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TRIAL REPORT

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CASTRATES, MALE PIGS AND IMMUNOCASTRATES – BEHAVIOUR & PENILE INJURIES

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Main conclusion

Male pigs and immunocastrates behaved more aggressively than castrates until the second vaccination, hereafter the immunocastrates behaved more like castrates. Recordings showed very low and no differences in penile injuries between male pigs and immunocastrates or in the prevalence of gastric ulcers between castrates, male pigs and immunocastrates.

Abstract

This report concerning behaviour, penile injuries and gastric ulcers is part of a comprehensive study with castrates, male pigs and immunocastrates. Analyses demonstrated that male pigs and immunocastrates exhibit significantly more aggressive behaviour and mounting behaviour than castrates until the second vaccination. From this point, immunocastrates started behaving more like castrates: they were less aggressive and there was less pushing and mounting than among the male pigs. The degree of penile injuries was very low and the same for castrates, male pigs and immunocastrates, but castrates had a higher occurrence of undeveloped penises. The prevalence of gastric ulcers did not differ between castrates, male pigs and immunocastrates.

As expected, male pigs were more active/more aggressive than castrates and immunocastrates after the immunocastrates had received the second vaccination, which is in line with findings in international studies. However, the overall low frequency of penile injuries and the lack of difference between male pigs and immunocastrates are rather surprising compared with several German studies that generally

found a very high prevalence (60-95%) of penile injuries in male pigs and a somewhat lower prevalence in immunocastrates (8-40%) [1,3].

This trial report solely concerns analyses of behaviour, penile injuries and prevalence of gastric ulcers. For an in-depth description of trial design and materials and methods, please see trial report no. 1219 [12].

Permission was granted by the Danish Centre for Animal Welfare to perform biopsies and blood sampling. Permission j.no. 2016-15-0201-01080.

Background

Production of male pigs includes a range of advantages compared with production of castrates:

1. No surgical castration (elimination of tasks related to castration, administration of pain relief and local anaesthesia, and lower mortality rates among male piglets).
2. Feed conversion ratio and lean meat percentage are better in male pigs compared with castrates.
3. Improved utilization of nutrients in the feed = lower carbon footprint.

Production of male pigs is generating increasing interest in several European countries for animal welfare reasons. However, several of the Denmark's main export markets still refuse to buy pork from male pigs due to, for instance, the risk of receiving meat with boar taint.

When male pigs approach sexual maturity their behaviour changes; they become more aggressive, they mount each other and exhibit explorative behaviour connected with sexual maturity, which increase the risk of injuries [3], such as injuries to legs and skin (rind-side damage which will negatively affect the carcass value). There is also a risk of penile injuries when the pigs mount/attempt to mate each other [1]. This challenge may be helped by either slaughtering the pigs before they reach sexual maturity or by introducing immunocastration that supresses the testicular function. Antibodies against anti-gonadotropin-releasing-hormone (GnRH) develop 4-14 days after the second vaccination by which point the testicle-hormone synthesis stops completely and changes in behaviour set in [4], [5]. There are also undocumented claims that male pigs have a higher prevalence of gastric ulcers than castrates and immunocastrates.

Male pigs are vaccinated twice with Improvac® containing GnRH at approx. 30 kg and 4-6 weeks before slaughter. As with all other vaccines, Improvac® (sold by Zoetis) triggers active immunisation against GnRH. This stops the secretion of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) in the pituitary gland thereby inhibiting the production testosterone and androstenone in the testicles. Skatole is indirectly reduced as skatole decomposition in the liver is not inhibited by androstenone [2].

Penile injuries

In castrates and male pigs, penis is fixed in the preputial sheath. When male pigs reach sexual maturity, they become able to erect their penis, as opposed to castrates. Studies made at German slaughterhouses found highly varying degrees of penile injuries: from small lesions to severe lesions and scars. This is reflected in the study made by Holinger *et al.* [6] who found penile injuries in 3% of the male pigs, whereas Isernhagen [7] found penile injuries in up to 82% of the male pigs examined. Analyses made by Weiler *et al*. [1] revealed penile injuries in 64-95% of the examined pigs with increasing prevalence with age. Severe penile injuries with infection or neurotized tissue were found in 9% of the male pigs [6]. Immunocastration may lower the frequency of penile injuries [8] [9], but some studies also found fresh lesions in immunocastrates [3]. The scars observed at slaughter in immunocastrates may be attributed to injuries inflicted before the 2nd vaccination.

Behaviour

Due to the elevated testosterone concentration, male pigs exhibit a significantly higher degree of sexual and aggressive behaviour. Mounting behaviour may cause injury to legs and skin of the victims. Furthermore, male pigs more frequently engage in fights for dominance, which primarily cause injury to the skin. This is typically observed during mixing of pigs [10].

Gastric ulcer

Very few studies have investigated the prevalence of gastric ulcers in male pigs, castrates and immunocastrates. One of these studies found no difference between the three genders [11].

The aim of this part of the study was to determine the effect of immunocastration on behaviour, penile injuries and gastric ulcers. This is analysed by

1) Analysing aggressive behaviour and mounting behaviour in all three genders
2) Determining the percentage of penile injuries
3) Determining the prevalence of gastric ulcers in castrates, male pigs and immunocastrates.

Results concerning productivity and boar taint in castrates, male pigs and immunocastrates, and economy are published in two separate trial reports [12] [13].

Materials and methods

The trial comprised six groups: castrates, male pigs and immunocastrates sired by high or low-androstenone boars, respectively (table 1).

Table 1. Trial design

Androstenone fathers	High	Low
Castrates	120	120
Male pigs	120	120
Immunocastrates	120	120

This trial report solely concerns analyses of behaviour, penile injuries and gastric ulcers. For an in-depth description of trial design and materials and methods, please see trial report no. 1219 [12].

This part of the trial included the pigs from the final insertion: 96 castrates, 58 male pigs and 59 immunocastrates.

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Permission to conduct the study was granted by the Danish Medicines Agency. Case 2018071166 concerning medicines experiments with animals.

Age

The pigs were approx. 82 days old (approx. 12 weeks) at transfer. The immunocastrates received the second vaccination when they were averagely 115 days old (approx. 16 weeks), and all the pigs were slaughtered when they were averagely 150 days old (approx. 21 weeks).

Behavioural observations

Behaviour among 96 castrates, 58 male pigs and 59 immunocastrates was filmed on three observation days:
1. One week after transfer (at the first vaccination of immunocastrates).

2. Four weeks after transfer (before the second vaccination of immunocastrates).
3. Seven weeks after transfer (3.5 weeks after the second vaccination of immunocastrates).

The pigs were filmed in the period 7 am to 7 pm with a camera suspended from the ceiling and all pigs were individually marked for identification purposes. Behaviour was recorded with frequency observation: a note was made every time one of the three pre-defined activities, aggressive behaviour, pushing and mounting behaviour, was observed. A detailed definition of the activities is provided in the ethogram shown in appendix 1.

Penile injuries

Penises were removed at the slaughterline and were immediately examined for injuries. All penises were photographed as documentation for the injuries. A few penises were too damaged by the slaughter process that it was impossible to determine whether there were any lesions.

Injuries were graded according to the below scale (images in appendix 2):

1. No injuries (figure 1)
2. Scars (figure 2)
3. Fresh injuries caused by biting (figure 3)
4. Severe injuries (figures 4, 5, 7, 8) or
5. Infection (figures 6, 7, 8)
6. Not developed – penis is stuck in the preputial sheath (figure 9).

If more than one score was assigned to one penis, only the highest score was included in the data analysis.

Gastric ulcer

At slaughter, stomachs were removed and forwarded to the Laboratory for Pig Diseases in Kjellerup for evaluation of gastric ulceration according to the below scale:

0. No visible keratinisation, no erosion or ulcers, no scar formation
1. Keratinisation <1 mm
2. Keratinisation >1 mm
3. Keratinisation is papillomatous
4. Erosion <½ cm in diameter
5. Erosion >½ cm in diameter
6. Small superficial ulcers <½ cm or slight scar formation
7. Medium ulcers ½-2 cm or less if they are deep or scar formation with slight fibrosis
8. Large ulcers >52 cm or less if they are deep or scar formation with clear fibrosis
9. Contracted oesophagus, but diameter of oesophagus >½ cm
10. Oesophagus diameter <½ cm.

For a detailed description, see appendix 3.

Statistical models

The trial was designed as a split plot trial on offspring of AI boars split according to AI boars' androstenone level (low = below 1.5 ppm or high = above 2.38 ppm) as whole-plot and three genders as sub-plot. All recordings were made on each individual pig, but a trial unit was constituted by the father (AI boar). In pens where behavioural observations were made, the pen constituted the unit.

The primary variables – penile injuries and gastric ulcers – were split into yes/no (penises with/without injuries, gastric ulcer above or below score 6) and subject to analysis in a generalised linear model

with gender and AI boar androstenone level as systematic effects and with regard made to repeat recordings on the same AI boar.

Behavioural recordings were subject to analysis in a generalised linear model with gender, AI boar androstenone level and pigs' age as systematic effects and with repeat recordings within a pen.

Results and discussion

Behaviour

The displays of aggressive behaviour, mounting and pushing dropped with increasing age for all three genders. The highest frequency of all three types of behaviour was observed at transfer in connection with fights to establish ranking. There were no differences in behaviour between male pigs and immunocastrates at transfer and before the second vaccination, but less aggressive behaviour and pushing were observed in pens housing castrates. After the second vaccination, fewer cases of aggressive behaviour, mounting and pushing were observed among castrates and immunocastrates than among male pigs (tables 2 and 3).

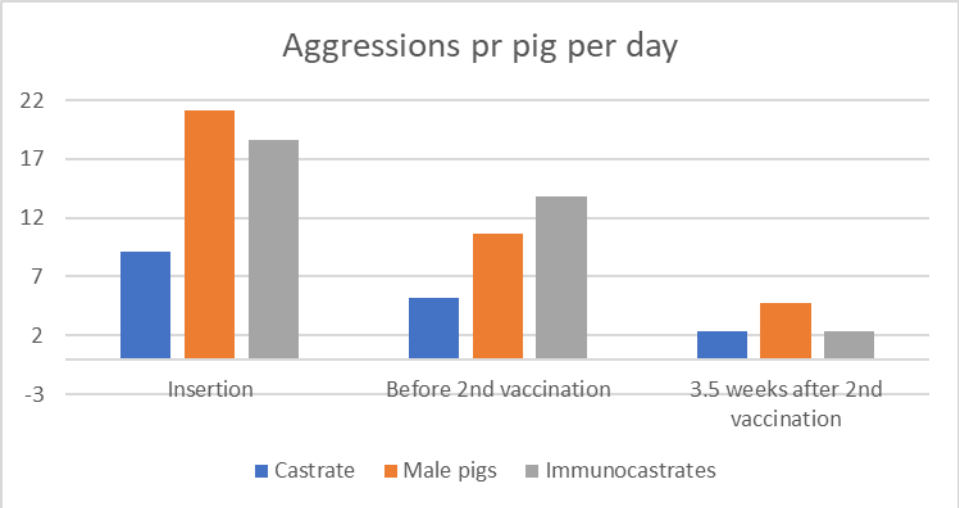

Figure 1. Aggressions per pig/day at transfer, before the second vaccination and 3.5 wks after the second vaccination of immunocastrates.

Table 2. Aggressions per day/pig at transfer, before the second vaccination and 3.5 wks after the second vaccination of immunocastrates.

Time of activity (age)	Castrate	Male	Immunocastrate	P-value, gender, across time
Transfer (82 days)	9.1[a]	21.1[b]	18.6[b]	p<0.01
2nd vaccination (115 days)	5.2[a]	10.7[b]	13.8[b]	
3.5 wks after 2nd vaccination (140 days)	2.4[a]	4.8[a]	2.3[a]	
P-value across gender	p<0.01			

a,b: Different superscripts indicate significant difference p<0.05.

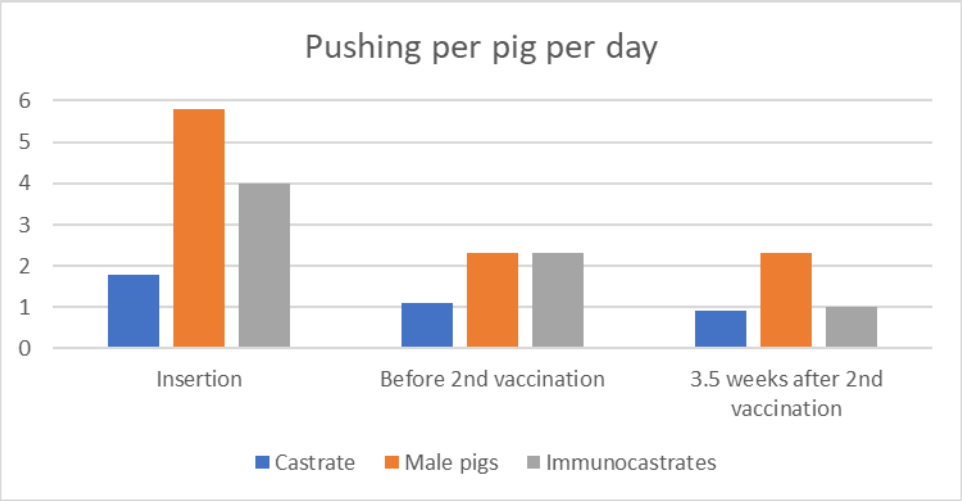

Figure 2. Pushing per pig/day at transfer, before the second vaccination and 3.5 wks after the second vaccination of immunocastrates.

Table 3. Pushing per pig/day at transfer, before the second vaccination and 3.5 wks after the second vaccination of immunocastrates.

Time of activity (age)	Castrate	Male	Immunocastrate	P-value, gender, across time
Transfer (82 days)	1.8[a]	5.8[b]	4.0[b]	p<0.01
2nd vaccination (115 days)	1.1[a]	2.3[b]	2.3[b]	
3.5 wks after 2nd vaccination (140 days)	0.9[a]	2.3[b]	1.0[a]	
P-value across gender	p<0.01			

a,b: Different superscripts indicate significant difference p<0.05.

The lowest occurrence of mounting behaviour was observed among castrates and the highest among male pigs and immunocastrates. At all three observation days, fewest displays of mounting behaviour were observed among castrates and the most displays among male pigs. For immunocastrates and male pigs, the frequency of mounting behaviour at transfer and before the second vaccination was identical, and 3½ weeks later it dropped to the same level for immunocastrates as for castrates and was thereby significantly less frequent than for the male pigs (table 4).

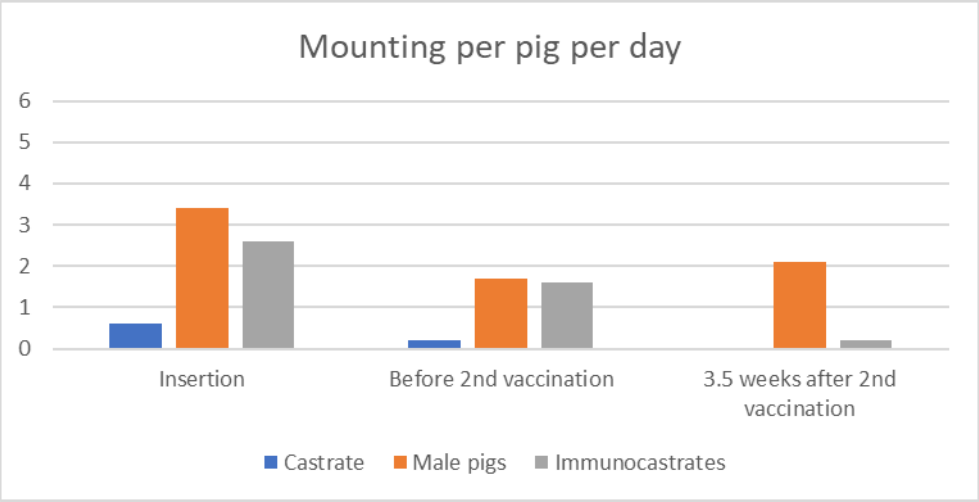

Figure 3. Display of mounting behaviour per pig/day at transfer, before the second vaccination and 3.5 wks after the second vaccination of immunocastrates.

Table 4. Display of mounting behaviour per pig/day at transfer, before the second vaccination and 3.5 wks after the second vaccination of immunocastrates.

Time of activity (age)	Castrate	Male	Immunocastrate	P-value, gender, across time
Transfer (82 days)	0.6[a]	3.4[b]	2.6[b]	p<0.01
2nd vaccination (115 days)	0.2[a]	1.7[b]	1.6[b]	
3.5 wks after 2nd vaccination (140 days)	0.02[a]	2.1[b]	0.2[a]	
P-value across gender	p<0.01			

a,b: Different superscripts indicate significant difference p<0.05

The occurrence of mounting behaviour is significantly correlated with the testosterone concentration of the male pigs and immunocastrates where high levels correlate positively to mounting behaviour.

Displays of aggression, pushing and mounting behaviour among male pigs drop as the pigs grow older as pigs initially engage in fights for ranking, and once hierarchy is established, aggressive behaviour is triggered by scarcity of resources such as lack of feed or water [14]. Overall, the behavioural observations are in line with findings in international studies from Holinger *et al.* [6] and Rydhmer *et al.* [14]. The rise in aggressive behaviour among male pigs compared with castrates was also seen in previous Danish studies observing behaviour in the slaughterhouse holding pens where 24% of the male pigs exhibited sexual behaviour. Furthermore, more male pigs (60%) than female pigs and castrates (43% and 28%) exhibited aggressive behaviour, but with large individual differences [15]. A Danish study comprising approx. 2 million slaughterings (166 herds with castrates and 25 with male pigs) found a significantly higher occurrence of skin lesions, abscesses on the fore end and on the middle on the carcass among male pigs than castrates [16] which is likely attributed to injuries caused by aggressive behaviour and mounting behaviour.

Behaviour in relation to hormone levels

In immunocastrates, testosterone concentrations above 0.5 ng/ml indicate suboptimum effect of the vaccine [5]. About 30% of the observed immunocastrates had testosterone concentrations above 0.5 ng/ml and performed ten times as many mounts as the pigs with testosterone concentrations below 0.5 ng/ml. Immunocastrates with testosterone concentrations above 0.5 ng/ml also displayed 1.7 times more aggressive behaviour. All male pigs had testosterone concentrations above 0.5 ng/ml.

By ranking the male pigs according to androstenone level (recorded at slaughter), approx. five times more mounts were recorded among high-androstenone male pigs (>2.0 ppm) than among low-androstenone pigs. The effect on aggressive behaviour was slightly lower as only 35% more aggressions were observed among high-androstenone male pigs.

Penile injuries

Results showed no effect of AI boar (high or low androstenone) or any interaction between AI boar and gender. There were no differences in the number of penises without injury between genders (table 5). An international study of three herds found 77-91% male pigs with injured penises [1], and another study found 71.2% penises with scars and 17.2% penises with lesions among male pigs and 44.8% penises with scars and 8.3% penises with lesions among immunocastrates [8].

The highest number of undeveloped penises was found in castrates (88%) versus only 4-5% among male pigs and immunocastrates, which is in line with findings in other studies [1]. Only 16 castrates were included in the analysis of penile injuries, as penis was either undeveloped or too damaged by

the slaughter process. The number of penile injuries and the percentage of each type of injury did not differ between immunocastrates and male pigs (table 6).

Table 5. Penile injuries, %.

Gender	Castrate	Male	Immuno-castrate
Number of penises collected	218	197	196
No injuries (number)	208	170	166
(%)	*87*	*89*	*92*
Not developed (number)	192	5	4
(%)	*88*	*3*	*2*
Lost – slaughter injury (number)	10	2	12
(%)	*5*	*1*	*6*

Table 6. Penile injuries recorded on male pigs and immunocastrates (castrates excluded as only 16 had a developed penis, of which 2 had an infection).

Gender	Male	Immuno-castrate
Lesion (number)	10	9
(%)	*5*	*5*
Scar (number)	10	11
(%)	*5*	*6*
Severe injury (number)	2	1
(%)	*1*	*1*
Infection (number)	8	2
(%)	*4*	*1*

Gastric ulcer

Recordings showed no differences in the prevalence of gastric ulcers between castrates, male pigs and immunocastrates regardless of whether this was determined as frequency over a certain cut-off value or as an average score. Analyses found no interaction between gender and AI boar and no effect of AI boar on the prevalence of gastric ulcer.

Conclusion

Analyses demonstrated that male pigs and immunocastrates exhibit significantly more aggressive and sexually oriented mounting behaviour than castrates until the second vaccination. From this point, immunocastrates started behaving more like castrates: they were less aggressive and showed less pushing and mounting behaviour than among the male pigs. The degree of penile injuries was the same for castrates, male pigs and immunocastrates, but castrates had a higher occurrence of undeveloped penises. The prevalence of gastric ulcers did not differ between castrates, male pigs and immunocastrates.

As expected, male pigs were more active/more aggressive than castrates and immunocastrates after the second vaccination, which is in line with findings in international studies. However, the overall low frequency of penile injuries and the lack of difference between male pigs and immunocastrates are rather surprising compared with several German studies that generally found a very high prevalence (60-95%) of penile injuries in male pigs and a somewhat lower prevalence in immunocastrates (8-40%). This is the first study investigating the prevalence of gastric ulcer in castrates, male pigs and immunocastrates.

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Participants

Technical assistance: Per Mark Hagelskjær, Henry Aalbæk & Peter Juhl Rasmussen.

Trial no. 1553

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Appendix 1

Ehtogram

Observations – each time one of the below activities was observed, activity and time were recorded for each individual pigs

BEHAVIOUR	No. + definition	No. + description
Aggressive / reactive social behaviour	1. Aggression – attacks 2. Aggression – receiver (victim) 3. Heavy push/shove - aggressor 4. Heavy push/shove – victim 5. Frequent push/shove – aggressor 6. Frequent push/shove – victim	1. Pig instigates fierce fighting, head knocking or biting another pig*. 2. Victim responds aggressively: fierce fighting, head knocking or biting another pig*. 3. Pushes/shoves another pig to make this pig move involuntarily. 4. Victim is pushed/shoved and moves involuntarily. 5. Pushes/shoves the same pig min. 4 times. 6. Victim is pushed/shoved min. 4 times by the same pig.
Sexual behaviour	7. Mounting - aggressor 8. Mounting – receiver (victim)	7. Mounting, mating behaviour 8. Is being mounted
* Sudden exhibit of aggressive behaviour between two or more pigs. If it is unclear which pig is the instigator, the pigs involved are classified as category 1.		

Appendix 2

Assessment of penis injuries:

1. No injuries (figure 1)
2. Scars (figure 2)
3. Fresh injuries caused by biting (figure 3)
4. Sever injuries (figures 4, 5, 7, 8) or
5. Infection (figures 6,7,8)
6. Not developed, ie. penis is stuck in the penile frenulum (figure 9).

Figure 1. Specimen without injuries (DB).

Figure 2. Specimen with multiple scars (DB).

Figure 3. Specimen with fresh injuries (DB).

Figure 4. Specimen with suppuration (DB).

Figure 5. Specimen with partial loss (DB).

Figure 6. Specimen with severe injury (DB).

Figure 7. Specimen with partial loss/necrosis (DB).

Figure 8. Specimen with hematomas and injuries (WB).

Figure 9. Not developed

Appendix 3

Gastric index	Evaluation the white part of the stomach	Description
0	No visible keratinisation No erosion or ulcers No scar formation	The white part of the stomach by the mouth of the oesophagus is white, shiny, smooth and elastic.
1	Keratinisation below 1 mm	Keratinisation: Mucosa around the mouth of the oesophagus gradually changes structure (keratinises)into cusp regeneration.
2	Keratinisation over 1 mm	
3	Keratinisation are papillomatous	
4	Erosion in < ½ cm in diameter	Erosion: the protective layer of mucosa has disappeared resulting in direct access to the underlying, sensitive tissue.
5	Erosion in > ½ cm in diameter	
6	Small superficial ulcers <½ cm or slight scar formation	Ulcer: Deep changes in the mucosa, possibly bleeding. Scar: Old injuries partially healed during scar formation. During scar formation, fibrous tissue (fibrosis) forms and the tissue turn inelastic and contracts.
7	Medium ulcers ½-2 cm or less if they are deep or scar formation with slight fibrosis	
8	Large ulcers >52 cm or less if they are deep or scar formation with clear fibrosis.	
9	Contracted oesophagus, but diameter of oesophagus >½ cm	Scar: Old injuries partially healed during scar formation. During scar formation, fibrous tissue (fibrosis) forms and the tissue turn inelastic and contracts. In the most severe degrees, the mouth of the oesophagus contracts to a narrow, inelastic aperture.
10	Oesophagus diameter <½ cm	

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