Reviving post-mortem diagnostics as a tool to increase porcine herd health and strengthen early detection of pig diseases – the PathoPig project 2014 - 2016

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Summary

Post-mortem diagnostics are an important tool for disease diagnosis and therefore early detection of (re-)emerging animal diseases and zoonoses as well as nationwide disease surveillance programs. To counteract the decline of porcine necropsies in Switzerland over the last ten years, the Federal Food Safety and Veterinary Office (FSVO) launched a national project in 2014 called PathoPig. Post-mortem examinations of pigs from herds with health problems were financially supported by the FSVO. During the first 3 years of the project, the number of pig necropsies increased by 195% (mean). An underlying cause of disease was identified in 74% of the cases. These findings resulted in specific recommendations by the attending veterinarians or by the Swiss Porcine Health Service. A follow-up survey revealed that herd health had improved in 90% of the farms implementing the recommendations.

Keywords

Early disease detection, necropsy, PathoPig, porcine herd health, post-mortem diagnostics

Introduction

Post-mortem diagnostics contribute to nationwide disease surveillance programs in veterinary medicine in Switzerland (Kimpfler et al., 2007; FSVO, 2010; FSVO, 2014; Graage et al., 2015) and are an important tool for early detection of (re-)emerging animal diseases and zoonoses. In Switzerland, currently approximately 6850 pig farms raise approx. 1,500,000 pigs, most of them located in the Swiss Midlands (Federal Statistical Office, 2017). In Switzerland, post-mortem diagnostics of pigs, including gross pathological, histopathological and microbiological investigations are performed at university, cantonal and private pathology laboratories. These examinations provide important information in cases of non-specific clinical signs or diagnoses, thus contributing to timely recognition and treatment of economically important diseases, zoonoses and emerging pathogens (Kimpfler et.al, 2007; Graage et al., 2015). Therefore, porcine post-mortem diagnostics are an important part of preventive veterinary medicine and veterinary public health.

Nevertheless, there has been a steady and marked decline in the number of porcine necropsies in Switzerland over the last years (Gurtner and Posthaus, 2013). Possible reasons for this are of financial (i.e. costs) or of logistic nature (e.g. transport, lack of equipment) and lack of awareness of the importance of necropsies as a diagnostic tool. To counteract this decline and strengthen pig necropsies in porcine health monitoring, the Swiss Federal Food Safety and Veterinary Office (FSVO) launched a project called PathoPig in 2014. The project implements the Swiss Animal Health Strategy 2010+, which aims at protecting and improving animal health FSVO, 2010). Laboratory costs for submission of animals to participating laboratories in case of herd health problems are financially supported by the FSVO. During herd investigations the Swiss Porcine Health Service (SPHS) motivated farmers to participate in the project and additionally carried out a survey on the herd health status of these farms.

The results of the pathological findings obtained during the first three years of the ongoing project are described and discussed in this paper. The main objectives of this study were to evaluate 1) the effect of the project on the number of porcine post-mortem examinations and 2) the effect of increased diagnostic efforts on health status of the individual herds, which participated in this program from 2014 to 2016. Furthermore, information on the most commonly diagnosed porcine diseases by post-mortem examinations in Switzerland is provided.

Animals, Materials and Methods

A total of seven Swiss pathology laboratories participated in the project: two university laboratories (the Institute of Animal Pathology, University of Bern (ITPA) and the Institute of Veterinary Pathology, University of Zurich (IVPZ)); two cantonal laboratories (Zentrum für Labormedizin (ZLMSG), St. Gallen, and the Institut Galli Valerio (GV), Lausanne); three privately owned laboratories: IDEXX Diavet Labor (IDEXX), Bäch; Labor Zentral AG (LZ), Geuensee, and the AG für Tiergesundheit (AGTG), Gunzwil (Fig. 1).

To participate in the project, farmers contacted the herd-attending veterinarian or the SPHS in case of herd health problems. Up to three animals could be submitted, if at least one of the following criteria were fulfilled: High morbidity and/or mortality, unusual clinical signs (e.g. tremor, poor doing, etc.), recurrent problems of unknown etiology resistant to therapy, or increased use of antimicrobials. If these criteria were met, laboratory costs were supported by the FSVO up to a predetermined amount of money, i.e. 200, 400, and 500 CHF for one, two and three submitted pigs respectively, and 400 CHF for breeding pigs. A standardized clinical history protocol, developed for this project, was sent with the pigs to one of the designated pathology laboratories. The clinical history protocol provideded information about observed clinical signs, percentage of affected animals, duration of the health problem as well as information about earlier herd health problems, management, vaccination status and therapy attempts. Upon submission of live animals or carcasses, a necropsy was performed in all cases. According to gross pathological findings, the laboratories performed or sent samples for specific further diagnostics (e.g. histopathological, bacteriological, parasitological and virological analyses) to various specialized laboratories (Institutes of Veterinary Bacteriology, Universities Bern and Zurich; Institutes of Parasitology, Universities Bern and Zurich; Institute of Virology, University of Zurich; Institute of Virology and Immunology in Mittelhäusern; IDEXX Diavet Labor, Bäch). Additionally, the federal Institute of Virology and Immunology tested all pigs for antibodies against the porcine reproductive and respiratory syndrome virus and classical swine fever virus as exclusion diagnostics with the aim of strengthening early detection of animal epidemics and diseases. In Switzerland, certain highly contagious notifiable diseases (e.g. Classical and African swine fever) can be tested by exclusion diagnostics without an isolation period of the farm.

Based on the results of all laboratory analyses, a final diagnostic report was generated. In addition, results for every case were filled in a standardized MS Excel file template and submitted electronically to the FSVO for data collection in a central Access data bank. This data was analyzed according to number of cases, number of submitted animals per case, submission criteria, and age categories of submitted pigs. Furthermore, the data was grouped into geographical distribution of participating farms, occurrence and etiology of disease. Every case was assigned to a predefined disease complex: gastro-intestinal tract (GIT), septicemia, musculoskeletal, polyserositis, insufficient weight gain, respiratory andcardiovascular. If none of these complexes applied to the case, the case was categorized under “other”. For each disease complex the most common underlying agents or diagnoses were documented. Within these analyses, the category “miscellaneous” summarizes diagnoses found in no more than one case per disease complex or of unknown etiology or multiple underlying agents. The data was further analyzed in order to obtain an overview of the current herd health of the Swiss porcine population of participating farms.

Based on the results of the final diagnostic report the herd-attending veterinarian made recommendations. To follow up on implementation and success of these recommendations, the SPHS contacted the farmers 3 to 6 months later by phone or visited the farm. A follow-up survey was performed of all cases in 2014 and 2015, and in 2016 of all cases submitted by the SPHS. The farmers were asked specific questions to evaluate their impression on the herd health and the success of the implementation of the initial recommendations. Implementation of recommendations was classified as: *a) Complete implementation, b) Partial implementation* and *c) No implementation*. In addition, herd health was classified as: *1) Much better, 2) Better, 3) Unchanged* or *4) Worse*.

The project was publicly announced in several journals (FSVO, 2014; Hadorn et al., 2014; Hadorn D., 2015; Hadorn D., 2016; Hadorn D., 2017), at various meetings (Balmer et al., 2014, Balmer et al. 2015, Schediwy et al. 2017) as well as in the FSVO's reports (FSVO, 2014; FSVO, 2015; Graage et al., 2015; FSVO, 2016).

Results

From January 2014 to December 2016, 149 veterinarians and 769 pig farms participated in the project, representing approximately 11% of all pig farms in Switzerland. Altogether, 1165 cases were submitted to the participating laboratories for post-mortem diagnostics. Due to the possibility of multiple animal submissions per case, this resulted in a total of 1897 pig necropsies. The annual number of porcine necropsies (*PathoPig* project necropsies in addition to porcine necropsies performed at both university laboratories beyond the project) in comparison to the number of necropsies in 2013 (n=428) showed an increase of 183% in the first, 202% in the second and 201% in the third year of the project (Fig. 2) with monthly fluctuations. Most cases were submitted from cantons of the Swiss Midlands Lucerne and Bern, followed by Thurgau, Aargau, St. Gallen and Zurich. This finding corresponds with the number of pigs kept in these cantons and reflects the pig densities in different regions of Switzerland.

The number of submitted animals per case is shown in Figure 3. Overall, 52% of the submissions included one animal, 33% two and 15% three animals. Compared among individual years, this distribution remained steady. Most cases submitted for post-mortem diagnostics belonged to the age category of suckling piglets (n= 379, 33%), followed by weaners (n= 328, 28%), growers (n= 258, 22%), breeding pigs (n= 92, 8%) and finishers (n= 80, 7%). In 2% (n= 28) of the submitted cases, the age category was unknown (Table 1).

The two university pathology laboratories performed the majority (n= 822, 71%) of the necropsied cases (Fig. 4), followed by the private laboratories (n = 225, 19%) and the cantonal cases (n=118, 10%). During 2014 and 2016 two of the private laboratories had to close down their necropsy service.

Multiple submission criteria were met in some cases and noted in the clinical history protocol. The main submission criteria between the years 2014 – 2016 were increased mortality and/or morbidity within the herd (40%), followed by unusual clinical signs (26%), recurrent problems of unknown etiology resistant to therapy (24%), and increased use of antimicrobials (10%).

In 74% of all cases, a conclusive pathological diagnosis was obtained, and the underlying cause of disease or death of the submitted animals was identified.

The most commonly diagnosed disease complex was GIT(n= 626 cases, 54% of all submitted cases) followed by septicemia (n= 175, 15%), musculoskeletal (n= 62, 5%), polyserositis (n= 51, 4%), insufficient weight gain (n= 46, 4%), respiratory (n=41, 4%) and cardiovascular(n= 34, 3%). 11% of all submitted cases (n=128) were categorized under the disease complex other (Fig. 5).

In the most commonly diagnosed disease complex GIT, *Escherichia coli* (*E. coli*) infections causing neonatal or post-weaning diarrhea predominated (n=242 cases, 38,7% of all GIT cases), followed by intestinal volvulus (n=111, 17.7%) (Table 2). Other infectious agents, such as a clinical manifestation of *Lawsonia intracellularis* (n=55, 8.8%) or porcine circovirus type 2 (PCV2, n=32, 5.1%) as underlying agents in GIT diseases were found less frequently. The animals affected by infection with PCV2 causing GIT problems were weaners and finishers.

The second most commonly diagnosed disease complex was septicemia in 175 cases (15% of all cases) (Fig. 5). Within this disease complex frequently isolated pathogens were *Streptococcus suis* in 55 cases (31% of all septicemia cases) and *E. coli* in 49 cases (28%). Less frequently isolated agents were *Erysipelothrix rhusiopathiae* (n=7, 4%), *Streptococcus dysgalactiae* (n=3, 2%) and *Trueperella pyogenes* (n=3, 2%). In 58 cases (33%) the etiological agent occurred not more than once or remained unknown and the tentatitve diagnosis was based on macroscopical and histological findings.

Musculoskeletal associated problems were diagnosed in 5% of all submitted cases (n=62). Most commoly diagnosed was polyarthritis (n=16 cases, 26%), followed by osteochondrosis dissecans (n= 8, 13%).

Within the respiratory disease complex (n=41 cases, 4%) bronchopneumonia was diagnosed in 68% (n=28), followed by rhinitis (n=2, 5%), embolic pneumonia (n=2, 2.5%), and interstitial pneumonia (n=1, 2.5%).

In the disease complex insufficient weight gain (n= 44 cases, 4%), the underlying cause of inadequate weight gain remained unclear.

The most common cardiovascular disease complex (n= 34 cases, 3%) finding was mulberry heart disease (n=5 cases, 15%), followed by endocarditis valvularis (n=4, 12%), pericarditis (n=2, 6%) and myocardial infarction (n=2, 6%).

In five cases, notifiable disease agents were identified and in four of those cases characteristic lesions of the respective diseases were detected. Samples from lesions were submitted to appropriate laboratories for culturing and subsequent serotyping or molecular testing (PCR) to identify the agent. In three cases of bronchopneumonia, lesions were compatible with contagious pleuropneumonia and *Actinobacillus pleuropneumoniae* was isolated from lung samples. In one case enzootic pneumonia, caused by *Mycoplasma hyopneumoniae*, was confirmed. Additionally, in one case with salmonellosis the animals showed chronic diarrhea but no macroscopical changes were seen. Non-specific histological changes were seen in the ileum and colon. Bacterial culture and serotyping identified *Salmonella enterica* subsp. *enterica* Serovar Brandeburg.

A follow-up survey was performed in 543 cases. Of those farmers fully implementing the recommended measures (n=357), herd health was assessed in 90% of the cases as "much better" or "better" (FSVO, 2016; Fig. 6).

Discussion

During the first three years of the PathoPig project, the aim to increase the number of pig necropsies performed in Switzerland was clearly achieved. In comparison to the number of porcine necropsies in 2013 (n=428), there was a definite increase over the following three years of the project (183% in the first, 202% in the second and 201% in the third year, Fig. 2). A clear clear association between regional pig densities and the number of submitted cases per canton was seen, with highest submission of cases from cantons with the highest number of pigs

(Lucerne and Bern). Despite closure of two private laboratory necropsy services submission numbers remained relatively stable with monthly fluctuations.

In approximatly ¾ of all cases an underlying cause for the disease was found and only in ¼ of all submitted cases within the project no underlying cause of disease was detected. This shows that post-mortem examinations are a valuable tool to obtain disease diagnoses in pigs. The results of the follow-up survey performed by the SPHS indicate that recommendations voiced by the submitting veterinarian following post-mortem examinations had a positive impact on herd health. Although a scientific evaluation of herd health status before and after submission of pigs for necropsy was not part of this study, farmers reported an improvement of herd health situation due to the specific measures taken. An important advantage of the post-mortem examination is the accurate diagnosis of infectious diseases. Infectious agents can be directly isolated from lesions and a causative relationship between pathogen and disease can be confirmed. These results can assure veterinarians in their clinical diagnoses and help determine optimal treatment options for entire herds, therefore preventing unnecessary or unsuccessful antimicrobial treatment, e.g. in cases of viral rather than bacterial infections. In addition, the increasing number of necropsies helps to reflect the current health status of Swiss porcine herds, as it specifically targets the sub-population of diseased animals in the entire pig population of a country. Possible reasons for detecting no underlying cause of disease in approximatly ¼ of the cases are that either submitted animals did not represent the herd health problem or the decay of the carcass after delayed shipment impaired post-mortem diagnostics. In other cases, the herd health problem, often defined as insufficient weight gain, could have been caused by management factors on the farm (e.g. housing, hygiene) and therefore no specific etiology was detected. Furthermore, in an attempt to limit financial losses, farmers might tend to submit poor doers and chronically diseased animals for necropsy instead of pigs in the acute stage of a condition displaying typical clinical signs. Poor doers are often affected by multiple conditions and it is difficult to obtain representative results for a herd problem. These problems demonstrate the key role of the attending veterinarian as a link between diagnostic laboratories and farmers. The veterinarian should identify suitable animals for necropsy and finally interpret the post-mortem results and diagnoses from the laboratories in the light of the housing and management as well as clinical presentation of the health problem on the farm. Through direct contact with the farmers, veterinarians can convince them to invest in disease diagnostics, monitoring, and prevention as well as targeted therapies, including the use of antibiotics.

Within the PathoPig project, most of the detected infectious diseases represent commonly known herd health problems in Switzerland, with GIT infections being the most prevalent problem. Diarrhea linked to infection with *E. coli* remains one of the most important diseases in suckling piglets and weaners in Switzerland (Graage et al., 2015; Brand et al., 2017). A similar situation is reported from Austria (Schoder, pers. comm.) and Germany (Löbert, pers. comm.).
Lawsonia intracellularis induced enteritis was diagnosed in 5% of all submitted cases even though the estimated prevalence of *Lawsonia intracellularis* among breeding herds was estimated to be around 90 % (SUISAG SGD, 2009). Likewise, diseases associated with PCV2 were rare (3% of all submitted cases). The low prevalence of health problems related to these two pathogens in our study material could be the result of the broad vaccination practice in Switzerland. Nevertheless, diseases linked to these agents are economically relevant regarding their economic impact and animal welfare issues. Due to the high prevalence of asymptomatic carriers of these agents, continuous monitoring of disease incidence and occurrence is indicated. Detection of *Lawsonia intracellularis* is often based on a highly sensitive semi-quantitative

positive chain reaction (PCR) assay (Burrough et al., 2015) in feces. However, due to the high prevalence of the pathogen in pig herds without clinical disease, it is recommended to associate the pathogen with pathological lesions during necropsy and subsequent histopathological examination (with possible immunohistochemistry) for a proper disease diagnosis. This approach allows testing for other etiological agents as well, based on pathomorphological findings.

Our results confirm that the respiratory health of Swiss pig herds appears to be good considering that only 4% of all submissions were diagnosed with primary respiratory diseases. To date, Switzerland is free from porcine reproductive and respiratory syndrome virus (FSVO, 2016). Due to the national eradication program for enzootic pneumonia and contagious pleuropneumonia between1996 and 2004, the prevalence of these diseases has dramatically decreased (Sidler et al. 2015). Thus, we consider the low abundance of primary respiratory diseases as a direct consequence of the low abundacy of these pathogens. It is important to keep this status by continously checking for (re-)emergenge of these pathogens as cause of disease. Through submissions within the PathoPig project, three cases of reportable pulmonary infections, in three cases *Actinobacillus pleuropneumoniae* (biovar 1 serovar 7,12) and, in one case, *Mycoplasma hyopneumoniae* were detected. In each case diagnosis allowed cantonal veterinary services to take appropriate measures to prevent the spread of the diseases, showing that especially in situations with very low disease prevalence, post-mortem analyses are an important tool for early disease detection.

The use of a central data bank at the FSVO containing results and information about the herds proved to be of advantage for surveillance, reporting and communication purposes and it is recommended to follow this approach and apply it to other species and potential data sets (e.g. clinical data) in the future.

Our experience in the PathoPig project shows that sufficient numbers of case submissions to diagnostic laboratories are crucial for maintaining diagnostic capabilities, knowledge about diseases and internal quality assurance. As a beneficial effect, this enables research on various infectious agents causing health problems in pigs. In the case of PathoPig studies on the involvement and zoonotic potential of endoparasites in pigs (Schubnell et al. 2016,) pathogenic *E. coli* (Schneeberger et al. 2017) and antibiotic resistance (Brand et al. 2017) have already been performed.

Besides many advantages of the PathoPig approach, several limitations of the post-mortem analysis in pigs in Switzerland became apparent during the 3-year period.
Although necropsy numbers almost doubled, the increase was lower than initially expected, given the fact that diagnostic investigations were financially supported by the FSVO. About 11% of all pig farms in Switzerland submitted animals within the project. Reasons for the relatively low participation rate are unknown and would have to be investigated in other surveys. Submission numbers remained relatively stable over the entire year, but showed monthly fluctuations. In 2014 and 2015, these fluctuations did not show any particular pattern, but a decrease of necropsies during the summer months was noted in 2016. This decline ran in parallel to the drop in pig meat prices (data not shown). This finding suggests that financial aspects play a predominant role for the farmer’s decision to submit animals for post-mortem diagnostics. Although costs for post-mortem diagnostics were subsidized and potentially covered, it is possible that farmers were not interested in a thorough diagnostic workup due to a low rentability of their operation. Another result pointing in the same direction is the relatively low rate of multiple animal submissions. Despite the obvious diagnostic and financial (due to higher

financial benefits) advantages of submitting two or even three animals, single animals were submitted in approximately 50% of the cases. In multiple animal submissions, suckling piglets and weaners predominated, followed by growers. In the categories of finishers and breeding pigs, the willingness to sacrifice more than one animal to investigate the herd health problem seemed to be much lower. Despite higher financial benefits, even farmers motivated to invest in visits by veterinarians and veterinary care of their animals, could not be motivated to sacrifice additional animals to secure a post-mortem diagnosis.

Pig herd sizes in Switzerland are rather small (less than 400 animals) compared to herds in other European countries as for example Germany, where 74% of the pig farms keep at least 1000 animals (Eurostat. Pig farming sector - statistical portrait, 2014; Deutscher Bauernverband, 2017). Therefore, the losses in a Swiss herd do not affect as many pigs as in the bigger herds abroad and farmers often opt for treating the sick animals. Furthermore, in Switzerland the porcine meat price is defined weekly by supply and demand. As a result, pig farmers hesitate to sacrifice pigs for post-mortem examination and therapy attempts (e.g antimicrobial agents) are often preferred instead.

Regarding costs, the financial support of post-mortem diagnostics by the government was the most effective way to counteract the decline in porcine necropsies. However, only a small amount of all pig farms in Switzerland participated in the project. If post-mortem diagnostics are to be re-established as a regular tool in herd health management, actions must be taken to increase the number of participants.

One of the main factors contributing to a good herd health and enabling quick action in case of (re-)emerging diseases is a high participation of pig farmers in diagnostic examination programs. Besides financial support, other options such as transportation services to pathology laboratories and on-farm necropsies by trained veterinarians could contribute to optimize some of the logistical problems present in Switzerland. Further training involving Swiss diagnostic laboratories could prepare veterinarians for such services. Despite this possibility, a sufficient number of diagnostic laboratories with trained pathologists and microbiologists will be needed to provide further diagnostic analyses and professional support, especially in cases where herd problems cannot be solved by this type of first line on-farm diagnostics.

A professional post-mortem diagnostic service requires adequate infrastructure, trained pathologists and technicians. The provision of such a service generates high costs, which cannot be covered by the revenues from the provided services. This problem was highlighted by two commercial laboratories in Switzerland recently terminating their necropsy service. Already without these closures, 71 % of all PathoPig necropsies were performed at the two university institutes. Here post-mortem investigations are an important part in the education of veterinary students and thus cross financed through teaching budgets. A positive side effect of this is that case submissions can be used for practical training of future generations of veterinarians. The downside of this is that, due to their focus on teaching and research, academic institutions cannot be primarily service oriented, and therefore tend to lack behind private and state run diagnostic laboratories in terms of customer compliance.

Therefore, sustainable long-term options concerning post-mortem diagnostics and all involved parties should be addressed in future to maintain high-standard necropsies without refraining from profitability

Conclusion

Initiation of the PathoPig project in 2014 led to an increase of porcine necropsies and to an improvement of pig herd health after implementation of recommendations based on the final post-mortem diagnostic report. Therefore, it is important to integrate post-mortem diagnostics as a routine tool in herd diagnostics, to support effective and broad monitoring and surveillance activities and contribute to a high herd health standard.

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Figures

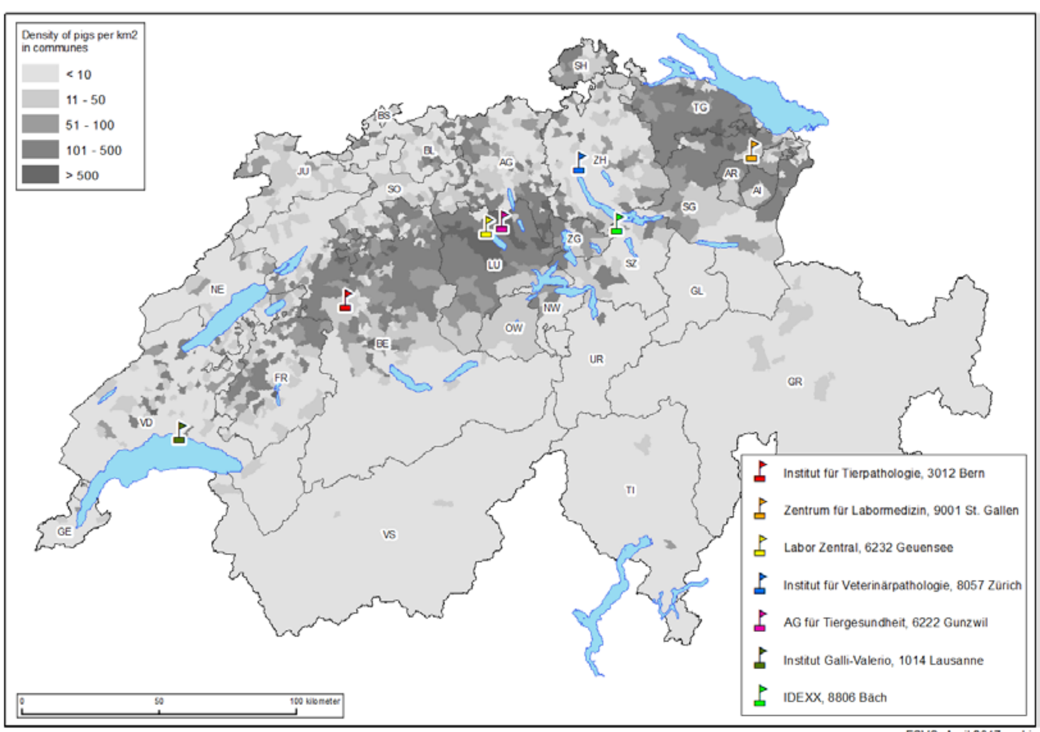


Figure 1: Geographic distribution of participating laboratories in the PathoPig project 2014 – 2016 and density of pigs in Switzerland in 2011.

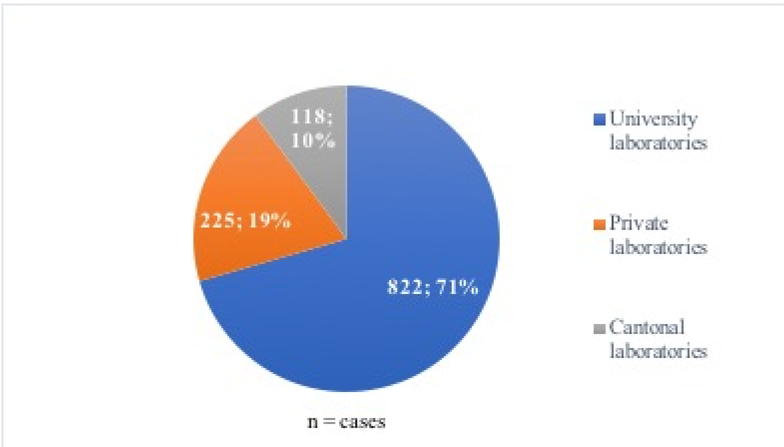


Figure 2: Porcine necropsies in all Swiss pathology laboratories, 2008 – 2016.

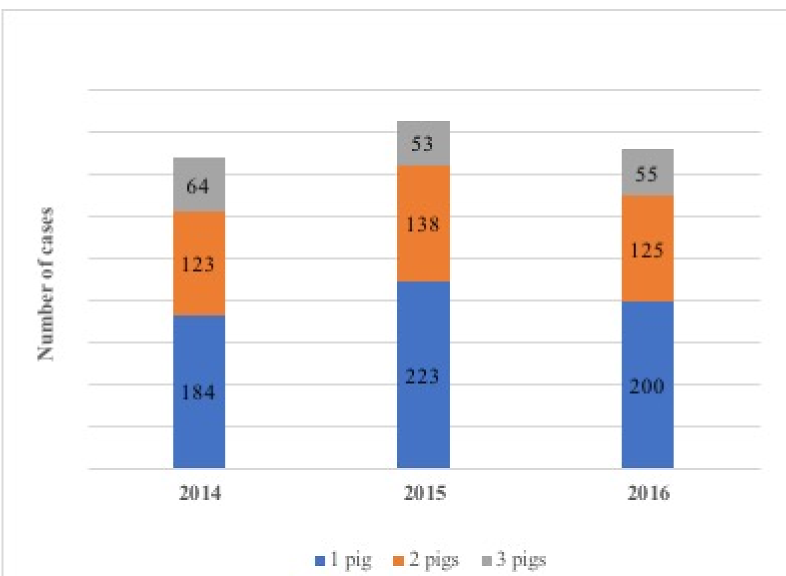
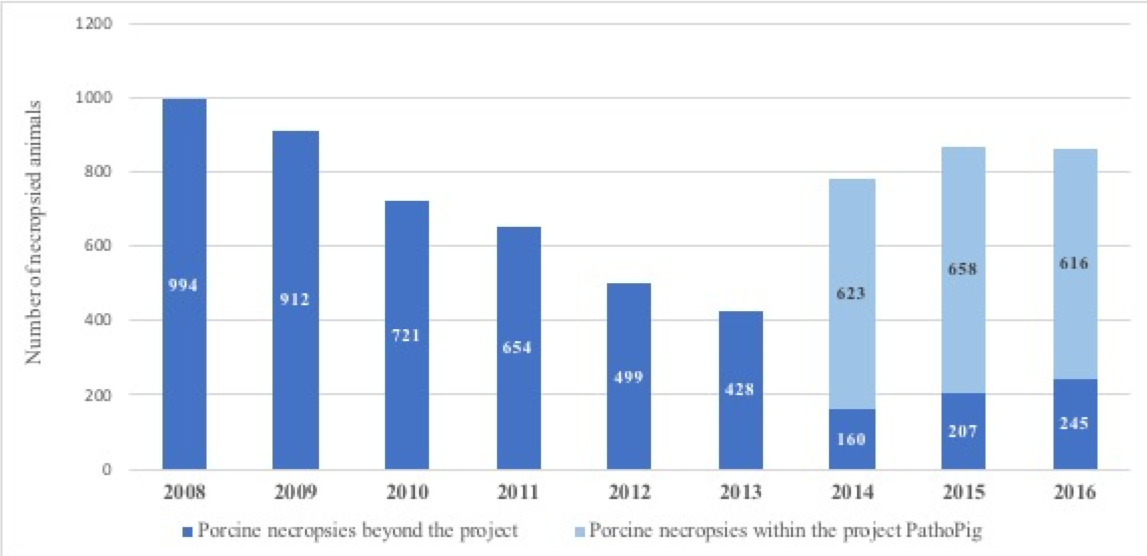


Figure 3: Number of pigs submitted per case (up to 3 animals from a farm showing the same herd health problems), 2014 – 2016.



470 **Figure 4:** Percentage of porcine necropsies performed by participating laboratories within the PathoPig project in addition to the cases from the university laboratories beyond the project, 2014 – 2016.

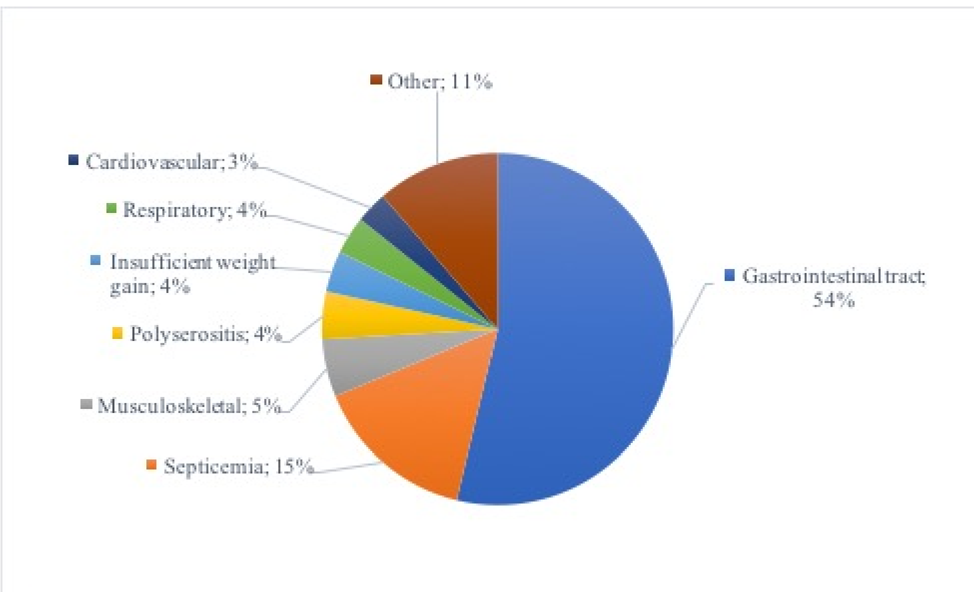


Figure 5: Frequency of disease complexes in the PathoPig project, 2014 – 2016 (n=1165 cases).

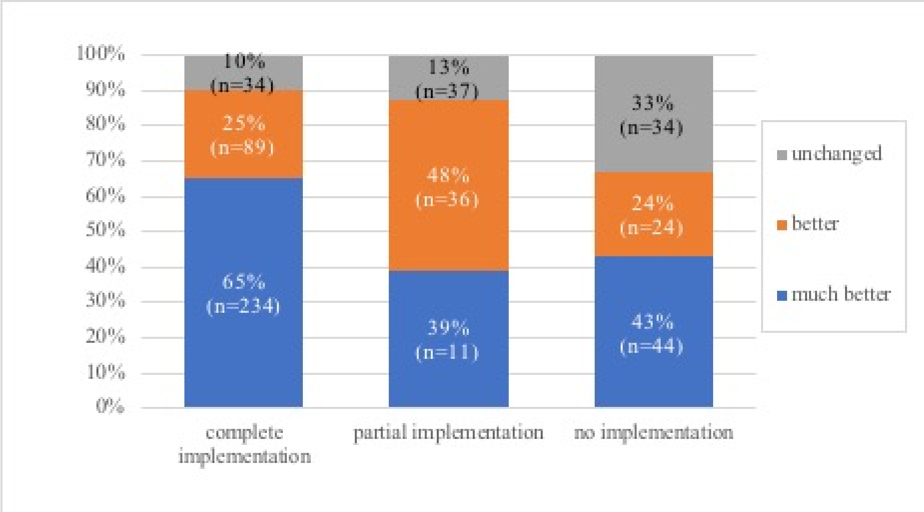


Figure 6: Results of follow-up survey on implementation and success of suggestions to improve herd health (n=543 cases), with full implementation of recommended measures (n=357) and improvement of herd health in 90% (n=321) of these case, 2014 – 2016 (FSVO, 2016).

Age categories (n = number of cases)	**2014**	**2015**	**2016**	**TOTAL**	**%**
Suckling piglets	122	120	137	379	33%
Weaners	107	125	96	328	28%
Growers	84	92	82	258	22%
Finishers	20	28	32	80	7%
Breeding pigs	28	38	26	92	8%
Unknown	10	11	7	28	2%
Total	371	414	380	1165	100%

Table 1: Number of submitted pigs by age category, 2014 – 2016.

Frequency of GIT diseases	**Number of cases**	**Frequency [%]**
E. coli	242	38,7%
Intestinal volvulus	111	17.7%
Lawsonia intracellularis	55	8.8%
PCV2	32	5.1%
Rotavirus diarrhea	30	4,8%
Necrotizing enteritis of suckling piglets (*Clostridium perfringens Type C*)	20	3%
Miscellaneous	136	21.7%
Total	626	100%

Table 2: Number and frequency of gastrointestinal tract (GIT) complex diseases, 2014 - 2016.