vhlevljeni in so dobivali hrano po volji. Zapisana je bila dnevna poraba krme, prašiči so bili med poskusom tehtani pri 12, 19 in 24 tednih starosti. Na klavni liniji so jim bili odvzeti in stehtani testisi in akcesorne spolne žleze. Dan po zakolu so bili odvzeti vzorci podkožnega maščevja za določitev koncentracije androstenona in skatola. Rezultati so pokazali, da je bilo cepljenje 100% uspešno, saj so bile koncentracije androstenona pod mejo detekcije laboratorijske metode. Koncentracije skatola so bile nizke in primerljive pri kirurških in imunokastratih, medtem ko so bile pri merjascih znatno višje. Cepljenje je močno vplivalo tudi na zmanjšanje teže mod in akcesornih spolnih žlez. Kar zadeva pitovne lastnosti so bili imunokastrati v prvem obdobju (do revakcinacije) po zauživanju in porabi krme ter dnevnem prirastu podobni merjascem, v drugi fazi rasti (po revakcinaciji) pa bolj podobni kirurškim kastratom. Pričujoča raziskava, narejena v slovenskih pogojih prašičereje, je pokazala pozitivne učinke imunokastracije na proizvodne rezultate. Prav tako je bila uspešna v smislu preprečitve tvorbe substanc odgovornih za nastanek vonja mesa po merjascu.

Ključne besede: prašiči; imunokastracija; rastnost; androstenon; skatol

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Original Scientific Article

EFFECT OF IMMUNOCASTRATION (IMPROVAC®) IN FATTENING PIGS II: CARCASS TRAITS AND MEAT QUALITY

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Summary: The effect of immunocastration (vaccination against gonadotropin releasing hormone using Improvac® vaccine) on carcass and meat quality traits of Slovenian commercial fatteners was studied. The experimental pigs were selected from 35 litters (2 castrates and 3-4 boars per litter) farrowed within two weeks period and assigned to three treatment groups: boars (n=25), immunocastrated (n=24) and surgically castrated males (n=25). Vaccination of pigs was performed twice; at 10 and 19 weeks of age. Pigs were slaughtered at 24 weeks of age (two slaughter batches in two consecutive weeks) according to the routine abattoir procedure. Various carcass and meat quality traits were assessed. Additionally, an olfactory evaluation of meat for the presence of boar taint was performed using a six member panel. Differences between treatment groups were detected for fat tissue measurements and ratio meat to fat in ham and on the cross-section at the level of last rib or neck. In comparison to the surgically castrated males (the fattest) and boars (the leanest), the immunocastrated males took an intermediate position. However, they were mainly closer to the boars as to the surgically castrated males, except in one case (neck fatness) where immunocastrates were closer to surgical castrates. No significant differences between treatment groups were noted for pH value, colour or drip loss. Regarding intramuscular fat which is important for sensory quality, we noted a significant difference between surgical castrates and boars, whereas the immunocastrates were either intermediate (*biceps femoris* marbling) or closer to boars (*longissimus dorsi* intramuscular fat). The present study provided additional evidence of the benefits of the immunocastration for carcass quality, with no major effect on meat quality.

Key words: pig; immunocastration; carcass traits; meat quality

Introduction

Review of the literature in regard to the prevention of boar taint and the effect of using the Improvac® vaccine has been presented in the first part of the article. Furthermore, the results of the present experiment on pig growth performance, size of the reproductive organs and the level of malodorous compounds responsible for boar taint have also been presented and discussed. This novel method of boar taint prevention is currently intensively tested in many European countries, since the vaccine was approved in the European Union in 2009, some of the tests have already been published (1, 2, 3, 4, 5). Besides its impact on growth, the consequences in terms of carcass leanness seem even more important. According to the published results, the immunocastrated pigs have leaner carcasses compared to the surgical castrates (3, 4, 5, 6), which represents an important advantage for pig producers. Namely, carcass lean meat content is the main criterion for the payment of commercial pig fatteners. However, a better and more complete picture on carcass and meat quality is necessary taking into consideration the demands of retailers and consumers. There is a need for more detailed data on the relative distribution or importance of muscle and fat tissue in valuable meat cuts as well as on the technological and sensory quality of meat. In view of this and to test the vaccine in the local conditions, the objective was to assess the effect of immunocastration on the selected carcass and meat quality traits by comparing three groups of pigs, the entire males, the surgically castrated males

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and the males vaccinated with Improvac®. In addition we performed an olfactory test for the presence of boar taint in all three groups of the pigs.

Material and methods

Olfactory evaluation of boar taint

The intensity of boar taint in pork was performed by a panel of six members of the Veterinary faculty staff selected for their ability to detect this specific odour. Five members were women and one was a man. Women have been shown to be more sensitive to androstenone. Meat samples (15 g) were put in a 250 ml flask, covered by a small amount of water, heated and left boiling for 10 minutes. After the heating, the samples were taken into the separated laboratory, where the intensity of the odour was estimated. Panellists had to choose among the three levels of odour intensity: none, slight or strong. All samples were individually assessed for boar taint by each member of the panel.

Measurements on the slaughter line

The origin and rearing conditions for the experimental pigs have been described in the first part of the present study. Shortly, experimental pigs (50% crosses of Duroc) were selected from 35 litters (2 castrates and 3-4 boars per litter) farrowed within two weeks period, and assigned to three experimental groups: boars (n=25), immunocastrated males (n=24) and surgically castrated males (n=25). The vaccinations with Improvac® were performed at the age of 10 and 19 weeks. During the trial, pigs were lodged individually. Pigs were slaughtered at the age of 24 weeks (in two slaughter batches within two consecutive weeks) according to the routine abattoir procedure *i.e.* CO_2 stunning, vertical exsanguination, vapour scalding, dehairing and evisceration followed by the veterinary inspection and carcass classification. Pigs were transported (app. 1 hour) and left to rest (app. 2 hours) before being slaughtered. At the slaughter line, pigs were classified according to SEUROP by official classification body, using a method approved for Slovenia (7) which consists of taking two measurements at the carcass split line; DM fat (minimal fat thickness over the *gluteus medius* muscle – GM) and DM muscle (shortest distance between cranial end of GM and dorsal edge of vertebral canal). Measurement of pH (pH45) in *longissimus dorsi* muscle (LD) was taken 45 minutes and 24 hours *post mortem* using a MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, 8603 Schwarzenbach, Switzerland) fitted with a combined glass electrode (InLab427) and previously calibrated at pH 4.0 and 7.0. The carcasses were cooled overnight by storage at 0-2 °C until the internal carcass temperature dropped below to 7 °C.

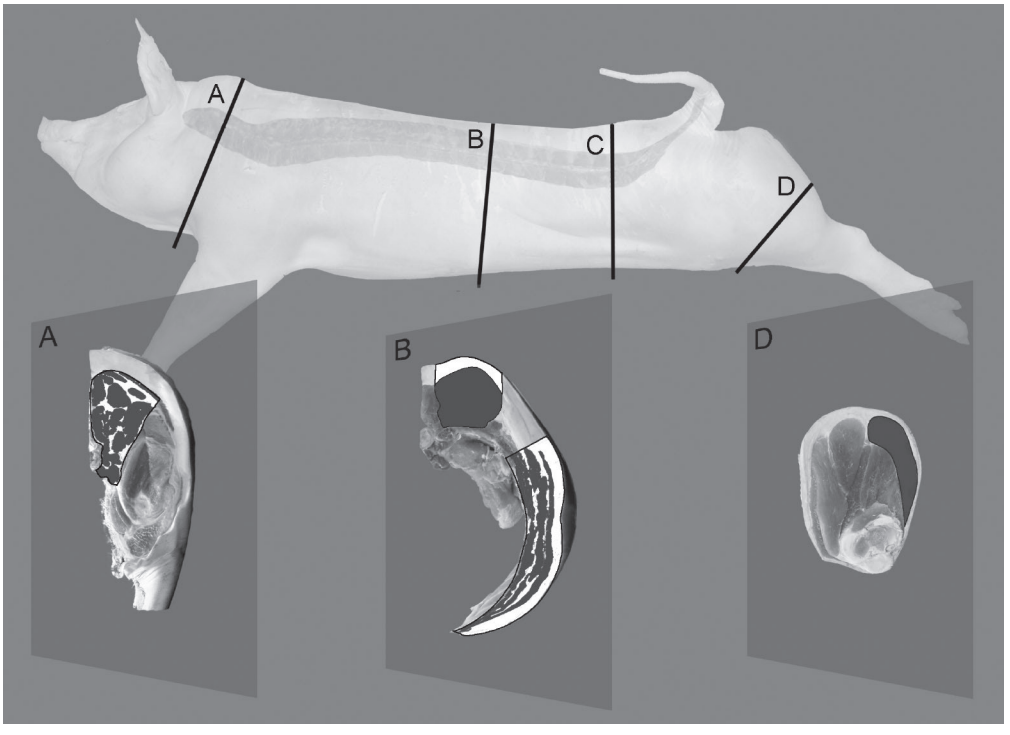

Figure 1: Locations of cross-sections for the evaluation of carcass properties

Carcass properties

A day following slaughter, additional carcass traits were assessed. The carcass was cut at four sites (see Figure 1) which included cutting between 3rd and 4th cervical vertebra (cross-section A), after last rib (cross-section B), between 6th and 7th lumbar vertebra (cross-section C) and through the knee joint (cross-section D). Images of the corresponding carcass cross-sections were taken using a digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). LUCIA.NET 1.16.5 software (Laboratory Imaging s.r.o., Prague, Czech Republic) was used for image analysis determining the neck fatness (%) on the cross-section A, loin eye or *longissimus dorsi* area (LD area) and corresponding fat area (fat over LD) on the cross-section B. The ratio between LD meat and corresponding fat (LD meat:fat ratio) was calculated. Additionally, LD marbling, belly leanness (cross-section B) and *biceps femoris* muscle (BF) marbling (cross-section D) were visually assessed using 1-7 point scale (1 denoting the lowest and 7 the highest intensity). The hind leg (portion between cross-section C and D) was weighed prior (ham weight) and after (ham muscle+bone) the removal of the skin and subcutaneous fat and ham leanness % (ham meat) calculated as the ratio between the two weights.

Meat quality measurements

The measurements of the colour and pH were taken on a freshly cut surface of LD (cross section B). Colour of LD was assessed using 1-6 point Japanese colour scale (8). Colour parameter measurements (CIE L*, a*, b*) were taken in triplicate using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11 mm aperture, D_{65} illuminant, calibrated against a white tile. Muscle pH (pH_{24}) was determined in two replicates in the central area of LD. Caudally from the level of last rib two 2.5 cm thick slices of LD were removed from the loin for the determination of drip loss, intramuscular fat and olfactory test of boar taint. Drip loss was determined according to the EZ drip loss method (9). Shortly, two cylindrical samples were excised from the central part of LD, weighed and placed in special plastic containers. The samples were reweighed after 24 hours and after 48 hours of storage at 4 °C. Drip loss was expressed as a difference (%) of initial sample weight. Samples of LD muscle were minced and intramuscular fat content (IMF) estimated using NIRS (NIR System model 6500 Spectrometer, Silver Spring, MD, USA) (10).

Statistical analysis

The effect of treatment group (*i.e.* surgically castrated, immunocastrated or boars) was analysed using a GLM procedure of statistical package SAS (SAS Inc., Cary, NC, USA). In the case of meat quality traits (pH_{45}, pH_{24}, colour, Minolta L*, a*, b*, drip24 and drip48) slaughter batch was added to the model as a random effect. Significant differences ($P<0.05$) in means between groups were compared using *Tukey* test.

Results and Discussion

Olfactory evaluation of boar taint

The results of the sensory test (Table 1) revealed that surgically castrated males had the lowest incidence and intensity of the unpleasant odour. A slightly higher incidence and intensity were detected for the immunocastrated males. On the other hand, in the case of boars, all samples were recognized as having an unpleasant odour (99.2 % incidence for a strong boar taint). It is somehow surprising that two samples of immunocastrated males were scored by panellists as having a strong boar taint, despite the fact, that all fat samples of immunocastrated males exhibited levels of androstenone which were below the detection limit of laboratory method and consumer thresholds (see Table 2 of part I of this study). The effectiveness of the immunocastration for the prevention of boar taint has been proven by many studies on the molecular level *i.e.* decreased fat tissue androstenone and skatole levels (5, 11, 12, 13, 14, 15) as well as by the sensory analysis using either consumers (1) or panel tests (2, 16).

Table 1: Incidence of boar taint in surgically castrated, immunocastrated males and boars

Degree of odour	Panel member							
	A	B	C	D	E	F	Average	Incidence, %
SURGICALLY CASTRATED MALES								
None	23	22	22	24	24	22	22.8	95.1
Slight	1	2	2	0	0	2	1.2	4.9
Strong	0	0	0	0	0	0	0.0	0.0
IMMUNOCASTRATED MALES								
None	22	22	22	21	22	21	21.7	90.4
Slight	0	1	0	0	0	1	0.3	1.3
Strong	2	1	2	3	2	2	2.0	8.3
BOARS								
None	0	0	0	0	0	0	0.0	0.0
Slight	0	0	0	0	0	1	0.2	0.8
Strong	25	25	25	25	25	24	24.8	99.2

Carcass properties

There was a significant effect of the treatment group on the majority of the measured carcass traits (Table 2). In the present experiment, boars tended ($P<0.10$) to have heavier carcasses (3.9 kg) as surgically castrated males, while the immunocastrated males were intermediate (1.7 kg lighter than boars and 2.2 kg heavier than surgically castrated males). No differences among treatment groups were found for dressing percentage. Contrary to our result, dressing percentage has sometimes been reported to be lower in immunocastrated males (3, 12). Several studies (3, 12, 17) reported heavier carcass weights for surgically castrated males and immunocastrated males as compared to boars, however, all of the mentioned experiments were conducted on group housed pigs, where growth performance of boars can be affected negatively due to their more aggressive social behaviour (18, 19).

Table 2: Carcass traits (mean ± se) in surgically castrated, immunocastrated males and boars

	SURGICALLY CASTRATED MALES	IMMUNOCASTRATED MALES	BOARS	*P-value*
Number of carcasses	24	24	25	
Carcass weight, kg	91.1 ± 0.85	93.3 ± 1.22	95.0 ± 1.55	0.095
Dressing, %	77.8 ± 0.31	77.1 ± 0.33	77.5 ± 0.39	0.412
Leaf fat, kg	1.3 ± 0.07^{a}	1.1 ± 0.05^{b}	0.9 ± 0.05^{c}	<0.000
DM fat, mm	18.3 ± 0.94^{a}	15.1 ± 0.67^{b}	13.2 ± 0.69^{b}	<0.000
DM muscle, mm	71.4 ± 0.99	73.5 ± 0.90	73.5 ± 0.71	0.167
DM meat, %	56.1 ± 0.72^{a}	58.8 ± 0.48^{b}	60.1 ± 0.52^{b}	0.000
LD area, cm2	47.8 ± 0.80	48.2 ± 0.88	47.5 ± 0.89	0.537
Fat over LD, cm2	17.5 ± 0.69^{a}	15.4 ± 0.49^{ab}	15.0 ± 0.68^{b}	0.014
LD meat : fat ratio	2.77 ± 0.12^{a}	3.20 ± 0.12^{ab}	3.36 ± 0.18^{b}	0.020
Ham, kg	10.9 ± 0.12	11.2 ± 0.18	11.2 ± 0.20	0.471
Ham muscle+bone, kg	8.7 ± 0.15^{a}	9.2 ± 0.17^{ab}	9.4 ± 0.17^{b}	0.014
Ham meat, %	79.7 ± 0.71^{a}	82.1 ± 0.59^{b}	84.1 ± 0.42^{b}	<0.000
Belly leanness (1-7)	4.6 ± 0.19^{a}	5.1 ± 0.12^{b}	5.2 ± 0.12^{b}	0.041
Neck fatness, %	26.0 ± 0.93^{a}	24.6 ± 0.69^{a}	21.6 ± 0.70^{b}	<0.000

LD – muscle *longissimus dorsi*; means with different letters within one row are significantly different ($P<0.05$). DM denotes the name of the Slovenian method for SEUROP classification.

As expected, surgically castrated males were fatter (P<0.05) than boars as demonstrated by several measurements on fat tissue (e.g. leaf fat, DM fat, fat over LD). On several anatomical locations (last rib, neck, ham) they also had significantly lower meat to fat ratio than boars. The immunocastrated males were positioned in between these two control groups. In case of leaf fat, the immunocastrated males were intermediate, differing (P<0.0001) either from surgically castrated males or boars. Such intermediate position of the immunocastrated males has also been demonstrated by Gispert *et al.* (3). For DM fat measurement, the immunocastrated males were closer to boars and they both exhibited lesser subcutaneous fat depot (P<0.0001) and consequently higher carcass meat % (P<0.0001) as surgically castrated males. It is worth mentioning that the difference in carcass meat % between surgically castrated and immunocastrated males was 2.7% points, which is economically important benefit in favour of the later. In the case of fat area over LD and LD meat:fat ratio, the immunocastrated males were also positioned closer to boars, however the difference was insignificant either in relation to boars or surgically castrated males. The immunocastrated males had only slightly lower belly leanness than boars, both groups having leaner belly (P<0.05) as surgically castrated males. The situation was, however different in the case of neck fatness, where the immunocastrated males had (insignificantly) lower fatness as surgically castrated males, whereas they both had fatter neck area as boars. There are several studies reporting on carcass properties of the immunocastrated males compared to the surgically castrated males or boars, however, due to the differences in slaughter weight and other experimental conditions, the results are inconsistent. Whereas one of the early studies (20) showed the immunocastrated males to be fatter from either surgically castrated males or boars, others found no differences between treatment groups (14) or reported the immunocastrated males to be closer to boars than to surgically castrated males (3, 4, 11). Additionally, two recent studies (5, 6) comparing only surgically castrated males against immunocastrated males confirmed the benefits of the later in terms of lower backfat thickness and better lean meat percentage. In summary, we could conclude, that the immunocastrated males are generally reported to be leaner than surgically castrated males and fatter than boars. It is clear that the age at vaccination and especially the timing of second vaccination is the key factor, since it determines the phase during which the animals can profit the anabolic potential of the entire male. It was, for example shown for subcutaneous fat depot (12) that the immunocastrated males, which were vaccinated earlier in life (15^{th} and 19^{th} week), were similar as surgically castrated males, whereas those vaccinated later (18^{th} and 22^{th} week) were closer to boars. Similar result was also obtained by Turkstra *et al.* (13), showing that the immunocastrated males which responded to the vaccination earlier (already after first immunization) had thicker backfat and lower carcass meat % (similar to the control surgically castrated males) as late responders (after second immunization), which were similar to boars. In the present experiment, the immunocastrated males had leaner hams than surgically castrated males and fatter hams (insignificantly) than boars. In regard to ham traits, the literature does not provide uniform data. No advantage of immunocastrated males over surgically castrated males was observed for ham weight (6), while in some cases similar ham weight was reported for immunocastrated males and boars (4, 11). As regards the ratio of fat to muscle tissue in the ham, similarly to our study, an intermediate position of the immunocastrated males has been shown in a recent experiment (3).

Meat quality

The comparison of the tested groups in regard to meat quality (Table 3) revealed significant differences only for intramuscular fat (BF marbling, LD IMF) and Minolta b*, whereas no differences were found for other parameters of meat technological quality. Regarding intramuscular fat content, the immunocastrated males exhibited similar content as boars in the case of low fat muscle like LD, whereas in the case of BF muscle, the immunocastrated males exhibited less marbling as surgically castrated males and more marbling as boars. This result corroborates with the differences observed for body composition. There was however no significant difference in LD marbling score, probably because marbling in that muscle was generally too low to detect a difference.

Table 3: Meat quality traits (mean ± se) in surgical castrates, imunocastrates and boars

	SURGICALLY CASTRATED MALES	IMMUNO-CASTRATED MALES	BOARS	*P-value*
Number of carcasses	24	24	25	
Marbling BF (1-7)	3.4 ± 0.25[a]	2.9 ± 0.15[ab]	2.6 ± 0.17[b]	0.036
Marbling LD (1-7)	1.3 ± 0.08	1.3 ± 0.07	1.2 ± 0.07	0.649
LD intramuscular fat, mg/g	19.8 ± 0.76[a]	15.8 ± 0.83[b]	15.6 ± 0.90[b]	0.001
pH_{45}	6.17 ± 0.05	6.27 ± 0.06	6.27 ± 0.06	0.306
pH_{24}	5.60 ± 0.03	5.62 ± 0.04	5.67 ± 0.04	0.375
Colour (1-6)	3.42 ± 0.10	3.46 ± 0.10	3.50 ± 0.11	0.861
Minolta L*	48.9 ± 0.59	49.2 ± 0.75	48.6 ± 0.76	0.799
Minolta a*	7.3 ± 0.19	6.9 ± 0.22	6.9 ± 0.26	0.405
Minolta b*	2.8 ± 0.23[a]	2.1 ± 0.19[b]	2.4 ± 0.26[ab]	0.049
Drip 24h, %	2.8 ± 0.43	3.7 ± 0.52	2.7 ± 0.53	0.273
Drip 48h, %	4.5 ± 0.52	5.4 ± 0.61	4.2 ± 0.64	0.304

BF – muscle *biceps femoris*; LD – muscle *longissimus dorsi;* pH_{45} – pH measured in LD 45 minutes after slaughter; pH_{24} – pH measured in LD 24 hours after slaughter; Colour (1-6) denotes use of Japanese colour scale; means with different letters within one row are significantly different (P<0.05).

Regarding the marbling score and IMF, an intermediate degree of marbling and intramuscular fat in immunocastrated males compared to leaner boars and fatter surgically castrated males was reported before (3), which is in accordance with the present study for the first, but not for the later trait. In the present study, the IMF of the immunocastrated males is closer to the one of boars. Although it is indicated in the literature, that due to their behaviour (higher level of aggressiveness and physical activity) boars could deplete muscle glycogen resulting in the effect on the ultimate pH and meat quality (18, 21, 22, 23, 24), this was not the case in the present study. In accordance with our results, no differences in pH (3, 4, 25), WHC or colour (4, 25) were reported between immunocastrated males, boars or surgically castrated males**.** However, there are studies, which reported immunocastrated males to have darker meat (L*) than surgically castrated males (26) and lighter meat than entire boars (3) and also lower drip loss than surgically castrated males (27).

Conclusions

The present study (in the local Slovenian conditions) provided additional evidence that the immunisation with Improvac® successfully reduces boar taint, improves carcass quality in comparison to surgically castrated pigs, without any major effect on meat quality.

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UČINEK IMUNOKASTRACIJE (IMPROVAC®) PRI PRAŠIČIH PITANCIH: II. KLAVNE LASTNOSTI IN KAKOVOST MESA

M. Škrlep, B. Šegula, M. Prevolnik, A. Kirbiš, G. Fazarinc, M. Čandek - Potokar

Povzetek: Preučevali smo vpliv imunokastracije (cepljenja proti gonadotropin sproščajočemu hormonu s cepivom Improvac®) na lastnosti klavnega trupa in kakovost mesa slovenskih prašičev pitancev. Poskusni prašiči (50% križanci pasme durok) so bili izbrani iz 35 gnezd (po 2 kastrata in 3-4 merjasci na gnezdo), rojeni v obdobju dveh tednov. Živali smo razdelili v tri poskusne skupine: merjasce (n=25), imunokastrate (n=24) in kirurške kastrate (n=25). Cepljenje z Improvacom ® smo opravili pri starosti 10 in 19 tednov. S poskusom smo pričeli pri starosti 12 tednov in je trajal do 24. tedna starosti, ko so bili prašiči zaklani (dve seriji zakola v dveh zaporednih tednih) po standardnem klavničnem postopku. Na klavni liniji oziroma na hladnih trupih dan po zakolu smo ocenili različne klavne lastnosti in kakovost mesa. Dodatno smo s pomočjo šestčlanskega panela ocenjevalcev izvedli senzorično ocenjevanje mesa na prisotnost vonja po merjascu. Pomembne razlike med poskusnimi skupinami smo opazili pri meritvah debeline podkožnega maščobnega tkiva, v razmerju med maščobo in mesom na stegnu in prerezu za zadnjim rebrom ter na vratu. V primerjavi s kirurškimi kastrati (najbolj zamaščeni) in merjasci (najbolj mesnati) so bili imunokastrati med obema skupinama, vendar večinoma bližje merjascem, le pri zamaščenosti vratu so bili bolj podobni kirurškim kastratom. Glede vrednosti pH, barve mesa in izceje nismo ugotovili nobenih razlik med poskusnimi skupinami. V primeru intramuskularne maščobe, ki je pomembna za senzorično kakovost mesa, smo ugotovili razlike med kirurškimi kastrati in merjasci. Imunokastrati so bili na sredini med obema skupinama v primeru marmoriranosti mišice *biceps femoris* oziroma bližje merjascem v primeru kemijsko določene intramuskularne maščobe v mišici *longissimus dorsi*. Rezultati naše raziskave so potrdili ugoden učinek imunokastracije na kakovost klavnega trupa brez posledic za kakovost mesa.

Ključne besede: prašiči; imunokastracija; klavne lastnosti; kakovost mesa

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Slov Vet Res 2010; 47 (2)

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