Transport stress - consequences for bacterial translocation, endogenous contamination and bactericidal activity of serum of slaughter pigs

Transportstress – Konsequenzen für die bakterielle Translokation, endogene Kontamination und bakterizide Aktivität im Serum von Schlachtschweinen

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Summary:
On transport and at the abattoir animals are confronted with a lot of stressors, such as sound/noise, crowding/mixing, pollutants and infectious agents that act on the organism. After transport stress an endogenous contamination is often seen in slaughter carcasses and presents a hazard for the consumer. These events are often correlated with a rise in endotoxin level (Misawa et al., 1995; Morales et al., 1992) and a modified immune response. Previous own investigations confirm this hypothesis (Zucker and Krüger, 1998, Seidler et al., 2000). The attempt was made to investigate the impact of selected stressors (short term transport (1 h), long term transport (7-8 hrs), high temperature, high humidity and intense handling/moving) on bacterial translocation, endogenous contamination, endotoxin levels and bactericidal activity of body fluids.

Keywords: Slaughter pigs, endogenous contamination, bacterial translocation, transport, endotoxin

Introduction
Bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal (GI) tract through the epithelial mucosa to other sites. Causes for this process are imbalances in homeostasis, like disruption of the indigenous flora, bacterial overgrowth, impaired host immune defence or physical disruption of mucosal barrier (Berg, 1995). As sources for translocation are known abscesses, profound wounds and the permanent priming by autochthonous flora, but also temporary translocation of transient pathogens.
A lot of defence mechanisms exist by macroorganism to protect against entry of bacteria - natural, indigenous GI flora, intact intestinal barrier and an intact immune system. Non-specific components are saliva, stomach acids and enzymes, bile salts, water and electrolyte secretion, mucus, epithelial barrier itself, peristalsis etc. On heat shock protein (hsp, able to restore denatured proteins) a strong correlation between gene expression and amount of intestinal colonisation is described. Macrophages (MØ) mediate phagocytosis, antigen presentation and cytokine induction, and their nitric oxid (NO) synthase inactivates bacterial key enzymes. Radical inactivators and lysozyme, complement and acute phase proteins prevent bacterial growth as well.

Specific components are presented by a large immune organ of the body - GALT presents more than 40 % of immune effector cells, distributed in three compartments: aggregates in Peyer’s patches (PP), lymph follicles and diffuse elements in mucosa. A special structure in dome-epithelium shows the M cell, forming an intraepithelial pocket to present antigens (ag) to lymphocytes and MØ. Some peculiarities in pig are two separate categories of PP (25 - 30 small all one's life in jejunum and proximal ileum, one large - 2,5 m - in terminal ileum involuted after one year) and the MHC II molecule expression only by cells of subepithelial lamina propria, not by intestinal epithelial cells. This demonstrates the impossibility to transfer results on antigen presentation from laboratory animals to domestics.

Two different hypothesised routes for bacterial translocation through GI epithelia are described (Berg, 1995). More common seems to be an intracellular passage \( \rightarrow \) lymphatic way \( \rightarrow \) mesenteric lymph nodes (MLN) \( \rightarrow \) blood stream \( \rightarrow \) heart \( \rightarrow \) systemic spread. Another way, especially after challenge is extracellular passage \( \rightarrow \) vascular route via portal vein \( \rightarrow \) liver \( \rightarrow \) back in bloodstream \( \rightarrow \) heart \( \rightarrow \) systemic spread.

An intestinal bacterial overgrowth (1.) spreads bacteria to MLN, whereas intestinal mucosal injury (2.) causes invasion of spleen and liver. Compromised immune defences (3.) are marked by translocation in bloodstream. A combination of the three mechanisms can lead to death. First mechanism is seen after starvation, use of oral antibiotics or gut atony, second can be caused by ischemia or reperfusion lesions. The third will be recognised after psychical/physical or climatic stress, etc. (Fink, 1994; Salzmann et al., 1994; Berg, 1995).

On transport animals are often confronted with a lot of stressors, additionally to the described crowding, sounds/noise, pollutants and infectious agents. In the past differences seen in catecholamines or glucocorticoids were interpreted as a reaction of exogenous stressors (Cort et al., 1990). Less is focussed on the organism himself - after local inflammation or antigen contact activated MØ liberate cytokines which act on higher brain centres, stimulate ACTH secretion and liberation of stress
hormones by adrenals - showing the bidirectionality of immune-neuroendocrine interactions (Olson et al., 1995; Delrue-Perolet et al., 1995; Savino and Dardenne, 1995).

In stress enhanced catecholamines cause increased sympathicotonus, sphincter contraction and atony of bowel (Meier-Hellmann and Reinhardt, 1995); increased levels in glucocorticoids are able to blockade or cut surface receptors on immune cells (Diez-Fraile et al., 1999). Consequences are ischemia, increased adherence and translocation rate of GI flora and an impaired clearance by cellular defence mechanisms (see also Figure 1).

As a result of non-satisfying answers by characterising stress situations on transported animals we are focussing on other parameters. In the late 80's a correlation between bacterial endotoxin, immune parameters and translocation of pathogens was shown on humans and laboratory rodents (O'Dwyer et al., 1988; Deitch et al., 1991). One component of the outer membrane of the cell wall of gram-negative bacteria is endotoxin (syn. LPS - lipopolysaccharide) an amphiphilic molecule, assembled on repeating units of sugar forming polysaccharides, a core region and lipid A.
LPS is responsible for a wide range of biological activities. Beside the common systemic reactions an internalisation in membranes was shown previously, disturbing fluidity and other functions of these biological structures. Last possible pathomechanism is the mimicry of ceramide, a second messenger mediating apoptosis (programmed cell death) and growth suppression - possibly acting as immune effector cell depression (Pushkareva et al., 1995; Wright and Kolesnick, 1995; Nakabo and Pabst, 1997).

Material and methods
In reflection to time dependence we surveyed short (1 h) and long (7-8 hrs.) time transports. To characterise extreme conditions we focussed in other trials on the influence of temperature, air humidity and carrying/moving. Blood samples of male slaughter pigs (~ 100 kg body mass) were taken intravenously before the onset of the stressor, during the action of the stressor and after the end of the stressor action. Detected parameters were bacterial translocation rate in lymph nodes (MLN) and organs (liver, spleen, kidney and quadriceps muscle), blood cell count (especially lymphocytes), free endotoxin concentration in plasma, the serum bactericidal activity and CH-50 values.

Results
We were able to confirm the effect of extreme temperatures as well as moving to increased endotoxin level and a higher bacterial contamination rate of body lymph nodes and different organs. Furthermore we were able to confirm the effect of
extreme temperatures and moving over a distance of 200 m to increased endotoxin level and the bacterial translocation rate (contamination rate $C_R$), shown in Figure 2.

On short term transports we could not demonstrate a correlation between bacterial endotoxin and bacterial translocation in the examined tissues. In contrast, long term transports showed this connection (Figure 3).

The serum bactericidal activity and CH-50 values decreased after severe stressor action. Vice versa by *in vitro* bacterial inoculation of serum samples of stressed animals - different bacterial strains were able to survive and even multiply in contrast to serum of non-stressed animals, where the serum still possessed a higher bactericidal potential.

In conclusion, severe stressful situations do not only increase the translocation rate of bacteria through the gut wall and increase the free endotoxin level but at the same time diminish immunological defence mechanisms in serum resulting in a higher contamination rate of different organs. Free bacterial endotoxin in plasma could be established as a potential parameter for stress and endogenous contamination after long term transports.

**References**


Nakabo, Y., Pabst, M. J. (1997): C2-ceramide and C6-ceramide inhibited priming for enhanced release of superoxide in monocytes, but had no effect on the killing of leukemic cells by monocytes. Immunology 90, 477 - 482.


Fig. 1: Effects of catecholamines and glucocorticoids on endogenous contamination

![Diagram showing effects of stress on intestinal flora](image)

consequences:
- ischemia
- increased adherence of intestinal flora
- increased translocation rate
- impaired clearance by cellular defence mechanisms

Fig. 2: Endotoxin/contamination rate in relation to environmental conditions

![Graph showing endotoxin/contamination rate](image)
Fig. 3: Endotoxin/contamination rate on short time transport

Fig. 4: Impact of stress-situations on the degree of endogenous contamination