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SHORT COMMUNICATIONS

Short communication: preliminary observations on the serum levels of HSP70 and its correlation with serum cortisol, thyroid hormones, and acute-phase protein concentration in cattle naturally infected with foot-and-mouth disease virus

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Abstract
The study aimed to explore the serum levels of HSP70 and identify its possible association with serum cortisol, thyroid hormones, and acute-phase protein concentrations in cattle naturally infected with foot-and-mouth disease (FMD) virus. After the FMD outbreak in an organized dairy cattle farm in India, blood samples were obtained from clinically infected ($n=40$) and apparently healthy ($n=30$) animals. Samples were processed and tested by an in-house DIVA assay for confirmation of FMD infection. Serum was analyzed for HSP70, cortisol, T4, T3, haptoglobin, and serum amyloid A by enzyme-linked immunosorbent assay (ELISA). HSP70 concentrations were significantly higher in the serum of clinically infected cattle ($p<0.01$) than the healthy group. To the best of our knowledge, this is the first report describing the elevated serum levels of HSP70 under infectious diseases of bovines. Cortisol ($p<0.05$), haptoglobin ($p<0.001$), and serum amyloid A ($p<0.05$) concentrations also markedly increased in the diseased animals; however, no differences ($p>0.05$) were found in T4 and T3 levels between healthy and infected cattle. Elevated HSP70 concentration correlated positively with high cortisol ($p<0.05$) and haptoglobin ($p<0.001$) levels suggesting an essential link between these acute events during clinical infectious phase of FMD.

Keywords Serum HSP70 · Cortisol · Thyroid hormone · Acute-phase proteins · Foot-and-mouth disease

Introduction

Foot-and-mouth disease (FMD) is one of the most contagious and highly infectious viral diseases of cloven-hoofed animals with a devastating impact on the livestock economy. Clinical signs of FMD are high fever, anorexia, hypersalivation, blisters on the foot and mouth, and lameness. The disease is rarely fatal in adult animals but often leads to high morbidity and decreased productivity that persists even after recovery from clinical infection (Chowdhury et al. 1993; Ferrari et al. 2014). In India, FMD is endemic and poses a constant risk to the livestock sector.

Heat shock proteins (HSPs) are a group of evolutionary conserved proteins present nearly in all cells in all life forms. In the last few decades, HSPs have received much attention for their intracellular chaperone activity during thermal shock. In addition, the involvement of HSPs in other stressful situations including bacterial and viral infections has also been confirmed (Ungar-Waron et al. 1996; Njemini et al. 2003). Although HSPs are primarily intracellular, they can be released into the extracellular milieu during stress (Vega et al. 2008). Within the family of HSPs, HSP70 is the most abundant and well-characterized protein chaperone (Hunt and Morimoto 1985). In farm animals, several studies have documented HSP70 levels in the blood (Kristensen et al. 2004) and other biological fluids (Lamy et al. 2017), and peripheral HSP70 levels have been reported to be elevated in cattle during heat stress (Mishra et al. 2011; Gaughan et al. 2013). Heat shock response following infection is one of the intriguing but less studied aspects. Study in human subjects

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has proposed extracellular HSP70 level as a criterion to distinguish between patients with infection and healthy individuals (Njemini et al. 2003). Previously, over-expression of HSP70 has been reported in FMD virus infected heart tissues of lambs (Gulbahar et al. 2011). In recent studies, HSP70 when used as an adjuvant in novel FMD vaccine formulations boosted immune response in mice and pigs (Sedeh et al. 2014; Lee et al. 2020). At this juncture, understanding impact of FMD viral infection on HSP70 response at the whole organism level may be insightful and informative.

Infection burdens are often accompanied by endocrine alterations and inflammatory responses. Cortisol is well-recognized as the major stress hormone in mammals (Martin et al. 2011). Many stressors including disease processes can activate the hypothalamic–pituitary–adrenal (HPA) axis. Activation of the HPA axis can mediate metabolic and immune system effects (Bailey et al. 2003). The published literature on the impact of FMD on serum cortisol levels is limited and the results are contradictory (Ghanem and Abdel-Hamid 2010; Barkakati et al. 2016). It has been further postulated that FMD viral infection interferes with the hypothalamic-pituitary-thyroid axis (Artz et al. 2011). Few researchers have investigated thyroid functionality during experimental FMD (Maddur et al. 2011; Saravanan et al. 2020); however, serum T3/T4 levels following natural FMD infection in cattle are still largely unknown.

Acute-phase proteins (APPs) are hepatocyte derived proteins whose blood concentrations change in response to infection, inflammation, or stress (Murata et al. 2004). APPs are used in veterinary medicine as additional biomarkers for animal disease diagnoses (Godson et al. 1996). Haptoglobin and serum amyloid A are the two major APPs (Horadagoda et al. 1999) in cattle. The degree of change in APPs was related to the clinical severity of FMD (Merhan et al. 2017). However, the association between acute-phase response protein and hormonal changes during FMD viral infection is poorly understood. Moreover, whether these changes are associated with circulating HSP70 concentrations is not known. Therefore, the present study aimed to investigate serum levels of HSP70 and its correlation if any with serum cortisol, thyroid hormones, and acute-phase protein concentration in FMD infected and clinically healthy cattle. To the best of our knowledge, changes in serum concentrations of HSP70 have not been explored under pathological conditions of bovines.

Materials and methods

After the FMD outbreak in natural conditions in an organized dairy (Holstein Friesian × Zebu) cattle herd in India, blood samples were collected from diseased ($n = 40$) and apparently healthy cattle ($n = 30$). The serum was separated after centrifugation at 3000 rpm for 10 min. Serological confirmation of FMD infection was done by in-house 3AB3 based indirect DIVA ELISA (Mohapatra et al. 2011). Concentrations of HSP70 (MBS7606199), cortisol (MBS028594), thyroxine (T4) (MBS017877), tri-iodothyronine (T3) (MBS014516), haptoglobin (MBS033026), and serum amyloid A (MBS041375) were estimated by commercial ELISA (MyBiosurce, SanDiego, California, USA) kits. Details regarding intra-assay and inter-assay coefficients of variation are presented in Supplementary Table S1. An unpaired t test was applied to compare the group means for various parameters. Pearson's correlation coefficient was used to find the association of HSP70 level with other serum parameters for each group. Scatter plot with simple linear regression was used to statistically describe the related variables. A p value < 0.05 was considered statistically significant. Graphpad prism software version 5 (GraphPad Software, Inc., San Diego, CA) was used for the statistical analysis of the data.

Results and discussion

The mean (± SE) concentrations of the blood parameters are presented in Table 1. In the present study, HSP70 was detectable in the sera of both group of animals and values are comparable with other observations for extracellular HSP70 concentration in cattle (Gaughan et al. 2013; Lamy et al. 2017). Serum levels of HSP70 were significantly higher ($p < 0.01$) in FMD infected animals than apparently healthy ones. Rise in circulating HSP70 possibly reflecting induced

Table 1 Comparison of blood serum parameters in healthy and FMD infected cattle

Serum parameter	Healthy (FMD negative)	Infected (FMD positive)	p value
HSP70 (ng/ml)	7.89 ± 0.43^{a}	13.45 ± 1.69^{c}	0.004
Cortisol (ng/ml)	76.79 ± 11.36^{a}	130.0 ± 19.14^{b}	0.019
T4 (nmol/L)	92.08 ± 11.20	108.60 ± 10.44	0.308
T3 (nmol/L)	0.47 ± 0.02	0.56 ± 0.08	0.242
Haptoglobin (µg/ml)	229.40 ± 23.74^{a}	506.72 ± 57.12^{d}	0.0005
Serum amyloid A (µg/ml)	6.86 ± 0.26^{a}	8.79 ± 0.34^{b}	0.012

Values with different superscripts differ significantly in a row $^{a,b}p < 0.05$, $^{a,c}p < 0.01$, and $^{a,d}p < 0.001$

intracellular production of this stress protein. However, the origin of free HSP70 in circulation is still not defined and implies multiple cellular provenances (Mambula and Calderwood 2006). There are also suggestions that fever in the face of acute infection can induce HSP70 in intracellular and extracellular space (Gupta et al. 2013). Increased circulating HSP70 during FMD progression presumably stimulate the host defense mechanism; however, further studies are warranted to establish whether elevated extracellular HSP70 level play pro-viral or pro-host function in FMD.

Stress imposed by any disease further impacts animal health and performance. Serum cortisol levels in FMD infected cattle were significantly higher ($p < 0.05$) in relation to healthy animals. High magnitude of systemic cortisol refers to disrupted homeostasis in FMD and this may be an outcome of increased stress on the animals due to infection. On the other hand, we observed a marginally higher serum T4 concentration in infected animals but the overall changes were not statistically significant ($p > 0.05$). Serum T3 levels also did not vary ($p > 0.05$) between healthy and infected group. Madur et al. (2011) reported a similar thyroid response in experimentally infected cattle while Saravanan et al. (2020) showed that T3 and T4 concentrations returned to pre-infection level after transient hyperthyroidism following the FMDV challenge in calves. The subtle variation in both the observations may be due to the difference in age of the experimental animals. Our data indicate that FMD virus infection does not affect thyroid gland activity.

In this study, we observed large interanimal variation in serum haptoglobin concentrations. Nevertheless, a markedly higher ($p < 0.001$) haptoglobin level was found in infected cattle sera compared to the healthy group. Serum amyloid A concentrations also increased significantly ($p < 0.05$) in the affected animals. Earlier, in an FMD virus challenge experiment, Stenfeldt et al. (2011) noticed that the rise in haptoglobin and serum amyloid A concentration coincided with the onset of clinical disease and the appearance of viremia. From our observations, haptoglobin is suggested as a more sensitive acute-phase reactant than serum amyloid A after natural FMD infection.

Scatter plot showing positively and significantly correlated parameters in the serum of FMD infected cattle. The solid lines represent the linear regression, *r* is the Pearson correlation coefficient and *p* is the significance level.

It is perhaps interesting to note a significant positive correlation of serum HSP70 levels with elevated cortisol ($p < 0.05$) and haptoglobin levels ($p < 0.01$) in infected animals (Table 2; Fig. 1 and Fig. 2). Cortisol modulation of tissue HSP70 levels has been earlier demonstrated in fish (LeBlanc et al. 2012). In this study, elevated circulating HSP70 appears to modulate infection related stress and

Table 2 Correlation of serum HSP70 level with serum cortisol, thyroid hormones, haptoglobin, and serum amyloid A levels in healthy and FMD infected cattle

Parameter	HSP70			
	Healthy (FMD negative)		Infected (FMD positive)	
	r	*p*	*r*	*p*
Cortisol	0.284	0.083	0.385	0.013*
T4	0.145	0.383	0.246	0.120
T3	0.217	0.188	0.262	0.090
Haptoglobin	−0.187	0.258	0.526	0.0004***
Serum amyloid A	−0.052	0.754	0.185	0.25

r indicates correlation co-efficient and *p* indicates level of significance *($p < 0.05$); ***($p < 0.001$)

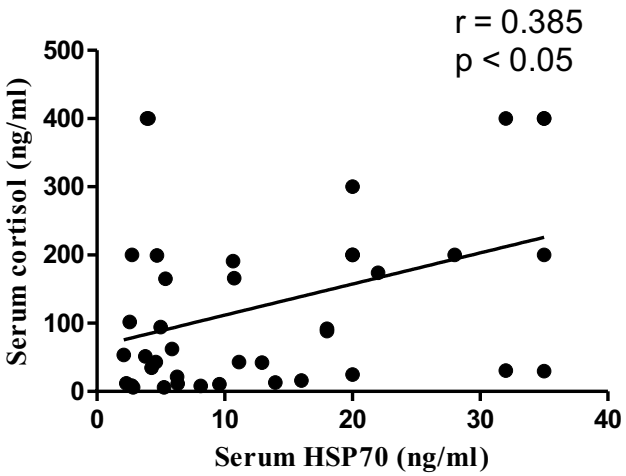

Fig. 1 A significant positive correlation of serum HSP70 levels with elevated cortisol ($p < 0.05$) in infected animals

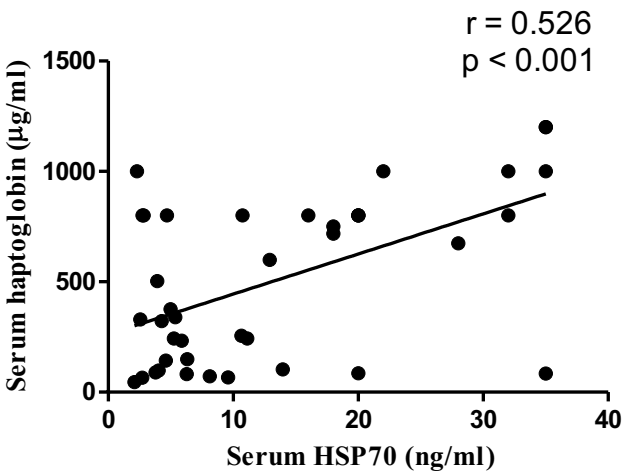

Fig. 2 A significant positive correlation of serum HSP70 levels with haptoglobin levels ($p < 0.01$) in infected animals

inflammatory response. Previously, corticosterone administration in broiler chickens induced an acute-phase response and HSP 70 expression (Zulkifli et al. 2014) while in human subjects, serum levels of HSP70 were closely related to inflammation status (Njemini et al. 2011). Our results corroborate these findings and substantiate the importance of serum HSP70 level as a danger signal to pathogen attack. No statistical significant, associations ($p > 0.05$) were observed for HSP70 concentration with other serum parameters in both the groups (Table 2).

In conclusion, serum concentrations of HSP70 increased in FMD infected cattle with a concomitant rise in cortisol, haptoglobin, and serum amyloid A levels compared to healthy cattle. No significant differences were observed for serum thyroid hormone concentrations between the groups. Higher circulating HSP70 levels correlated with cortisol and haptoglobin levels in infected animals indicating that this stress protein may have implications in the disease pathology.

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Data availability Data are available from the corresponding author on request.

Declarations

Ethics approval As per the institutional policy, no ethical clearance is required from the institutional ethics committee in disease outbreak investigations.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Barkakati, J., Sarma, S., Kalita, D.J., Goswami, J., Sharma, K., 2016. Activity of different serum enzymes and hormone profile in foot and mouth disease affected cattle. Indian Journal Of Animal Research, (50), 826-827.

Bailey, M., Engler, H., Hunzeker, J., Sheridan, J.F., 2003. The hypothalamic-pituitary-adrenal axis and viral infection.Viral Immunology, 16, 141-157.

Chowdhury, S.M.Z.H., Rahman, M.F., Rahman, M.B., Rahman, M.M., 1993. Foot and mouth disease and its effects on morbidity, mortality, milk yield and draft power in Bangladesh. Asian-Australasian Journal of Animal Sciences, 6, 423-426.

Ferrari, G., Tasciotti, L., Khan, E., Kiani, A., 2014. Foot-and-Mouth Disease and Its Effect on Milk Yield: An Economic Analysis on Livestock Holders in Pakistan. Transboundary and Emerging Diseases, 61, e52–e59.

Gaughan, J.B., Bonner, S.L., Loxton, I., Mader, T.L., 2013. Effects of chronic heat stress on plasma concentration of secreted heat shock protein 70 in growing feedlot cattle. Journal of Animal Science, 91, 120–129.

Ghanem, M.M., Abdel-Hamid, O.M., 2010. Clinical, haematological and biochemical alterations in heat intolerance (panting) syndrome in Egyptian cattle following natural foot-and-mouth disease (FMD). Tropical Animal Health and Production, 42(6), 1167-73.

Godson, D.L., Campos, M., Attah-Poku, S.K., Redmond, M.J., Cordeiro, D.M., Sethi, M.S., Harland, R.J., Babiuk, L.A., 1996. Serum haptoglobin as an indicator of the acute phase response in bovine respiratory disease. Veterinary Immunology and Immunopathology, 51(3-4), 277-92.

Gulbahar, M.Y., Kabak, Y.B., Karayigit, M.O., Yarim, M., Guvenc, T., Parlak, U., 2011. The expressions of HSP70 and αB-crystallin in myocarditis associated with foot-and-mouth disease virus in lambs. Journal of Veterinary Science, 12, 65–73.

Gupta, A., Cooper, Z.A., Tulapurkar, M.E., Potla, R., Maity, T., Hasday, J.D., 2013. Toll-like receptor agonists and febrile range hyperthermia synergize to induce heat shock protein 70 expression and extracellular release. Journal of Biological Chemistry, 288 (4), 2756-2766.

Horadagoda, N., Knox, K., Gibbs, H., Reid, S., Horadagoda, A., Edwards, S., Eckersall, P., 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Veterinary Record, 144, 437.

Hunt, C., Morimoto, R.I., 1985. Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc Natl Acad Sci U S A, 82(19), 6455-9.

Kristensen, T.N., Løvendah, P., Berg, P., Loeschcke V., 2004. Hsp72 is present in plasma from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and age classes. Cell Stress Chaperones, 9(2), 143–149.

Lamy, E., Jurkovich, V., Rodrigues, L., Geraldo, A., Cachucho, L., Silva, F., Pereira, A., (2017). Detection of 70 kDa heat shock protein in the saliva of dairy cows. Journal of Dairy Research, 84(3), 280-282.

LeBlanc, S., Höglund, E., Gilmour, K.M., Currie, S., 2012. Hormonal modulation of the heat shock response: insights from fish with divergent cortisol stress responses. American Journal of Physiology- Regulatory Integrative and Comparative Physiology, 302 (1), R184-92.

Lee, M.J., Jo, H., Park, S.H., Ko, M.K., Kim, S.M., B., Kim Park, J.H., 2020. Therapeutic perspectives in Advanced Foot-And-Mouth Disease Vaccine Platform for Stimulation of Simultaneous Cellular and Humoral Immune Responses. Vaccines, 8, 254.

Maddur, M.S., Rao, S., Chockalingam, A.K., Kishore, S., Gopalakrishna, S., Singh, N., Suryanarayana, V.V., Gajendragad, M.R., 2011. Absence of heat intolerance (panting) syndrome in foot-and-mouth disease-affected Indian cattle (*Bos indicus*) is associated with intact thyroid gland function. Transboundary and Emerging Diseases, 58, 274-9.

Mambula, S.S., Calderwood, S.K., 2006. Heat induction release of Hsp70 from prostate carcinoma cells involves both active secretion and passive release from necrotic cells. International Journal of Hyperthermia, 22, 575-585.

Martin, L.B., Andreassi, E., Watson, W., Coon, C., 2011. Stress and Animal Health: Physiological Mechanisms and Ecological Consequences. Nature Education Knowledge, 3(6), 11.

Merhan, O., Bozukluhan, K., Kiziltepe, S., Gokce, H.I., 2017. Investigation of Levels of Haptoglobin, Serum Amyloid A, Ceruloplasmin and Albumin in Cattle with Foot-and-Mouth Disease. Israel Journal of Veterinary Medicine, 72, 14-17.

Mishra, A., Hooda, O.K., Singh, G., Meur, S.K., 2011. Influence of induced heat stress on HSP70 in buffalo lymphocytes. Journal of Animal Physiology and Animal Nutrition, 95 (4), 540-544.

Mohapatra J K, Pandey L K, Sanyal A and Pattnaik B. 2011. Recombinant non-structural polyprotein 3AB-based serodiagnostic strategy for FMD surveillance in bovines irrespective of vaccination. Journal of Virological Methods, 177, 184-92.

Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. The Veterinary Journal, 168, 28-40

Njemini, R., Lambert, M., Demanet, C., Mets, T., 2003. Elevated serum heat-shock protein 70 levels in patients with acute infection: use of an optimized enzyme-linked immunosorbent assay. Scandinivian Journal of Immunology, 58(6), 664-9.

Njemini, R., J Smitz, C., Sosso, D.M., Mets, T., 2011. Circulating heat shock protein 70 (Hsp70) in elderly members of a rural population from Cameroon: association with infection and nutrition Archives of Gerontology and Geriatrics, 53(3), 359-63.

Saravanan, S., Umapathi, V., Priyanka, M., Hosamani, M., Sreenivasa, B. P., Patel, B. H. M., Narayanan, K., Sanyal, A., Basagoudanavar, S. H., 2020. Hematological and serum biochemical profile in cattle experimentally infected with foot-and-mouth disease virus. Veterinary World, 13(3), 426-432.

Sedeh, F. M., Yazdanpanah, S., Soleimanjahi, H., Mahravani, H., Shafaee, K., Razavi M.H., Rezaee, A., 2014. Enhancement of Immune Responses against Iranian Isolate of FMD-type O/IRN/1/2010 Based on VP1 and Human HSP70 Genes and Comparison with Conventional Vaccine. Acta Scientiae Veterinariae, 42, 1208.

Stenfeldt, C., Heegaard, P.M., Stockmarr, A., Tjørnehøj, K., Belsham, G.J., 2011. Analysis of the acute phase responses of serum amyloid a, haptoglobin and type 1 interferon in cattle experimentally infected with foot-and-mouth disease virus serotype O. Veterinary Research, 42, 66.

Ungar-Waron, H., Brenner, J., Paz, R., Moalem, U., Trainin, Z., 1996. γδ T-lymphocytes and anti-heat shock protein reactivity in bovine leukemia virus infected cattle. Veterinary Immunology and Immunopathology, 51(1–2), 79-87.

Vega, V.L., Rodríguez-Silva, M., Frey, T., Gehrmann, M., Diaz, J.C., Steinem, C., Multhoff, G., Arispe, N., De Maio, A., 2008. Hsp70 Translocates into the Plasma Membrane after Stress and Is Released into the Extracellular Environment in a Membrane-Associated Form that Activates Macrophages. The Journal of Immunology, 180 (6), 4299-4307.

Zulkifli, I., Najafi, P., Nurfarahin, A.J., Soleimani, A.F., Kumari, S., AnnaAryani, A., O'Reilly, E.L., Eckersall, P.D., 2014. Acute phase proteins, interleukin 6, and heat shock protein 70 in broiler chickens administered with corticosterone. Poultry Science, 93, 3112-3118.