

Input for updated online description of pilot chain 3

1) What is the main aim of the pilot chain?

The main objective of pilot chain 3 is the development and validation of a rapid test used in field for the determination of positive acute phase proteins (APP), focusing on the benefit that these systems of testing will have on animal health, welfare and food safety. APPs are plasma proteins that modify their concentration following infection, inflammation, trauma or stress. The circulating concentrations of these proteins can provide an objective measure of the health status of an animal and are increasingly being used as markers for animal health and welfare in farm animals such as pigs or cattle.

Pilot chain 3 focuses on the development of a rapid test for the positive APP Pig-MAP. This acute phase protein has been widely studied by the business partner (PigChamp) of pilot chain 3 and is considered a good marker for animal health and welfare with the advantage of showing relatively low variation in normal state. The objective of this pilot chain is to obtain a product adapted to the user needs which can be utilised for health screening at farms. Pilot chain 3 works also in the implementation of the rapid test in the industry for on-farm usage. The objective is to develop procedures for the use of rapid methods in the production chain, advancing towards the incorporation of routinely APP monitoring into integrated quality systems aimed to improve animal health and welfare at farms. In addition, pilot chain 3 will explore also the possible relationship between APP levels and meat quality traits.

2) Please explain the status quo of the activities within this pilot chain

Rapid test development

A rapid test for the detection of abnormally elevated levels of the positive APP pig-MAP has been developed. The test can be performed with serum or whole blood. The method has the advantage of not needing laboratory equipment which makes it possible to use it directly at the farm. The kit consists of a vial containing a buffer where the exact amount of sample is added as well as and the reactive strip. After adding the sample to the buffer and mixing the mixture is incubated for 5 minutes. Then, the stick is introduced in the vial, immunocromatography is developed and the results are observed after 10 minutes. Appearing of a pink line in the reaction zone, together with the blue control line, indicates that the sample is positive. The limit of the detection of the test can be adapted changing the amount of sample added to the vial.

The technical performance of the test has been evaluated by comparing the results obtained with the dipstick with the quantitative results obtained by ELISA, used as reference method. Figure 1 shows the results of the first evaluation of the test, after measuring 372 samples. The test shows good agreement with the reference method, with sensibility and specificity values higher than 97% (Figure 1). An exhaustive evaluation has been made with the results obtained in all the samples collected during the implementation of the test at farm level, with similar results.

		Comparative method (ELISA)		
		Conc. >1.5 mg/mL	Conc. <1.5 mg/mL	Total
Test to evaluate (Stick PigMAP)	Positive	131	4	135
	Negative	4	233	237
	Total	135	237	372

SENSITIVITY 1: 97.0 %

SPECIFICITY 2: 98.3 %

1 % of positive results in the samples with Pig-MAP concentration above the cut off value (> 1.5 mg/mL, as determined by ELISA).
 2 % of negative results in the samples with Pig-MAP concentration lower than the cut off value (< 1.5 mg/mL as determined by ELISA).

100% of samples < 1mg/mL (165) were negative
99% samples > 2 mg/mL (107) were positive

Figure 1: Evaluation of the performance of the rapid test with serum samples

Implementation of the test at farm level

Implementation of the rapid test at farm level is being performed at commercial farms from the region of Segovia, in central Spain, following standardised protocols which also include an evaluation of critical points at the farm based on a model developed by the University of Bonn. The testing programme includes taking blood samples for animals of different age groups (half males and half females) to get a picture of the situation of the farm at the time of sampling (Figure 2). The study includes the evaluation of the farm at periodical intervals to evaluate the effect of season on APP values. Differences are observed between farms, according to health status, as well as between ages, highlighting the presence of problems in these age groups. A higher number of positive samples has been obtained in the sampling points performed in autumn-winter, coinciding with higher incidence of disease.

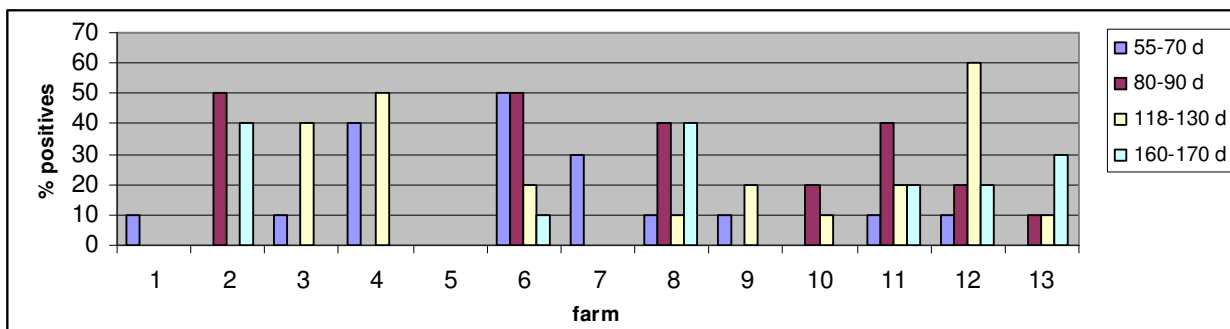


Figure 2: Percentage of positive samples in some farms studied, for the different groups of age analysed (days of live, d). Sampling performed in winter.

The results obtained so far indicate that the test is useful for its incorporation into a continuous health surveillance programme (Figure 3). An example is shown in Figure 4, where the results obtained in three farms of different health states at two sampling points are compared.

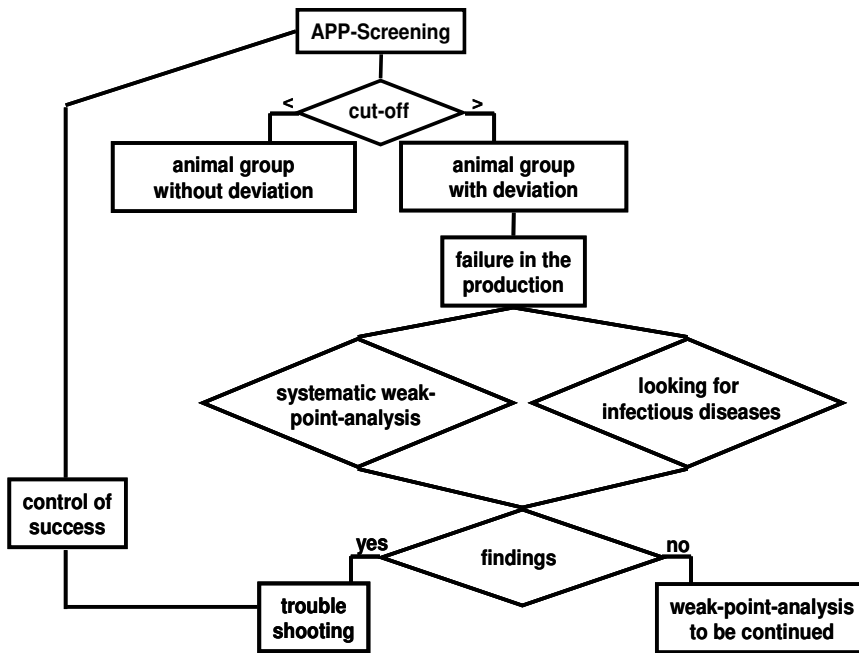


Figure 3: Scheme for APP monitoring in health surveillance programmes

The model combines monitoring APP together with a systematic weak point-analysis. The acute phase proteins are used as screening parameters. Are the determined levels below the cut off, the pig group is without deviations. Show the pigs elevated levels, the pig group is with deviation and there must be a failure in the production. A systematic weak point analysis should be performed and the vet should look for infectious diseases. If there are findings, a trouble shooting is necessary and a control of success should be done with the next pig group. If there are no findings, the weak point analysis should be continued.

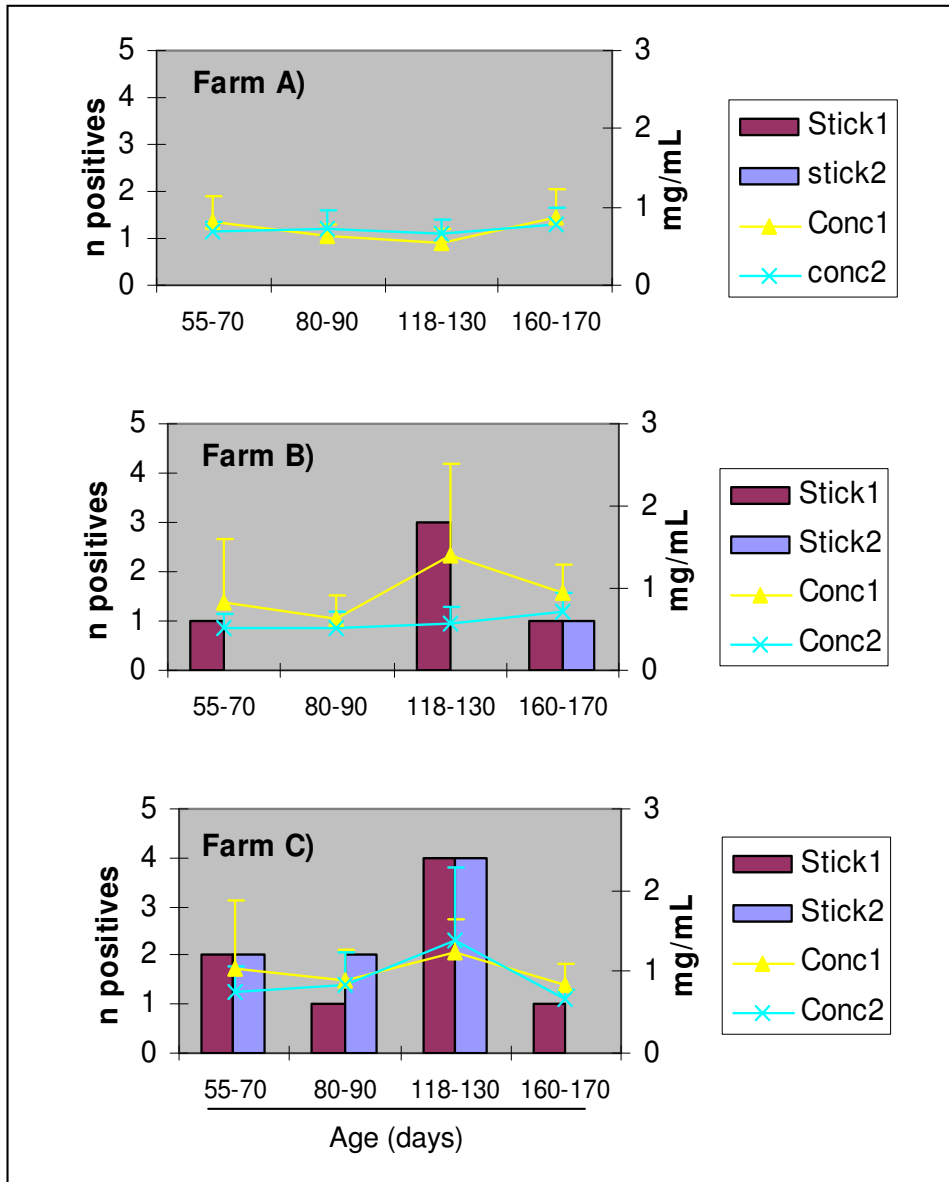


Figure 4: Use of the rapid test in health surveillance programmes

In each farm 10 animals per age group were sampled (serum) and samples analysed with the help of the rapid test (stick) and by ELISA to determine the concentration of pig-MAP (conc). The graphs show the results obtained in two consecutive sampling points (1, first sampling point time; 2, second sampling point time). Farm A) was a high health status farm, with mortality rate lower than 5%, no positive samples were obtained in any sampling point. Farm B) and C) had worse health states, with mortality rate higher than 8%. Farm B) implemented measures for improving health states and decreased the APP levels. Farm C) continues showing the same pattern in the second sampling point.

The rapid test can be also used for monitoring the state of new animals arriving at the farm (supplier oriented receiving inspection). This may be of special interest if the pigs are being transported for long distance which can affect their welfare and subsequent performance at the farm. In a second study, a monitoring programme for replacement gilts was implemented in farms from the region of Segovia, Spain. Most of the farms which were included in the study received gilts from England which arrived at the farm after a 2-3 days trip. 20% of the gilts of every new group arriving at the farm were sampled the day after their arrival and evaluated (serum) with the rapid test. Percentage of positive samples ranged from 0-40%. A season effect was observed with higher percentage of positive samples on the months of winter and summer.

3) Which results can be expected in the further course of the project (as far as you can estimate them at this point of time)?

Two new studies were initiated which results will be available at the end of the project. A collaborative study has been initiated with pilot chain 1 partners (EGO group). This trial aims to study the use of the rapid test at rearing farms, as a way to categorise farms and to evaluate the performance in the fattening period according to the APP values in the rearing farm.

Another trial is being conducted in collaboration with Module II to gain more information about the relationship between APP levels, productive performance and meat quality traits. In this longitudinal study the pigs will be evaluated during their lifespan and information about health status, growth performance and meat quality traits will be collected for each individual animal. The pigs will be examined every week from birth to slaughter to identify every change in their animal health status. The slaughtering will take place in a commercial slaughter house following the regular processes. Measurements of meat quality parameters will parallel take place. In this regard, blood and meat juice will be collected for APP measurement. This research activity will provide information for a possible implementation of APP measurement into quality management systems. Therefore, the diagnostic value of APP measurement at different time points during the life of fattening pigs will be investigated. The predictive value to expected changes of sustainability parameters and meat quality will be checked and the necessary amount of samples will be evaluated.

4) How can these results be realised (e.g. which technology is used etc.)?

The studies being performed include evaluation of the relationship of different factors such as the effect of sex, season/animal batch, health status, pig performance and the levels of APP/results of the rapid test. The technical performance (reliability) of the test is being evaluated by comparing the results obtained with the quantitative value obtained by ELISA, considered as a reference method.

Average data about productive performance parameters are available at the farms. A more detailed analysis is being performed in some of the studies, including obtaining data for individual animals during their lifespan (longitudinal studies).

5) What are the main steps which remain until the end of the project/runtime of pilot chain?

During the running time of the project the different ongoing studies will be concluded and results evaluated in a final report. Conclusions about the usability of the test for different applications, technical performance and guidelines for implementation at farm level will be included.

The results of pilot chain 3, regarding the use of APP for health and welfare monitoring, will be integrated into a new model for improving the HACCP (Hazard Analysis Critical Control Points) concept at farm level, to be developed in Module II.

6) Which are the innovative aspects of the pilot chain compared to the status quo of the pig and pork sector?

The rapid test developed is the first dipstick method available worldwide for the detection of elevated levels of APP in pigs. The method developed has the advantage of a simple use that does not require laboratory equipment. Furthermore, the results can be obtained fast, facilitating the work of veterinarians and consultants.

The studies performed during the project will result in a new concept for HACCP at farm which will include regular testing of APP. The implementation of these programmes can help to detect problems at the farm and to evaluate the effectiveness of the corrective measures implemented. The advantage of the APP testing is based on its property of being rapid, general and unspecific biomarkers of disease (possibility of detecting all types of problems). Thus, a single analysis can give an overview of the situation at the farm in terms of health and welfare. Presence of elevated levels of APP indicates that animals are under a situation

(disease, bad management causing stress) compromising its welfare and the productivity at the farm.

7) Please explain the collaboration e.g. with other modules within the project

Collaborations with other Modules are summarised in Figure 5. In this regard, pilot chain 3 is linked to Module II. The results obtained in pilot 3, regarding the APP testing at farm level will be incorporated into a new model for HACCP at farm level to be developed in Module II. Module V provided some samples for studying the relationship between APP and meat quality traits. As previously indicated, a collaborative study is being performed with pilot chain 1 partners (EGO) for implementing the rapid test at rearing farms of the group. In collaboration with Module B, activities of pilot chain 3 have been disseminated in different events.

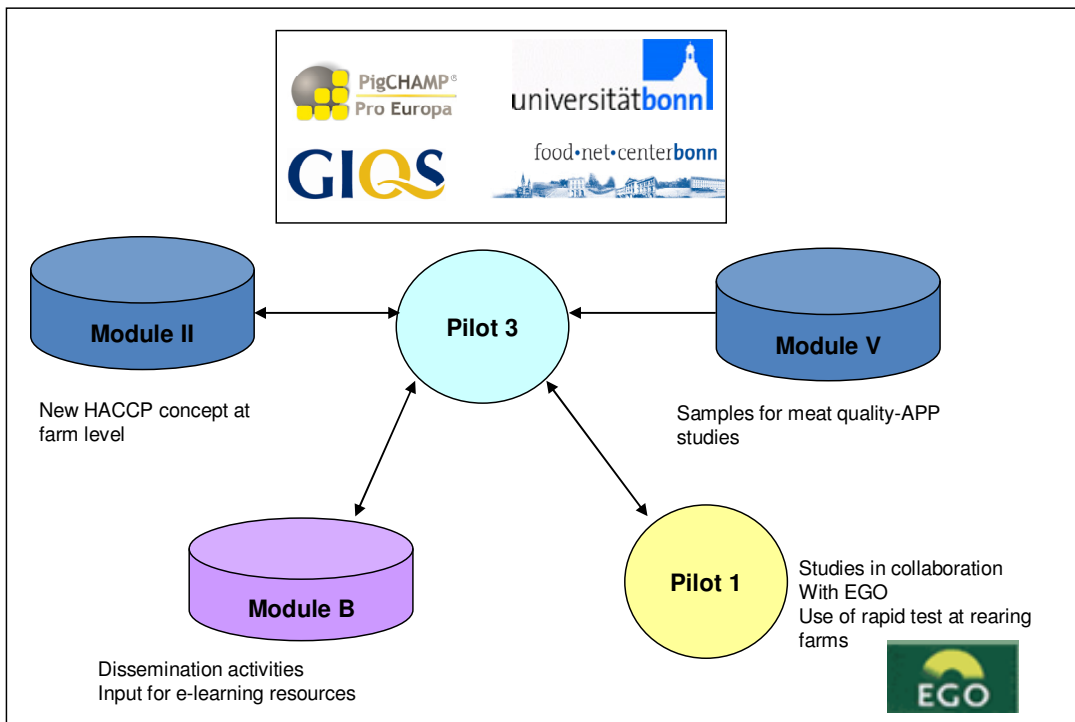


Figure 5: Pilot chain 3 collaborations in the frame of QPC activities

8) What are the main benefits during the daily business?

The main benefits of participating in a project such as QPC which offers so many possibilities of collaboration are not only the opportunities of taking part in new research activities, but also the possibilities of networking, developing new business lines, learn about new technical possibilities and future trends, finding new customers and collaborators etc.

9) What could have been improved in comparison to the status quo in the beginning of the pilot chain?

10) Could management decisions have been improved – if yes in which way? (advantages/disadvantages)?

The APP rapid test offers a good possibility to support management decisions on farm by knowing more about the health status of the animals kept. With these information in mind, an improvement of the information content is being guaranteed and, therefore, a facilitation in the daily business.

11) Please explain the impact of the project in its industry or research sector

The project will provide new tools for quality management at farm level. Counting with a rapid test for evaluating the health status of the pig herds at the farm will benefit the industry. As a result of the pilot activities our knowledge about the possibilities of including an APP monitoring programme in the production chain at farm level has increased. The results obtained in pilot chain 3 will be incorporated into a new concept for HACCP at farm level, offering the industry the possibility to advance towards quality programmes focused on improving animal health and welfare and the sustainability of primary pig production.