WuXi AppTec

WHITEPAPER

THE BAMA MINIPIG IN TOXICOLOGY

TOX Whitepaper 2.9.23

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WHITEPAPER

INTRODUCTION

Domestic swine have been used in medical research for decades but their large size and the difficulty in housing and handling the animals always limited their use. In the second half of the 20th century, breeding programs were set up in various countries to develop a smaller, more manageable pig. Today, a variety of miniature swine (minipig) breeds are commercially available for medical research.

The use of the minipig as a non-rodent species in non-clinical research[1,2,3,4,5,6,7] has continued to increase and although its use is still considerably less than that of the dog or the non-human primate (NHP), for certain areas of research the physiological similarities between swine and humans make it the species of choice. A recent European RETHINK project[8,9,10] has taken place to assess minipigs (in general not as a specific species) as models for the toxicity testing of new medicines and chemicals and concluded there is great utility in their use in various areas of research.

The most commonly used minipig in non-clinical research is the Göttingen, available in Europe, the USA, and Japan. Other strains include the Yucatan, Sinclair, and the Hanford minipigs used almost exclusively in the USA. There is a wide variety of literature available on individual strains and comparing combinations of these strains in terms of physiology, anatomy, metabolism, clinical pathology, and pathology.[11,12]

In China, the Guangxi University introduced a colony of pigs from Bama County in Guangxi Province and adopted a closed breeding programs to select small pigs for experiments. Subsequently animal breeders obtained pure bred Guangxi Bama miniature pigs and continued to operate breeding programs to produce todays Bama minipigs with a clear genetic background and that meet the required quality standards required for non-clinical research.

To date there is limited comparative data between the Bama minipig and other minipig strains. In this paper we will present control data on body weight, clinical pathology, electrocardiography, organ weight, and histopathology from Bama minipigs; draw comparisons with publicly available data on the Göttingen minipig (Ellegaard) and, where available, other strains of minipig; and discuss the applicability of the Bama strain to non-clinical research.

BREEDING AND HEALTH SCREENING[11,13,14]

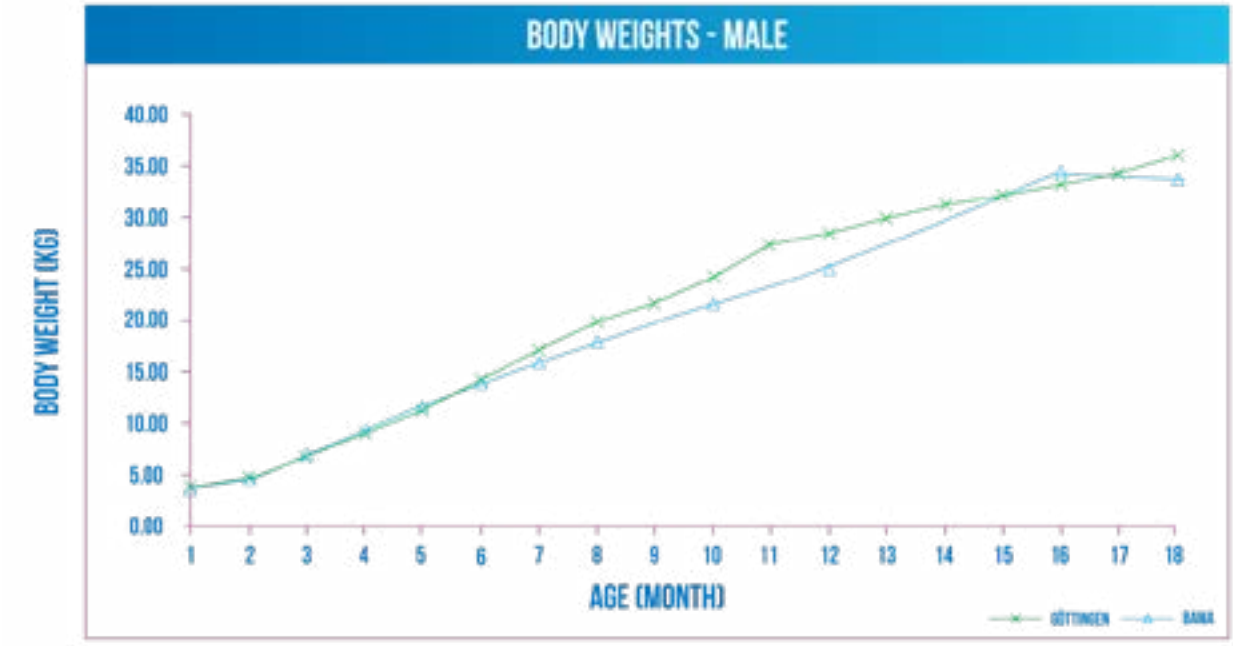
All the strains of minipigs mentioned above originated from breeding programs designed to select animals which were smaller in size and therefore easier to house and handle than conventional pigs.

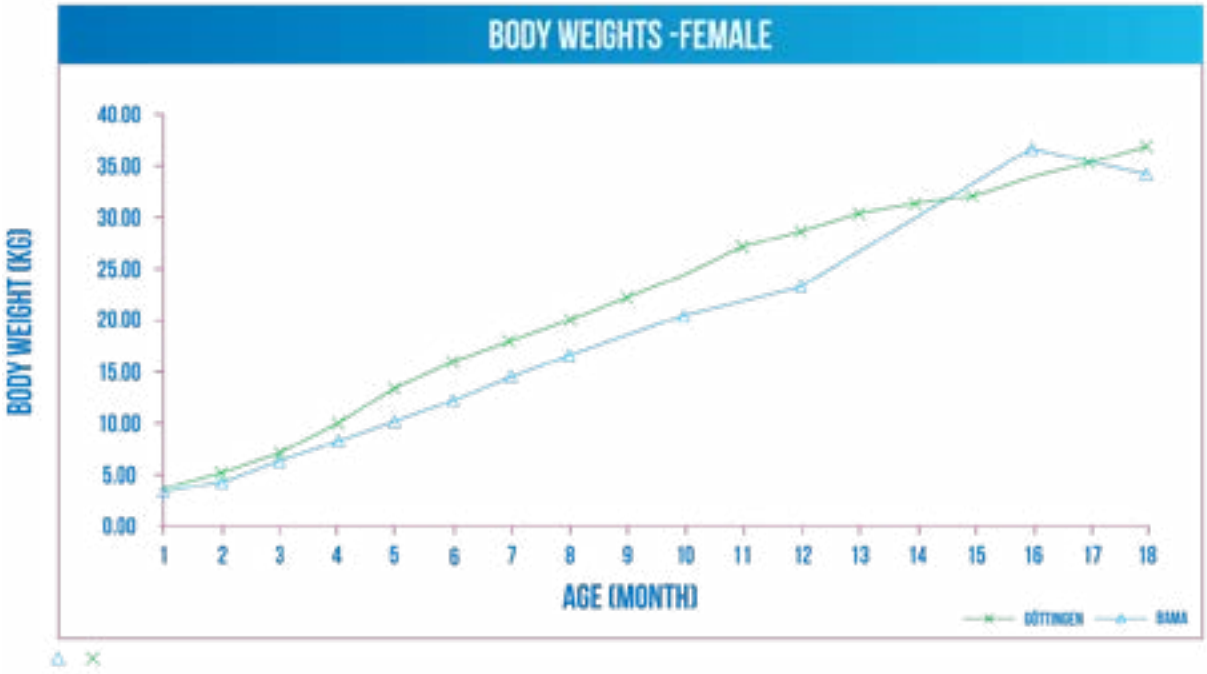
The breeding program for each strain is closely controlled to avoid inbreeding, maintain genetic stability, and maintain the health status of the breeding colony.

Animal care, veterinary care, facility operation, and colony management at the breeding facilities complies with both local and international requirements. Breeding colonies of Göttingen, Sinclair, Hanfordm, and Yucatan animals are AAALAC accredited. The Bama breeders are not currently AAALAC accredited, operating to national guidelines only.

Animals from all breeding programs undergo regular health screening for bacterial, viral, and parasitic infections according to local and international requirements along with vaccination programs.

BODYWEIGHT AND GROWTH CURVES[12,13]





The body weight of male Göttingen and Bama minipigs is comparable up to approximately 6 months of age after which the Göttingen body weight is slightly heavier than the Bama. In females, the body weight of the Bama strain is consistently lower than that of the Göttingen until approximately 14 months old.

HAEMATOLOGY [12,13]

MALES

		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLAT	RETIC #	RETIC %
Bama	Mean	21.47	8.14	12.8	39.9	49.0	15.8	32.3	722	0.06	0.77
	SD	5.74	0.56	1.0	3.2	2.9	0.8	1.0	148	0.03	0.35
	N	119	119	119	119	119	119	119	113	119	118
Gott	Mean	9.84	8.3	8.3	40.7	49.5	16.3	20.5	438	0.1	1.11
	SD	2.05	0.95	0.67	3.25	6.03	1.93	0.27	63	0.02	0.27
	N	17	17	17	17	17	17	17	17	17	17

		NEUT	NEUT %	LYMP	LYMP %	MONO	MONO %	EOS	EOS %	BASO	BASO %
Bama	Mean	7.36	33.3	12.30	59.0	0.99	4.3	0.24	1.2	0.15	0.7
	SD	4.10	12.6	2.80	11.7	0.49	1.5	0.13	0.7	0.08	0.3
	N	118	119	119	119	118	113	116	117	118	117
Gott	Mean	3.16	31.4	6.37	65.2	0.08	0.8	0.19	2.0	0.05	0.6
	SD	1.56	10.6	1.42	10.2	0.05	0.4	0.09	1.0	0.02	0.3
	N	17	17	17	17	17	17	17	17	17	17

FEMALES

		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLAT	RETIC #	RETIC %
Bama	Mean	21.45	8.56	13.4	42.0	49.1	15.7	31.9	652	0.07	0.81
	SD	4.98	0.51	0.9	2.9	2.8	0.8	1.1	176	0.03	0.35
	N	116	117	117	117	117	117	117	114	115	113
Gott	Mean	9.46	8.89	8.7	42.5	47.9	15.8	20.5	381	0.10	1.16
	SD	2.66	0.74	0.6	2.4	3.4	1.1	0.3	123.0	0.04	0.49
	N	17	17	17	17	17	17	17	17	17	17

		NEUT	NEUT %	LYMP	LYMP %	MONO	MONO %	EOS	EOS %	BASO	BASO %
Bama	Mean	5.36	25.0	14.10	65.8	1.10	5.2	0.39	2.0	0.18	0.9
	SD	2.54	9.0	3.41	8.6	0.43	1.6	0.18	1.0	0.08	0.4
	N	115	117	117	117	115	116	113	117	115	116
Gott	Mean	2.56	26.2	6.59	70.4	0.07	0.8	0.19	2.0	0.05	0.6
	SD	1.30	8.7	1.84	8.7	0.05	0.5	0.09	1.1	0.02	0.2
	N	17	17	17	17	17	17	17	17	17	17

Table 1 – Haematology values of control Göttingen and Bama minipigs

The total white cell count of male and female Bama minipig along with the absolute neutrophil, lymphocyte, eosinophil, and basophil counts are significantly higher than that of the Göttingen minipig. However, the percentage values for these cells are comparable between the two strains. The absolute and percentage monocyte counts are significantly higher in the Bama minipig. The values for Hanford, Yucatan, and Sinclair strains lie between the values observed in the Göttingen and Bama strains.[11]

The red blood cell count of the Bama and Göttingen strains are comparable while the haemaglobin concentration (HGB) and mean corpuscular haemaglobin concentration (MCHC) of the Bama strain is significantly higher than the Göttingen strain. The HGB and MCHC values in the Hanford, Yucatan, and Sinclair strains are comparable with the Bama values.

The platelet count of Bama minipigs is higher than that of all the other strains.

COAGULATION

MALES

		PT	APTT
Bama	Mean	13.2	9.6
	SD	0.7	1.2
	N	94	94
Gott	Mean	13.85	72.82
	SD	0.9	22.5
	N	17	17

FEMALES

		PT	APTT
Bama	Mean	13.0	10.0
	SD	0.7	1.2
	N	96	96
Gott	Mean	13.5	71.4
	SD	1.0	25.9
	N	16	16

Table 2 – Coagulation values of control Göttingen and Bama minipigs

While prothrombin time is comparable between the two strains, there is an apparently significant increase in the activated partial thromboplastin time in the Göttingen strain. The Göttingen value is so different from the values recorded in the other strains and in other research species[12,13] that although the data has been verified with the breeder it is considered that the difference is a result of the analytical technique or equipment rather than a true physiological difference.

CLINICAL CHEMISTRY

MALES

		ALT	AST	AP	GGT	CK	TP	ALB	A/G	TG
Bama	Mean	53	43	186	65	787	70.4	34.6	1.00	0.22
	SD	14	24	67	13	630	5.3	3.2	0.23	0.06
	N	110	109	110	109	103	110	110	110	110
Gott	Mean	78	38	153	54	1720	53.0	57.1	1.34	0.31
	SD	82	44	41	13	3900	11.76	2.19	0.20	0.10
	N	17	17	17	17	17	17	17	17	17

		Na	K	Ca	Cl	P	GLU	UREA	CRE	TCHO	TBILI
Bama	Mean	144	5.5	2.46	101	2.75	4.24	1.82	52	2.14	2.51
	SD	2.0	0.7	0.13	2.0	0.29	0.74	0.62	11	0.45	0.78
	N	110	110	110	110	110	110	109	110	109	110
Gott	Mean	131	4.54	2.32	91	2.20	4.48	1.66	77	1.32	0.40
	SD	16.6	0.8	0.45	12.1	0.48	0.91	0.46	13	0.35	0.90
	N	17	17	17	17	17	17	17	17	17	17

FEMALES

		ALT	AST	AP	GGT	CK	TP	ALB	A/G	TG
Bama	Mean	55	37	206	56	775	69.5	33.6	0.95	0.30
	SD	15	14	62	9	588	5.3	3.6	0.17	0.09
	N	107	106	107	106	104	107	107	107	106
Gott	Mean	64	30	138	54	1963	60.2	54.9	1.26	0.48
	SD	39	27	54	22	3984	14.0	1.9	0.23	0.15
	N	17	17	17	17	17	17	17	17	17

		Na	K	Ca	Cl	P	GLU	UREA	CRE	TCHO	TBILI
Bama	Mean	145	5.4	2.50	102	2.76	4.45	2.21	58	2.84	2.92
	SD	2	0.8	0.12	2	0.27	0.89	0.57	11	0.52	0.91
	N	107	107	106	107	107	107	107	106	107	105
Gott	Mean	133	4.45	2.41	91	2.35	4.75	2.34	74	2.09	1.44
	SD	18	1.0	0.43	13	0.54	1.19	0.68	21	0.48	1.24
	N	17	17	17	17	17	17	17	17	17	17

Table 3 – Clinical chemistry values of control Göttingen and Bama minipigs

The creatine kinase values of male and female Göttingen minipigs appear significantly higher than the values in Bama animals. However, the very large SD value of the Göttingen strain[13] would suggest that the apparent difference is possibly a result of analytical variability.

There are variations in the total protein, albumin, triglyceride, and total cholesterol concentrations between the Göttingen and Bama strains. There is also variation in these parameters in the Hanford, Yucatan, and Sinclair strains,[12] suggesting that differences in the constituents of the diet the animals receive is the most likely cause of the variation.

CARDIOVASCULAR/ELECTROCARDIOGRAPHY

A total of 6 standalone telemetry studies and 26 general toxicology studies have been performed on the Bama minipig at our Toxicology facility. Examination of ECG recordings from control animals in all these studies showed a very low incidence of ventricular premature complexes (VPCs) or atrial premature complexes (APCs) (Table 5). No other abnormalities were found on examination of the ECG recordings. This data compares favourably with published data on cardiac abnormalities in the Göttingen minipig.[15]

	No of Animals	NO OF ANIMALS WITH VPCS	No of VPCs
Male	18	0	0
Female	20	3	5
Total	38	3	5

Table 4 – Number of animals with Ventricular Premature Complexes and the number of VPCs in standalone cardiovascular telemetry studies

	No of Animals	NO ANIMALS WITH VPCS	No VPCs	No Animals with APCs	No APCs
Male	143	3	4	2	4
Female	131	0	0	1	2
Total	274	3	4	3	6

Table 5 – Number of animals with Ventricular Premature Complexes or Atrial Ventricular Complexes and the number of VPCs or APCs in general toxicology studies (Schiller)

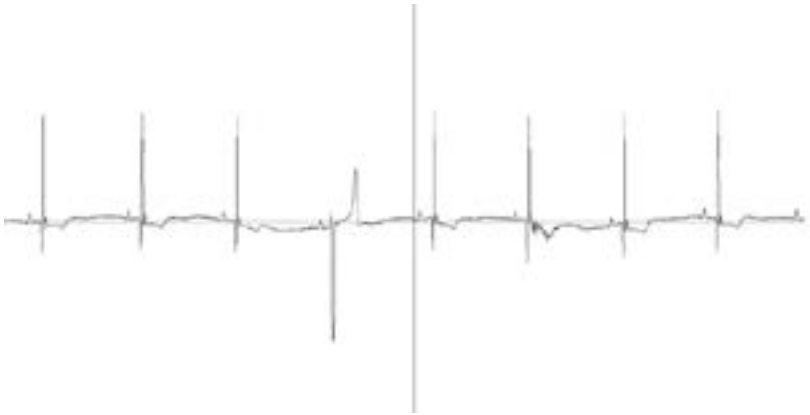


Figure 3 – Example of VPC (telemetry study)

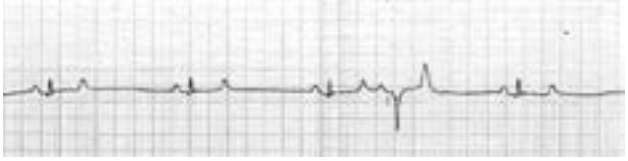


Figure 4 – Example of VPC (general toxicology study)

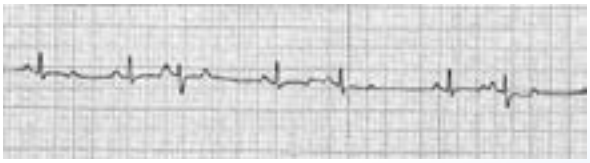


Figure 5 – Example of APC (general toxicology study)

ORGAN WEIGHTS

MALES ABSOLUTE

		BW	ADRENALS	HEART	LIVER	KIDNEY	SPLEEN	PITUITARY	THYROID	TESTES
Bama	Mean	16.2	1.902	96.49	327.26	97.35	39.76	0.121	2.353	49.07
	SD	4.2	0.338	23.67	76.54	28.28	8.83	0.027	0.915	15.35
	N	18	18	18	18	18	18	15	15	18
Gott	Mean	14.2	1.133	73.72	237.03	66.44	22.29	0.095	0.982	42.05
	SD	1.6	0.185	5.85	25.78	9.34	4.01	0.018	0.479	8.44
	N	20	20	20	20	20	20	20	20	20

RELATIVE TO BW

		ADRENALS	HEART	LIVER	KIDNEY	SPLEEN	PITUITARY	THYROID	TESTES
Bama	Mean	0.0139	0.603	2.05	0.599	0.398	0.0007	0.018	0.308
	SD	0.0025	0.10	0.34	0.089	0.088	0.0002	0.007	0.098
	N	18	18	18	18	18	18	18	18
Gott	Mean	0.0081	0.524	1.68	0.470	0.158	0.0007	0.007	0.298
	SD	0.0014	0.50	0.16	0.060	0.027	0.0001	0.004	0.062
	N	20	20	20	20	20	20	20	20

FEMALE ABSOLUTE

		BW	ADRENALS	HEART	LIVER	KIDNEY	SPLEEN	PITUITARY	THYROID	OVARIES	UTERUS
Bama	Mean	15.5	2.112	83.95	306.94	83.95	37.42	0.99	1.863	4.63	18.86
	SD	2.25.0	0.683	20.79	55.66	20.79	9.07	0.16	0.541	0.85	9.46
	N	18	18	18	18	18	18	15	15	18	15
Gott	Mean	14.6	1.130	59.76	218.24	58.66	20.58	0.100	0.971	2.08	83.04
	SD	2.2	0.183	8.98	33.69	11.16	4.26	0.17	0.202	1.08	57.42
	N	20	20	20	20	20	20	20	20	20	20

RELATIVE TO BW

		ADRENALS	HEART	LIVER	KIDNEY	SPLEEN	PITUITARY	THYROID	OVARIES	UTERUS
Bama	Mean	0.0139	0.548	2.10	0.563	0.254	0.0007	0.012	0.011	1.203
	SD	0.0025	0.077	0.441	0.099	0.055	0.0002	0.003	0.005	0.692
	N	18	18	18	18	18	18	18	18	18
Gott	Mean	0.0079	0.412	1.50	0.401	0.141	0.0007	0.007	0.014	0.576
	SD	0.0016	0.056	0.14	0.048	0.025	0.0001	0.001	0.007	0.395
	N	20	20	20	20	20	20	20	20	20

Table 6 – Organ and organ to bodyweight values of control Göttingen and Bama minipigs

In general, the organ to body weight ratio of principle organs correlated well between the two species.[13] Differences in diet and potentially age/maturity of the animals are considered to be the primary sources of the minor variations seen

HISTOPATHOLOGY – SPONTANEOUS FINDINGS[12,16,17,18]

Focal accumulations of mononuclear inflammatory cells in various tissues are the most common finding in the Göttingen minipig. Other lesions are generally of a mild and focal nature. The nature of spontaneous lesions in the Bama minipig is similar with minimal focal mononuclear cell infiltration being the most common observation in this strain also.

HISTOPATHOLOGY – SKIN

Swine skin shows many similarities to human skin. This has resulted in the extensive use of swine in burn and radiation research as the skin of other research animals such as rodents and dogs differ significantly from that of man.

The epidermis of these animals is much thinner with a flat epidermal–dermal interface which does not have rete ridges and papillary projections, with relatively loose dermal structures and an underdeveloped vascular system.

In comparison, swine skin is composed of three layers: epidermis, dermis, subcutis. The epidermis contains the 5 strata seen in human skin while the dermis contains superficial venules and arteriolar plexuses, collagen, elastic fibers, hair follicles, sebaceous glands, and apocrine sweat glands and the subcutis contains loose connective tissue and fat tissue.

A comparison of skin samples taken from the back of 16 Göttingen and Bama minipigs showed that the structure of the two strains was very similar. These numbers are adequate for evaluating the histology of skin and the comparison was complete. The only difference of note was a slight difference in thickness of the dermis (slightly thicker in the Göttingen animals), but this may be related to the age of the animals examined. These two breeds have similar microscopic morphology, architecture, and elements in the skin. These observations support the validity of using Bama pigs in skin toxicity as an alternative for Gottingen pigs.

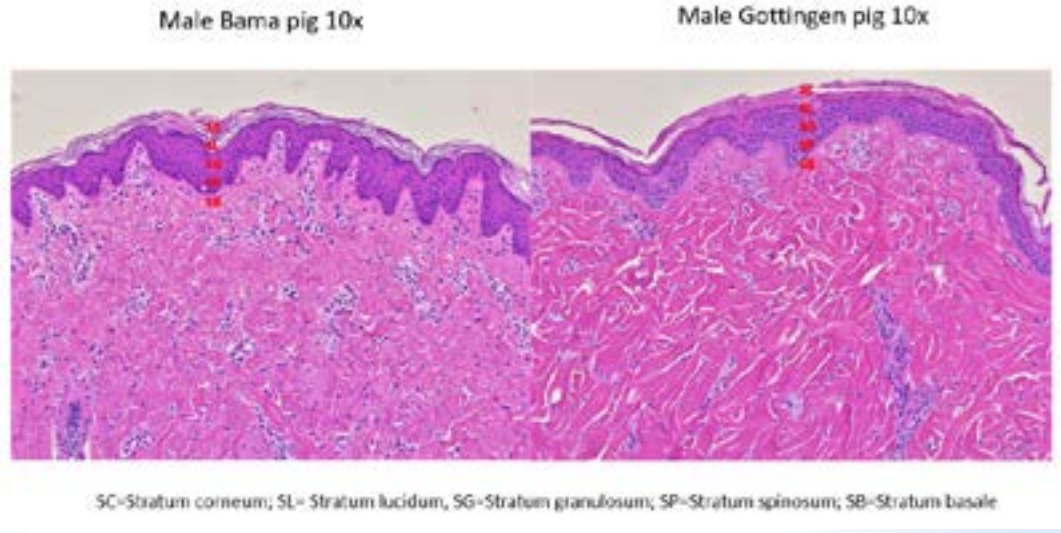


Figure 6 – Cross section of skin from control Göttingen and Bama minipigs

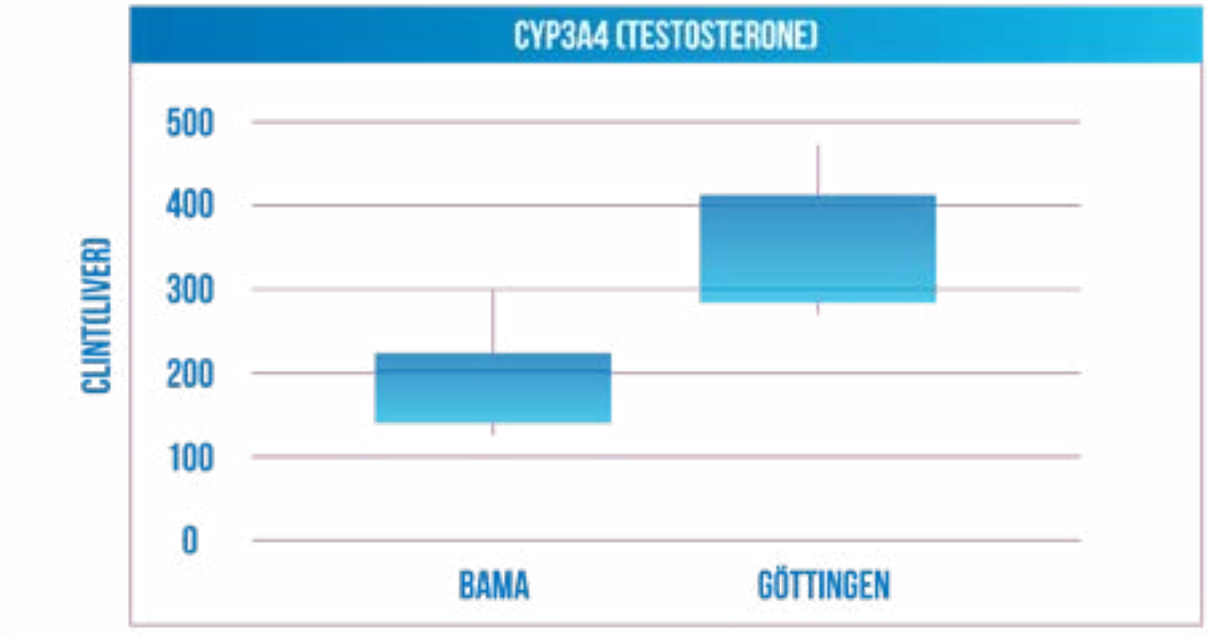
ADME/DMPK

WuXi AppTec compared several test compounds in the Bama and Göttingen test systems for microsomal and hepatocyte clearance as well as plasma protein binding.

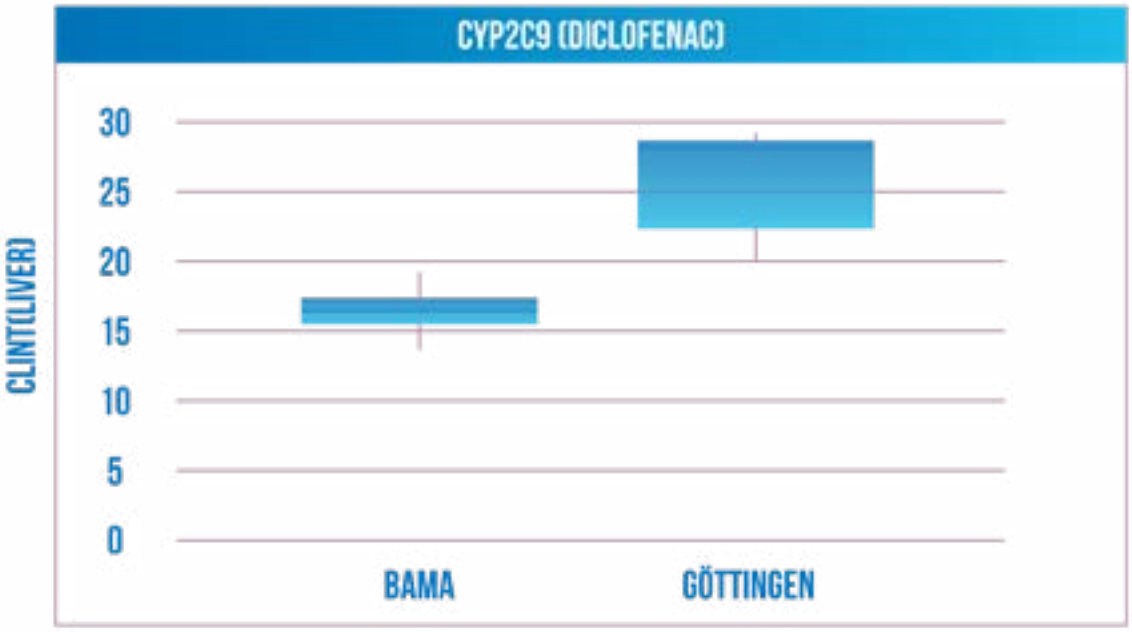
Only minor differences were found for the three positive control compounds used in the microsomal clearance assay (diclofenac, testosterone, and propafenone) (Figure 7).

In the hepatocyte clearance assays, 7-ethoxycoumarin (7-EC) and 7-hydroxycoumarin (7-HC) were used as positive controls for phase 1 and phase 2 biotransformation. While there was good agreement between both species for 7-HC, turnover of 7-EC appeared to be considerably higher in the Bama hepatocytes compared with Göttingen hepatocytes (Figure 8).

In plasma protein binding, the unbound fraction of the positive control warfarin was consistent for both breeds of pigs (Figure 9).



ADME/DMPK



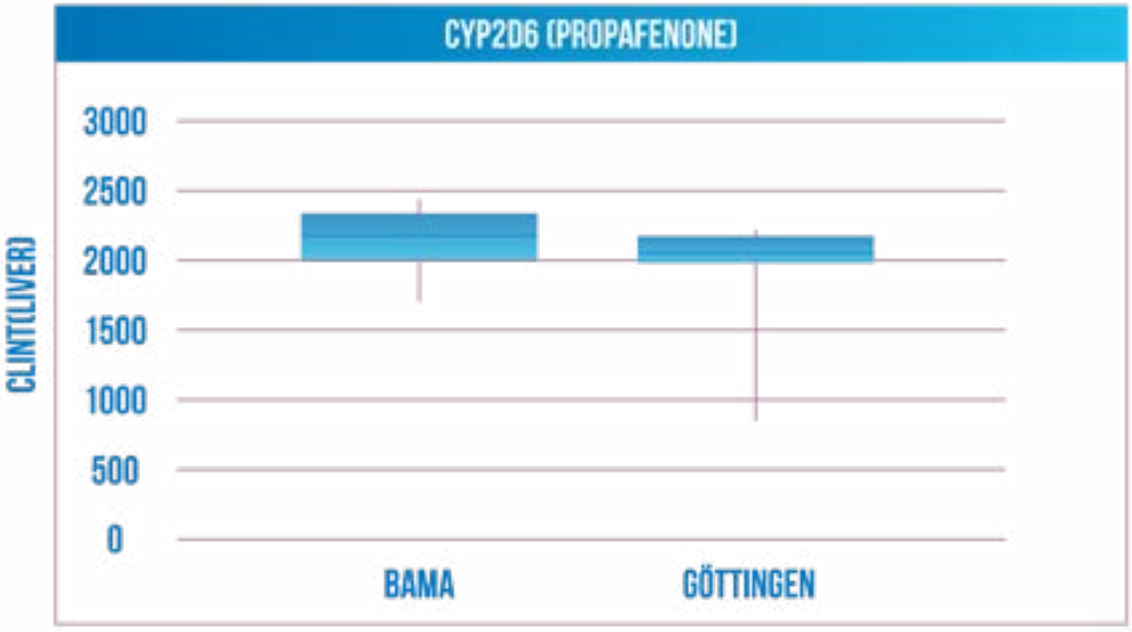


Figure 7: Metabolic clearance (ml/min/kg) of diclofenac, testosterone and propafenone in Göttingen and Bama pig microsomes (trends of > 6 independent experiments)

ADME/DMPK

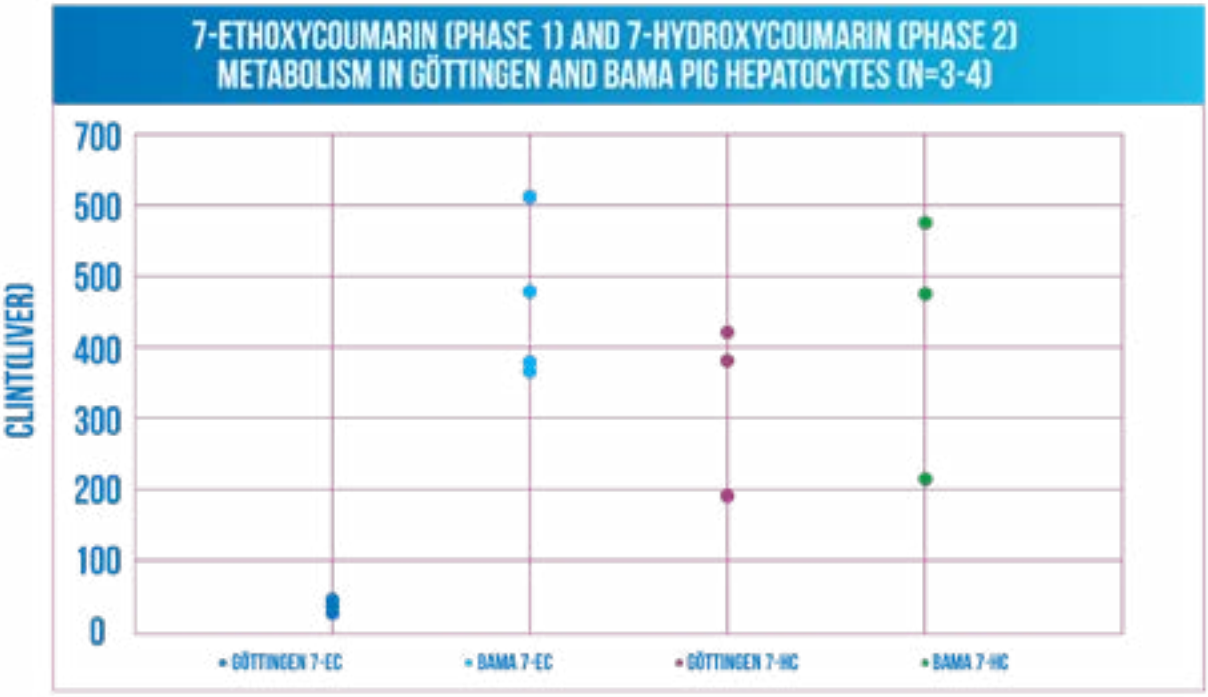


Figure 8: Metabolic clearance (ml/min/kg) of diclofenac, testosterone and propafenone in Göttingen and Bama pig hepatocytes

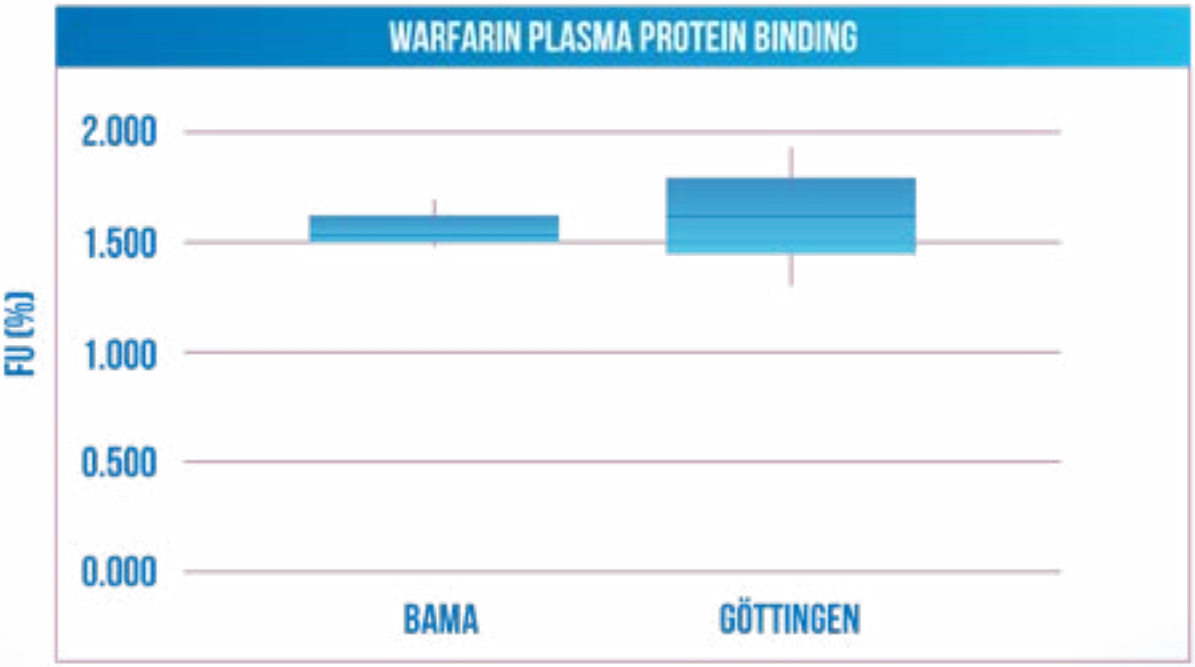


Figure 9: Plasma protein binding of warfarin in Göttingen and Bama pig plasma (trends of > 12 independent experiments)

DISCUSSION

Pigs have been used for decades in medical research. The domestic swine originally used were large and difficult to handle. Consequently, breeding programs were established to engineer smaller animals which were more docile and easier to handle and house. With growing ethical concerns about the use of non-human primates and dogs, with the minipig is seen as an ethically more acceptable animal[8,9,10].

Many aspects of the physiology, anatomy, and biochemistry of swine resemble that of man, and it is considered to be a very good model for research in to the cardiovascular, digestive, and the integumentary systems in particular.[20, 21,22,23] In the field of medical device testing, ISO guidelines indicate that the pig is a highly suitable model because of its haematological and cardiovascular similarities to man.[24] Pigs are also widely used in the area of radiation research, both for whole-body effects and local skin-burn effects.

The swine heart is anatomically similar to that of man and the heart-to-body-weight ratio is comparable. The growth of the heart and the great vessels in swine is comparable to that in children.

When compared to other laboratory species, swine are relatively hairless and the skin is generally less pigmented than that of dogs and non-human primates, making changes in the skin following treatment more easily detectable. The skin is tightly attached to the subcutaneous tissues in swine and there are similarities in the epidermal thickness, cellular composition, and cutaneous blood supply between swine and man, making swine a more suitable model for determining dermal effects than either the dog or the non-human primate.

The anatomy and physiology of the gastrointestinal tract of swine has more similarity to man that that of other non-clinical species. While rodent models of GI tract disease are widespread, they generally fail to mimic the pathologic features of disease in man. The pig and man are monogastric and omnivorous while the digestion and metabolism of carbohydrates, lipids, and amino acids produce similar metabolites. The epithelial cell population of the small intestine in swine is similar to that of man, the villus structure is finger-like, and the colon of the pig and man both possess sacculations and longitudinal muscular bands along their length, resulting in similar transit times and thus comparable digestive physiology in the intestine.[20] As with other research animals, no guidelines specify the strain which should be employed. It is solely the suitability/applicability of the strain that is to be justified. The aim of this paper is to demonstrate that the Bama minipig is suitable for use as a toxicological model by drawing comparisons between this strain and the other, more commonly used and accepted strains in terms of various physiological and anatomical parameters.

In terms of clinical pathology and electrocardiography, variations between the strains are apparent but these variations can be accounted for by differences in the breeding environment, housing, diet, and analytical equipment. Differences in organ weights are reflective of the differences in body weight.

Histological examination of skin samples from Bama and Göttingen minipigs showed very good comparability. In addition, Liu et al[19] directly compared skin from Bama minipigs and humans in terms of morphology, ultrastructure, immunohistochemistry, and collagen physicochemical properties and concluded that although some differences were evident, the Bama minipig was a suitable animal model for human skin.

The metabolism of a drug in the non-clinical species in comparison with the human system is fundamental to the selection of the most suitable species for the conduct of the non-clinical studies. There is considerable literature available on Cytochrome 450 activities in Göttingen minipigs [26, 28] but little available for the Bama strain.[25] In the limited evaluation carried out in our laboratory it is evident that there are similarities and differences between the two strains of minipig. However, this also occurs in different strains of rodents, dogs or NHPs, showing that it is important to determine relevant endpoints when selecting your non-clinical species and to use the selected strain throughout your project.

CONCLUSION

While differences in a variety of parameters measured within non-clinical studies are seen between the Bama and Göttingen minipigs, the differences are no more pronounced than those between different strains of other non-clinical species and do not preclude the use of the Bama minipig as a valuable model for research. There are no differences between the strains that would indicate that the Bama minipig is less suitable than the Göttingen minipig for non-clinical research via any dose route. However, the data does highlight the need to use the same strain of animal throughout the development of a compound.

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