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Animal Health

Disease dynamics in piglets in government breeding and backyard farms in Bhutan

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ABSTRACT

The seroprevalenceof porcine circovirus type 2 (PCV2), swine influenza virus H1N1 (SIV, H1N1), and Hepatitis E Virus (HEV) were previously found to be significantly higher in the government pig breeding farms than in the backyard farms. Therefore, there could be a risk of transmission and spread of these diseases (PCV2, SIV H1N1 and HEV only, not other diseases) to the backyard pig farms from government breeding farms through distribution of piglets. The main aim of this study was to to assess the dynamics of these diseases in piglets that are supplied from government breeding farms, with possible difference in infection dynamics between piglets being supplied to backyard farms and piglets retained in the breeding farms as replacement stocks. A survival analysis revealed no differences in overall seroconversions between piglets raised in government farm or in backyard farms, even though seroprevalence for PCV2 and HEV were significantly higher in piglets raised in government herds. Government breeding farms supplying piglets for fattening to backyard farms could promote the spread of diseases that are endemic in these farms. Depending on the damages these endemic diseases may cause in the backyard system, an increased level of disease control and biosecurity measures is warranted.

INTRODUCTION

Pig farming constitutes the livelihood of rural poor to socio-economically disadvantaged people, especially in developing countries. The majority of farmers in developing countries raise a small number of pigs in their backyards, primarily supplementing feed with kitchen waste (Furukawa et al. 2013). Bhutan is no exception, where the pig production system is mostly in the form of traditional backyard farms and is fed with locally available resources including kitchen wastes.

Poor biosecurity, uncontrolled pig movements, and limited knowledge on pig health and diseases among backyard pig farmers in developing countries can significantly influence the spread of infectious diseases (Paton and Greiser-Wilke 2003; FAO 2010). In view of the importance of pig farming in terms of its contribution to rural poor and possible potentials for pig rearing, many developing countries have initiated measures to promote pig farming. Local indigenous pigs are replaced by exotic pigs to help in the development from traditional systems of backyard raising to a more specialized production that will increase production and give farmers a higher income. The supply of pigs from large breeding farms with better biosecurity and hybridized pigs might help in the development of traditional systems of backyard raising into more specialized production and increased production providing farmers a higher income. However, there may be a risk of disseminating infectious agents if they are present in the breeding farms. A study by Monger et al. (2014) raised a concern on the risk of transmission and spread of diseases like Porcine Circovirus Virus type 2 (PCV2), Swine Influenza Virus H1N1 (SIV, H1N1), and Hepatitis E Virus (HEV) to the backyard pig farms through distribution of piglets. These diseases were found to be significantly higher in the government pig breeding farms than in the backyard farms. Therefore, the main aim of this study was to to assess the dynamics of Classical Swine Fever (CSF), SIV (H1N1), PCV2, and HEV infection in piglets originating from large government breeding farms. Of specific interest was to test the possible difference in infection dynamics between piglets being sold to backyard farms and piglets retained in the breeding farms, thus exposed to a different environment.

MATERIALS AND METHOD

Study area and sampling strategy

Selection of piglets

Three batches of piglets from two government breeding farms, Thimphu and Sarpang, were selected for the study (Table 1). The piglets in batch 1 and 3 were selected from Regional Breeding Farm, Thimphu. The farm now has been named as National Swine Nucleus Breeding Centre (NSNBC). The piglets in batch 2 were selected from National Pig Breeding Center, Sarpang. The study was carried out from August 2011 to January 2012. Within each batch, individual pigs were either sold to a backyard farms at the age of approximately 70 days or remained in the government farm as replacement breeding stock. The pigs in batch 1 and 2 were sampled five times and in batch 3 four times. The mean age of these pigs on each sampling was respectively 21, 70, 84, 132, and 242 days.

Table 1 Piglets from two government breeding farms grouped into three batches for longitudinal study.

Batch	Government Farm, Group	Total animals
1	Thimphu, Backyard	10
	Thimphu, Government	17
2	Sarpang, Backyard	10
	Sarpang, Government	15
3	Thimphu, Backyard	20
	Thimphu, Government	20

Sample collection
Blood samples were collected, using Sarstedt Monovette syringes from all selected pigs. Serum was obtained by centrifugation of the coagulated blood samples and stored at -20^{o} C until testing.

Laboratory tests

Antibodies against classical swine fever virus (CSFV)
The serum samples were tested in virus neutralisation test (VNT) against a cell culture adapted Alfort/187 strain (Dahle and Liess, 1995) of CSFV as described by (Terpstra et al., 1984), with validated sensitivity of 93% and specificity of 100% at Central Veterinary Institute (CVI), the Netherlands. The cell-adapted C-strain was chosen as most representative for the C-strain vaccine from Bhutan. Serum samples were considered to have antibodies against CSFV if it was positive to the CSFV strain in the VNT with titres ≥ 10.

Antibodies against swine influenza virus (SIV)
Serum samples were tested in a haemagglutination inhibition (HI) test using A/Netherlands/602/2009 (pandemic H1N1) antigen (Munster et al. 2009). The HI test was performed according to the standard procedures (OIE, 2008) and standardized using four haemagglutinin units (HU) per well. The starting dilution for testing sera was 1:10. Sera with a titre ≥20 were considered positive.

Antibodies against porcine circovirus type 2 (PCV2)
Serum samples were tested in a commercially available indirect ELISA (Porcine Circovirus 2 Antibody Test Kit; BioChek BV, The Netherlands) according to the manufacturer's instruction. The sensitivity and specificity of the kit were previously estimated at 85% and 95.6% respectively (Štukelj et al., 2014). The presence or absence of antibody to PCV2 was determined by calculating the test sera-to-positive (S/P) ratio. Samples were considered positive if the S/P ratio was ≥ 0.5.

Antibody detection against HEV
Antibody against HEV (IgG) in serum samples was measured using an enzyme-linked immunosorbent assay (ELISA) kit, as described earlier (Van der Poel et al., 2014). This essay has an estimated sensitivity and specificity of 84% and 89% respectively. Samples with Percent Positivity (PP) values < 25 PP were negative and samples with PP values ≥ 25 PP, positive.

Data analysis
A cox proportional hazard survival model was used to find out the effect of group (backyard and government) and batch (1, 2 and 3) on the seroconversion of specific disease (CSF, SIV, PCV2 and HEV) over time. The dependent variable was the outcome of a specific disease (CSF, SIV, PCV2 and HEV) (1 = positive, 0 = negative) and the independent variables were the group (backyard = 1, government = 2 farm) and batch (Thimphu 1 = 1, Sarpang = 2, Thimphu 2 = 3). The statistical analysis was done using SPSS and R (version 3.0.0; R Foundation for Statistical Computing, Vienna, Austria).

For each of the piglets in the study, the approximate moment of seroconversion was determined. Observation of seroconversions was hampered by the presence of maternal antibodies. Antibodies against each of these diseases in the first sampling were assumed to be maternal antibodies and not as a result of a sero-conversion. Thereafter, the pigs were considered to have seroconverted as soon as they fulfilled one of the following criteria;

1) A pig with a negative test result in any of the samplings becoming positive in the subsequent sampling
2) A pig with a positive test results in any of the samplings, showing a significant rise in titre in the subsequent sampling (for CSF and SIV defined as a fourfold increase and for PCV2 and HEV an increase of the S/P and PP by 0.3 and 0.2, respectively).
3) A pig with a positive test result in the first sampling, showing at least the same titre (CSF, SIV), S/P (PCV2) or PP (HEV) in the second sampling. This criterion assumes that titres of maternally derived antibodies should significantly be reduced between the first and second sampling, with a lack of such a reduction suggesting an active immune response.

RESULTS

Seroprevalence of CSF, SIV, PCV2 and HEV in piglets

CSF
Except for the batch 1 (backyard group) and batch 3 (government group), the seroprevalence of CSF was constant (Table 2), there was significant difference in the seroprevalence of CSF between batches. The seroprevalence differ between repeated sampling time. However, there was no significant difference in the seroprevalence between backyard farms and government breeding farms.

SIV (H1N1)
There was significant decrease in the seroprevalence over time between batches, with no seropositive animals in the last sampling (Table 3). There was no significant difference in the seroprevalence between backyard group and government group.

PCV2
The seroprevalence of PCV2 was significantly higher in the government group than backyard group. The animals in the government breeding farms were 2.23 times likely to be seropositive against PCV2 than animals in the backyard farm. The seroprevalence increased over time (Table 4). The seroprevalence was significantly higher in the third batch than other two batches.

HEV
The seroprevalence of HEV differed significantly between the backyard and government group. The animals in the government breeding farms were 2.77 times likely to be seropositive against HEV than animals in the backyard farm. The proportion of seropositive animals increased significantly over time (Table 5). The seroprevalence in three batches differed significantly. The proportion of seropositive animals increased significantly over time.

Sero-conversion to CSF, SIV, PCV2 and HEV in piglets
The seroconversion against CSF, SIV (H1N), PCV2 and HEV in batch 1, 2, and 3 are shown in Figure 1.

CSF

The seroconversion in batch 1, batch 2 and batch 3 were 15% (4/27), 16% (4/25) and 37% (14/38). There was significant difference in the seroconversion between three batches, with animals in the batch 3 taking shorter time (p=0.001) to seroconvert compared to batch 1 and 2. The group (backyard and government) did not have any effect on the seroconversion to CSF. There was no significant difference in the sero-converted animals between backyard and government group.

SIV (HN1)

There were not many sero-conversion against SIV (H1N1) except for five animals in batch 3.

PCV2

Overall, the seroconversion in batch 1, batch 2 and batch 3 were 89% (24/27), 92% (23/25) and 92% (35/38), respectively. Pigs in batch 3 had significantly high risk of getting infected by getting infected earlier against PCV2 than other batches. The

Table 2 Seroprevalence against PCV2 in different batches of piglets in a longitudinal study. The total period of sampling in batch 1 and 2 are five times and in batch 3 is four times.

Batch	Time				
	1	2	3	4	5
	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI
Backyard					
Batch 1	80% (10), 44-97%	50% (10), 27-73%	70% (10), 35-93%	80% (10), 44-97%	80% (10), 44-97%
Batch 2	100% (10), 69-100%	70% (10), 35-93%	60% (10), 26-88%	56% (9), 21-86%	88% (8), 47-100%
Batch 3	55% (20), 32-77%	35% (20), 15-59%	84% (19), 60-97%	86% (14), 57-98%	No sampling
Overall	73% (40), 56-85%	48% (40), 32-64%	74% (39), 56-85%	76% (33), 58-89%	83% (18), 59-96%
Government					
Batch 1	88% (17), 64-99%	35% (17), 14-66%	47% (17), 23-72%	47% (17), 23-17%	88 (17), 64-99%
Batch 2	80% (15), 52-96%	87% (15), 60-98%	93% (15), 68-100%	60% (15), 32-84%	93% (14), 66-100%
Batch 3	94% (18), 73-100%	88% (17), 64-99%	100% (15), 78-100%	100% (15), 78-100%	No sampling
Overall	88% (50), 76-95%	71% (49), 57-93%	79% (47), 64-89%	64% (47), 49-77%	90% (31), 74-98%

Table 3 Seroprevalence against HEV in different batches of piglets in a longitudinal study. The total period of sampling in batch 1 and 2 are five times and in batch 3 is four times.

Batch	Time				
	1	2	3	4	5
	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI
Backyard					
Batch 1	60% (10), 26-88%	30% (10), 6-65	40% (10), 12-74	100% (10), 69-100%	100% (10), 69-100%
Batch 2	60% (10), 26-88%	0% (10), 0-30%	10% (10), 0.2-45%	0% (9), 0-34%	50% (8), 16-84%
Batch 3	25% (20), 9-49%	10% (20), 1-32%	63% (19), 38-84%	93% (14), 66-100%	No sampling
Overall	43% (40), 27-59%	13% (40), 4-27%	44% (39), 29-60%	70% (33), 51-85%	78% (18), 52-94%
Government					
Batch 1	59% (17), 33-82%	35% (17), 14-66%	29% (17), 10-56%	100% (17), 75-100%	94 (17), 71-100%
Batch 2	40% (15), 16-68%	27% (15), 08-55%	7% (15), 0-32%	93% (15), 68-100%	100% (14), 77-100%
Batch 3	72% (18), 47-90%	100% (17), 80-100%	100% (13), 75-100%	100% (12), 74-100%	No sampling
Overall	58% (50), 43-72%	55% (49), 40-69%	42% (45), 28-58%	98 % (44), 88-100%	97% (31), 83-100%

Table 4 Seroprevalence against SIV (H1N1) in different batches of piglets in a longitudinal study. The total period of sampling in batch 1 and 2 are five times and in batch 3 is four times.

Batch	Time				
	1	2	3	4	5
	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI
Backyard					
Batch 1	80% (10), 44-97%	60% (10), 26-88%	60% (10), 26-88%	0% (10), 0-31%	0% (10), 0-31%
Batch 2	80% (10), 44-97%	20% (10), 2-56%	0% (10), 0-30%	0% (9), 0-34%	0% (8), 0-37%
Batch 3	40% (20), 19-64%	40% (20), 19-64%	32% (19), 13-57	0% (14), 0-23%	No sampling
Overall	50% (40), 34-66%	30% (40), 17-47%	31% (39), 17-48%	0% (33), 0-11%	0% (18), 0-19%
Government					
Batch 1	100% (17), 80-100%	94% (17), 71-100%	71% (17), 44-90%	0% (17), 0-20%	0% (17), 0-20%
Batch 2	67% (15), 38-88%	40% (15), 16-68%	0% (15), 0-22%	0% (15), 0-22%	0% (14), 0-23%
Batch 3	56% (18), 31-78%	33% (18), 13-59%	40% (15), 16-68%	0% (15), 0-22%	No sampling
Overall	74% (50), 60-85%	82% (50), 68-91%	38% (47), 25-54%	0% (47), 0-7%	0% (31), 0-11%

Table 5 The seroprevalence against CSF in different batches of piglets in a longitudinal study. The total period of sampling in batch 1 and 2 are five times and in batch 3 is four times.

Batch	Time				
	1	2	3	4	5
	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI	% (n), 95% CI
Backyard					
Batch 1	100% (10), 69-100%	100% (10), 69-100%	100% (10), 69-100%	100% (10), 69-100%	80% (10), 44-97%
Batch 2	100% (10), 69-100%	100% (10), 69-100%	90% (10), 56-100%	100% (10), 69-100%	100% (8), 63-100%
Batch 3	100% (20), 83-100%	100% (20), 83-100%	74% (19), 49-91%	86% (14), 57-98%	No sampling
Overall	100% (40), 91-100%	100% (40), 91-100%	85% (39), 69-94%	94% (34), 80-99%	89% (18), 65-99%
Government					
Batch 1	100% (17), 80-100%	100% (17), 80-100%	100% (17), 80-100%	100% (17), 80-100%	100% (17),80-100%
Batch 2	100% (15), 78-100%	100% (15), 78-100%	100% (15), 78-100%	100% (15), 78-100%	86% (14), 57-98%
Batch 3	100% (18), 81-100%	94% (18), 81-100%	80% (15), 52-96%	100% (15), 78-100%	No sampling
Overall	100% (50), 94-100%	98% (50), 89-100%	94% (47), 82-99%	100% (47), 92-100%	94% (31), 79-99%

median age for the infection was 80 days. There was no significant difference in seroconversion against PCV2 between backyard and government group.

HEV

The seroconversion HEV in batch 1, batch 2 and batch 3 were 100% (27/27), 76% (19/25) and 86% (32/37. The pigs from batch 3 had higher risk of getting infected with median age of seroconversion at 82 days. The backyard and government group did not have any effect on the seroconversion to HEV. There was no significant difference in the seroconversion time between backyard and government group.

DISCUSSION

The main aim of this study was to to assess the dynamics of SIV (H1N1), CSF, PCV2 and HEV infection in pigs through serological study. For this purpose, a field study was carried out in which 90 piglets were selected from two government breeding farms and divided into two groups (backyard and government) within three batches (1, 2 and 3) which were then followed over time to see dynamics of infection against CSF, SIV (H1N1), PCV2 and HEV. However, due to the practice of vaccination against CSF in the government farms, no conclusions could be made regarding the circulation of CSFV from this study. Most seropositive are the result of the

vaccination. In the backyard group, the animals were followed up in the government breeding farms until 70 days and thereafter at the backyard farms until the age of 242 days, whereas in the government group the animals were followed up in the government farm throughout the study period.

The CSF vaccination in the government breeding farms is done soon after the piglets are weaned at 45-50 days, before they are sold to the farmers in the backyard farms. The seroprevalence in the first and second batch of piglets in the backyard and government group varied over time, with decline towards the last sampling (Table 2). This could be because of delay in the vaccination due to unavailability of vaccine at that time. Most SIV infections take place after the decay of maternal antibodies which occurs after 10 weeks of age (Loeffen et al. 2009; Loeffen et al. 2003). In this study, it was found that, the antibodies waned over the period of time, as the piglets grew older (Table 2). By the end of the study (last sampling), none of the animals had antibodies. It reveals that the piglets had maternally derived antibodies against SIV initially which eventually declined. The study also showed that, there was hardly any seroconversion taking place in the piglets, indicating no new infection. However, in the longitudinal study results in sows indicated that the prevalence of SIV (H1N1) was consistent in sows. This could be attributed to close contact between human (pig handlers) and pigs, as the SIV (H1N1) circulating in pigs in Bhutan have been found to be of human origin (Monger et al. 2014). There has been reports of pandemic H1N1 in humans in Bhutan (Wangchuk et al. 2012).

Among the three batch groups, the pigs in batch 3 had high risk of seroconversion against CSF, PCV2 and HEV and there was early seroconversion compared to batch 1 and 2. In our study, the pigs were known to be infected by 246 days, against PCV2, which were at high risk of shedding the virus, as the PCV2 is shed in similar amounts by nasal, oral and faecal routes at least until 209 days post-farrowing (Patterson et al. 2011).

HEV infection in pigs generally occurs at about 2-4 months of age (Meng 2003). In this study, the infection of piglets took place between the age of 82–134 days. All the piglets were already seropositive in the beginning of the study with maternal

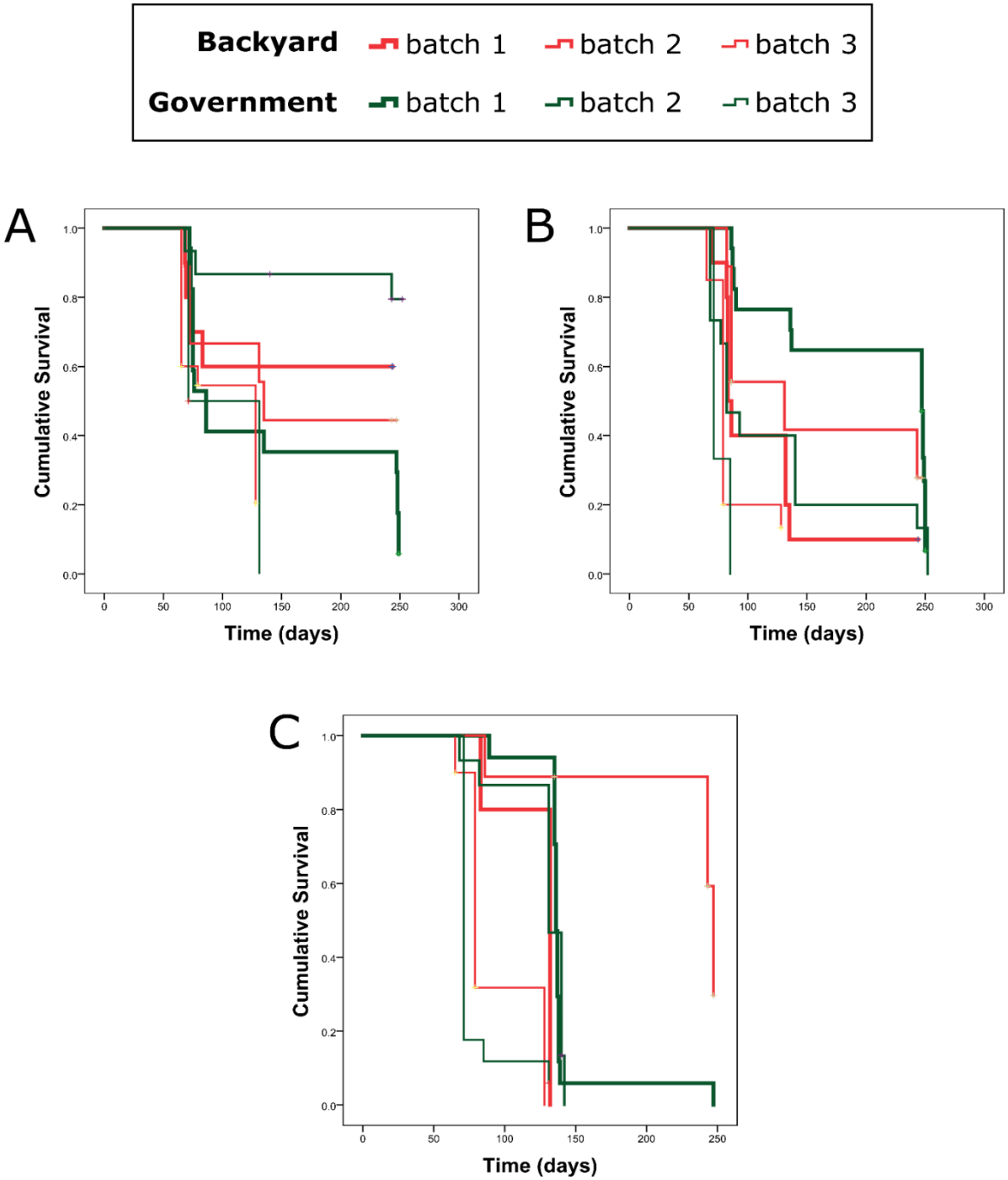

Figure 1 The survival curves for batch 1, 2 and 3 raised in backyard vs those raised in government farms.

antibodies. There was a brief decline at about 70 days, and then there was rise in antibodies until the end of the study, which clearly indicates infection after the decline of maternally derived antibodies. Slight differences in antibody dynamics of HEV infection were found in the three batches. Batch 3 showed evidence of HEV infection at 82 days, whereas batch 1 and 2 pigs started to develop antibodies late at 134 and 159 days of age. The study indicated that animals are infected during early life and could still be infected at slaughter age (8-9 months), representing a risk for food security. Further study on pigs as potential sources of human exposure to HEV would be important by testing of faecal samples from pigs by PCR, which would help in further identifying the strains, circulating in pigs. The study showed that animals in the government farms are endemic for PCV2 and HEV, indicating that the government breeding farms are more likely to be source of infection in the backyard farms. Once in the backyard farms, it is likely to be aggravated further due to poor biosecurity and hygiene. Therefore, it is important to regularly monitor the disease dynamics in government farms prior to distribution to the farmers and also improve the farm biosecurity at the village backyard farms.

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